Scientia Herpetologica

Papers submitted from 7th O.G.M. of Societas Europaea
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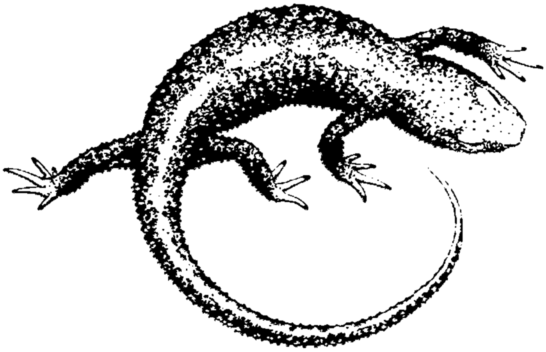

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CONTENTS

Systematics, Taxonomy and Evolutionary Trends

Anatomy and Morphology

Biology and Ecology

Physiology

Parasitology

Distribution

Conservation

PRESENTATION

The initial idea of this book was a meeting occurred in September 1993 in the city of Barcelona: the 7th Ordinary General Meeting of the Societas Europaea Herpetologica. During 5 days, more than 200 herpetologists from all the European countries and other world countries discussed about many ideas around the biology of the Amphibians and Reptiles.

The organization of this meeting was charged to the herpertologist team of the *Departament de Biologia Animal* (*Vertebrats*) of the University of Barcelona, Spain. Two years ago of that event, the Organizing Commitee want to express its gratitude to all the participants for their tolerance and good humour, the personal of the Biology Collegue for their good work, the Asociación Española de Herpetologia and Societas Europaea Herpetologica for financing grants to some people and a lot of anonomous people for their very important help.

As a result of the work of all the herpetological participants, *Scientia Herpetologica* was born, a book which aim is show the last scientific investigations studies on different subjects of the live of the Amphibian and Reptiles species.

Scientia Herpetologica is composed by 72 papers which indicated the high confidence in us of the herpetologist who participate in Barcelona. We are grateful to all authors for their participation in this book, for their patience in seeing it in print and for the revision of their papers to obtain finally a uniform format in all the papers.

We thank many investigators of our Department for the review of the papers.

Finally, we extend our sincere gratitude to Asociación Herpetológica Española and Societas Europaea Herpetologica for financing this publication.

The Editors

SPEECH OF THE PRESIDENT OF THE S.E.H.

Authorities! Ladies and Gentlemen! ¡*Queridos amigos y colegas*!

It is for me a great pleasure and a much greater honour to have the undeserved privilege to open the **7th Ordinary General Meeting of the Societas Europaea Herpetologica**.

Since León, 1983, this is the second time that the congress of our Society takes place *en Tierra de España*. This is further proof that my closing words uttered in Budapest about the Spanish people were right: once their doors had opened to democracy they took giant steps in every field, and, of course, in herpetology as well. Although I am -as everybody knows- a citizen of the world, let me however be proud to acknowledge that this time even the richest and most advanced nations may actually look up with confidence and hope to a Latin Country. Richer nations, for sure, but may be not so much rich in that sort of humanity which here seeps out of any lump of earth and materialized in any handshake and any glance.

I am very ashamed to confess that I am setting foot in Spain after more than thirty years. This has happened not through indifference but because my life circumstances decided differently. On the other hand I should blush even more to admit that I know Galápagos, Tierra del Fuego and Somalia better than my Tuscany... Anyway, like most of my foreign Colleagues, I hope to be able to come to Spain as soon as possible to taste again your warmth and beauties. *¡Ojalá fuese verdad!* All the more so because, according to Cervantes, "*El andar tierras y comunicar con diversas gentes hace a los hombres discretos*".

Our scientific papers, even the most valuable ones, will sooner or later become out of date, but the memories of this stay in Spain will always live with us. For this reason and not only for their organizing efforts, I would like to thank -also on behalf of SEH- all the people who enabled us to participate in this congress.

Thanks again to all of you !

Benedetto Lanza.
President of the Societas Europaea Herpetologica

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Amphibian cytogenetics, evolutionary biology, and developmental biology

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Abstract: In the last 20 years, amphibian cytogenetics has been greatly improved by several banding techniques and radioactive and nonradioactive *in situ* hybridization methodologies, through which the 2 basic criteria of karyotyping, namely chromosome identification and recognition of homologs, result to be quite unambiguous. Also the study of giant lampbrush chromosomes has been renewed by the new techniques: accordingly, the conventional maps, built in several species mainly on the basis of morphological diagnostic markers detected on lampbrush chromosomes (such as location of centromeres, peculiar loops, nucleolar organizers, number and localization of spheres; number and localization of chiasmata ecc.), are at present enriched with data on location of important genes, as well as of chromosome- and/or loop- specific proteins revealed by immunostaining with monoclonal antibodies: the last approach has been proved extremely valuable in examining genome organization and establishing more precise homologies between chromosomes and/or segments of chromosomes belonging to karyotypes of related species. Therefore, cytogenetics of amphibians has been given in the last few years a relevant contribution in order to clarify the cytological mechanisms of peculiar modes of reproduction, namely that characterizing the European green water frogs of the hybrid form, "*R. esculenta*". Moreover, amphibian cytogenetics, paralleling or encouraging the researches with isozyme electrophoresis, the studies with restriction enzymes, as well as DNA cloning and sequencing, has greatly contributed to improve the body of knowledge of chromosome mechanisms of speciation and evolution at molecular level. Finally, the use of monoclonal antibodies has resulted to be important in highlighting nature and complexity of the genetic bridge connecting oogenesis to both early development and cytological differentiation by means of the characteration of chromosomal and nuclear (maternal) proteins which migrate from oocyte into developing embryo at fertilization.

Key words: chromosomes, genomes, genes, development, speciation, evolution.

*This paper, read during the 7th S.E.H. Ord. Meet. in Barcelona, is dedicated to the memory of Prof. H.G. Callan, F.R.S. and Prof. Emeritus of the University of St. Andrews, Scotland, who died on November 3, 1993. Prof. H.G. Callan was one of the greatest students of lampbrush chromosomes in the last few decades and played a primary role in the transition from conventional to molecular amphibian cytogenetics. I first met him in 1961-62 when I was in St. Andrews for learning the ways of studying lampbrush chromosomes, and I was impressed very much by not only his experience and expertise but also his outstanding personality and friendship. Since then, he often provided me with precious advices and encouragements. Prof. Callan will remain in my memory.

INTRODUCTION

In a recent survey of the first hundred volumes of "Chromosoma", CALLAN (1992) has evaluated the importance for cell biology of some of the work on chromosomes published in that journal, since it first appeared in 1939. The review points out the astonishing contribution given to nuclear cytology over the past 50 years by the cytogenetic studies performed on the two types of giant chromosomes known in animal cells, namely the polytene chromosomes in the salivary glands of the dipteran larvae and the lampbrush chromosomes of amphibian oocytes.

If one could piece together in a single review all the results of amphibian cytogenetic studies published over the same period also in other specialized journals, a far vaster and more complete set of information would be available. In particular, the "tremendous impact" of the several banding and *in situ* hybridization techniques on cytogenetics would be placed in greater evidence, because the present methodologies have actually made the two basic criteria of karyotyping, that is chromosome identification and recognition of homologs, absolutely unambiguous.

However, the present amphibian cytogenetics does not mean only karyotyping, because the progress of cytogenetics, ensured by the continuous advances made in molecular biology techniques, is more and more leading to studying structure of chromosomes and chromosomal nucleic acids and proteins. These techniques include the use of restriction enzymes, DNA cloning and sequencing, as well as the use of monoclonal antibodies to identify both chromosomal proteins and their coding genes. Effective nonradioactive *in situ* hybridization systems, which imply the use of DIG-(digoxigenin), biotin-, or fluorescein nucleotides for direct as well indirect fluorescent *in situ* hybridization experiments (FISH technique), have provided safe technical conditions and have greatly increased the accuracy and speed of results. Such techniques can be used to identify chromosomes, detect chromosomal abnormalities, or determine the chromosomal location of specific sequences. With FISH it is possible to detect RNA species. The potential simultaneous detection of DNA and RNA of individual genes makes FISH a powerful technique in the study of the nuclear architecture (Hulspas & Bauman, 1992). Then, FISH technique plays an increasingly important role in a variety of research areas, including chromosome and gene mapping in amphibians, since introduction of such technique in the late 1970s.

At last, also the use of confocal laser scanning microscopy must be considered rich in pōtential, as it is the best method to obtain quantitative information on concomitant 3-D localization of genes and chromosomal proteins, associated to computer methods in image analysis.

The introduction of sophisticated molecular techniques in chromosome studies has created the new branch of amphibian molecular cytogenetics, which has conserved the same interests towards naturalistic cytogenetics which traditionally deals with amphibian phylogeny (such as searching for an evidence supporting lissamphibian monophyly or polyphyly of the Lissamphibia), while widening its scope to the detection of the several genetic functions related to single chromosomes and single transcription units.

A complete picture of all chromosome studies carried out so far in amphibians cannot be illustrated here; regrettably the present paper cannot include all the authors who have contributed, in no mean way, to the progress of amphibian cytogenetics, improving the general knowledge of phylogenetic relatedness, karyophylogenies and the basic mechanisms of speciation and chromosome evolution by means of every possible molecular technique on DNA, nucleic acids and chromosomal proteins.

FROM CONVENTIONAL TO MOLECULAR AMPHIBIAN CYTOGENETICS

The most salient improvement of cytogenetic investigations has been developed in the following nine research areas:

1-Chromosome numbers; banded mitotic karyotypes; meiosis
2-Giant lampbrush chromosomes
3-Extensive and minute rearrangements
4-Sex-chromosomes
5-Genome size values
6-Polyploidy
7-Microchromosomes
8-Supernumerary chromosomes (B-chromos.)
9-Spontaneous and natural hybridization

Area no. 1 - In most amphibian species, mitotic and meiotic chromosomes are relatively large and easy to submit to banding and molecular techniques: therefore, chromosome numbers and C-banded karyotypes are known in a good number of species of the three orders (about half of urodele species; about one/fourth of the total number of amphibian species) (Table I). The cytological mechanism of meiosis was described long ago, and reasonably it is shared by all amphibian species (cf. White, 1973). Meiosis is a very delicate stage of the biological cycle of the organisms, and can be extremely important for differentiating populations and defining the process of chromosome speciation (Mancino *et al.*, 1979a; Mancino, 1990, 1991).

Table I

Approx. number of species investigated	Chromosome numbers*
Orders	
- Caecilians (Gymnophiona):	
16 out of 163 (= 9.8%)	20 - 42
- Salamanders (Urodela):	
155 out of 357 (= 43.4 %)	22 - 78
- Frogs (Anura):	
833 out of 3521 (= 23.6 %)	14 - 104
- Total:	
1004 out of 4041 (= 24.8 %)	

*After Olmo (1990), King (1990), and Green & Sessions (1991). In subsequent years, the number of banded karyotyped has increased, but there has not been significant variation in chromosome numbers.

However, chromosome studies must be increased especially in Caecilians where the growth of knowledge has lagged far behind that of both salamanders (Caudata) and frogs (Anura) (Nussbaum, 1991). Almost all aspects of caecilian biology are poorly studied, and caecilian cytogenetics is no exception. In fact, caecilians are restricted to the tropics and are difficult to find; then chromosome studies relate to few available specimens and are restricted to few groups (Wake & Case, 1975; Wake, 1987; Wake *et al.*, 1980). However, it is possible to discuss chromosomal evolution of caecilians in an evolutionary framework despite the lack of a stable phylogeny and classification of caecilians makes it difficult to interpret the limited data on variation in caecilian chromosomes (Nussbaum, 1991). Knowledge of cladistic and phylogenetic relationships within the group is still scarse or problematic and based mainly on morphological ground. However, a convincing analysis of the evolutionary history of caecilians is at an interesting and stimulating starting point after the work recently published by Jenkins and Walsh (1993).

Chromosome studies need to be improved also primitive families of salamander and frogs (cf. Kohno *et al.*, 1991), to add information to the knowledge of phylogenetic relations and macroevolutionary cytological patterns (such as changes in band position; Robertsonian fissions/fusions, being centromeric fusion and fissioning the dominant process of karyotype evolution of amphibians; conserved linkage groups throughout the vertebrates; evolution of sex-determining mechanisms), which can be reciprocally informative. Also the extent of chromosome abnormalities at meiosis in species hybrids can be used for evaluating the "cytogenetic distances" between parental species (Mancino *et al.*, 1978, 1979a and b; Mancino, 1990, 1991).

Area no. 2 - Ovarian oocytes of amphibian species contain giant lampbrush chromosomes, the fine morphology of which can be studied in detail. Therefore, the single chromosomes can be schematically mapped on the basis of conventional diagnostic cytogenetic markers, such as peculiar lateral loops, centromeres, granules and globules, chiasmata, and other organelles (such as chromosomal spheres and nucleoli). These maps, constructed especially in the 1960's, 1970's and the first half of the 1980's in several species of newts and salamanders (cf. Callan & Lloyd, 1975; Callan, 1986), can now easily be brought up to date with identification and location of the genes for 18S+28S and 5S ribosomal RNAs, 4S RNA, and of certain repetitive DNA families (cf. de Lucchini *et al.*, 1993). Regrettably, only occasionally other important genes can be detected and localized by means of *in situ* hybridization with specific probes. Angelier *et al.* (1984, 1986, 1990), and N'Da & Angelier (1990) were able to visualize the transcriptionally active chromatin at the level of lateral lampbrush loop markers by means of electron microscopy spreads according to Miller & Beatty (1969) or by scanning electron microscopy, in standard or in thermic stress conditions.

At present, the up-dating process of mapping is going ahead with the detection and localization of chromosome- and loop-specific proteins recognized by monoclonal antibodies directed against germinal vesicle proteins, which have been used to acquire additional information on structure, organization, and possible modifications of the single genetic units of lampbrush chromosomes and to reveal and map specific sites on lampbrush chromosomes of several congeneric species (Ragghianti *et al.*, 1987).

The monoclonal antibodies used can be roughly grouped into three categories, according to the staining pattern on lampbrush chromosomes given by each mAb tested so far (Table II).

Table II

Some examples of mAbs tested for cytogenetic purposes*

1st group: mAbs which stain all the transcription units (loops), aside from the so-called giant fusing loops and the loops with fibrillar matrix (i.e. A33/22).

2nd group: mAbs which stain only a few loops and other lateral structures on a restricted number of chromosomes, so contributing to discriminate among individual genomes and single transcription units (i.e. A1/5).

3rd group: it includes B24/3 mAb, which stains only the so-called spheres both free in the nucleoplasm and attached to the sphere-organizing sites on chromosomes.

* See text for further explanation and bibliography

The first group has a modest cytotaxonomic and cytogenetic value, because the mAbs it contains discriminate only between the great majority of lateral loops which are positive towards mAbs belonging to this category and two other types of lateral loops (giant fusing loops and fibrillar loops), which are negative (e.g. $A_{33/22}$ mAb).

The second group of mAbs is considered very useful for cytogenetic purposes, because the mAbs it contains, discriminate among single chromosomes of a lampbrush genome and among single loops of any morphology (e.g. $A_{1/5}$ mAb).

The third group of mAbs is typically represented by $B_{24/3}$ mAb, which identifies peculiar structures, such as the spheres in *Triturus* and *Notophthalmus* (LACROIX *et al*., 1985; RAGGHIANTI *et al*., 1987; ROTH & GALL, 1987, 1989) and, in addition to the spheres, also the so-called mass M in *Pleurodeles* (LACROIX *et al*., 1985). $B_{24/3}$ mAb and similar mAbs are significant for chromosome mapping.

Therefore, the use of monoclonal antibodies has proved the most interesting development in the field of cytotaxonomy and cytogenetics, since it is extremely valuable in identifying individual chromosomes and single lateral structures on the basis of information about the localization of chromosome- and loop-specific proteins. Moreover, this methodology is highly efficient also in establishing homologies between chromosomes of related species and therefore it greatly contributes also in establishing possible phylogenetic relashionships, as pointed out also by MACGREGOR *et al*. (1990).

Area no. 3 - The karyotype of amphibians is subject to minute and extensive chromosome rearrangements, which can give rise to chromosome polymorphisms, and are not necessarily connected to short- and medium-term speciation process, although they can contribute to erect incipient isolating reproductive barriers. The evolutionary significance of chromosome polymorphisms can be highlighted by a comparison with enzymatic polymorphisms, as it was done in *Triturus italicus* by RAGGHIANTI & WAKE (1986).

Area no. 4 - Sex-chromosomes have been recognized in a number of urodelan and anuran species due to the presence, or absence of a band or a heterochromatic cap on a given chromosome pair of the complement, or even through a more marked chromosome heteromorphism (cf. SCHMID *et al*., 1991).

The best known case of sex-chromosome differentiation is still that studied by Kezer and coworkers in Neotropical bolitoglossines, which despite extensive diversity in ecology and organismal morphology, present only modest variation in chromosome morphology and all species have 13 pairs of chromosomes (KEZER *et al*., 1989). Four genera - namely *Dendrotriton, Nototriton, Oedipina,* and *Thorius* - have highly differentiated sex-chromosome heteromorphism of the XY male/ XX female type, not seen in any of the remaining genera that have been examined, although *Chiropterotriton dimidatus* presents a weakly differentiated WZ chromosomal heteromorphism (cf. SESSIONS & KEZER, 1991).

Recent work on sex-determination in amphibians has revealed a wealth of information that should be applicable to studies on amphibian phylogeny (SCHMID *et al*., 1991). NARDI (1991) and BATISTONI *et al*. (1991) have started reinvestigating the possible phylogeny of plethodontid genus *Hydromantes,* which represents a model particularly suitable for evolutionary studies, because it contains a limited number of species and subspecies, the range of which is strictly defined. European species are restricted to small areas in southern France, northcentral Italy and Sardinia, while non-European species live in California. Such a range is considered to be a classic example of disjunct locations of relict populations of the once widespread *Hydromantes* ancestral group, probably unable to adapt to Tertiary climatic and ecological changes and survived only in a few rather specialized habitats (WAKE, 1966). Then, NARDI (1991) and BATISTONI *et al*. (1991) have combined the studies on the degree of differentiation of karyotypes and sex-chromosomes with those on molecular characterization of the individual specific genomes, evidencing the absence of repetitive DNA families from the genome of American *Hydromantes* shastae and their occurrence in the genomes of European species, although according to a species-specific and/or a chromosome distribution pattern. NARDI (1991) and BATISTONI *et al*. (1991) have proved the separation of the eastern Sardinian species from both the western Sardinian *H. genei* and the continental species, *H. italicus* and *H. ambrosii* and put forward a possible hypothesis of karyophylogeny inside the European *Hydromantes* on the unique basis of the cytogenetic data. Regrettably, the lack of hybridological results has avoided the evaluation of the importance and extent of reproductive isolating mechanisms (including ecological and ethological barriers) among European *Hydromantes* species and between European and Californian species. The hybridological method is believed to represent a necessary tool for verifying the biological compatibility still existing among different related taxa after their separation, as the results obtained in the genus *Triturus* largely demonstrate (cf. MANCINO, 1991). The lack of genetic (hybridological) and cytogenetic evidence does not permit so far to accept the proposal of the division of the genus *Hydromantes* into two separate genera (the European *Speleomantes* and the Californian *Hydromantoides*), as proposed or accepted by some authors (cf. LANZA & VANNI, 1981; DUBOIS, 1984; EWALD, 1989). Moreover, beyond any question concerning nomenclature and priority, the name of *Hydromantes* should be maintained as largely known and meaningfull. One can refer to SMITH & WAKE (1993) for better focusing the specific question.

Area no. 5 - Most amphibians are known to possess vastly more DNA in their genome than is needed for genes. In salamanders most of the genome consists of middle repetitive DNA. Amphibians represent therefore a typical example of the "C-value paradox", in so that, generally, genome size does not correspond to the amount of DNA needed for protein-coding functions. As stressed in various chapters of the book edited by GREEN & SESSIONS (1991), it is a paradox only under the expectation that genome size should be equal or proportional to gene number and should therefore increase with organismal complexity.

The present amphibian cytogenetics requires information regarding the genome size value of each species studied, in addition to chromosome number and banded karyotype (Table III), as extensive changes in genome size have obviously been an important aspect of amphibian genome evolution.

Table III

Genome size (DNA value pg/diploid nucleus)*	
Orders:	
Apoda:	9.4 - 27.9
Caudata:	18.2 - 181.8
Anura:	2.0 - 18.7

*After OLMO (1990), KING (1990), and GREEN & SESSIONS (1991).

A large genome is believed to be potentially maladaptive in that it leads to slow rates of development and constraints on body size. For this reason, in this area, molecular cytogenetics is still expected to give a larger contribution, because the origin and functional significance of genome size have been a source of controversy and speculation (CAVALIER-SMITH, 1985; SESSIONS & LARSON, 1987), as well as its possible involvement in genome evolution. In fact, the genome size values have allowed new evolutionary hypotheses to be put forward, in so far as a large amount of DNA affects cell size, and therefore there could be a correlation between genome size and rates of development, cell cycle time, and histological organization, then interfering any cytological mechanism of evolution. However, genome size in bolitoglossine salamanders, as in other plethodontids, surprisingly varies not only with taxonomic group, but also with geographic distribution as to furtherly complicate the comprehension of such cytological phenomenon. Secondary simplification of the salamander brain appears to result from paedomorphosis, or retention of juvenile or embryonic morphology into adulthood. Paedomorphosis is correlated with an increase in genome size, that in turn is positively correlated with cell size, but negatively correlated with cell proliferation and differentiation rates. Although increasing genome size and paedomorphosis tend to compromise the function of the salamander brain, compensating mechanisms have evolved that may restore or even enhance brain function (ROTH *et al*., 1993).

Area no. 6 - Cases of spontaneous polyploidy are known, which are more widespread among anurans than among urodeles. There is no evidence of polyploidy or aneuploidy in caecilians (NUSSBAUM, 1991). In 1991, ANDERSON, BOGART, and TYMOWSKA autonomously reviewed and discussed speciation mechanisms by auto- or allo-polyploidization. In *Xenopus*, as in a few other groups, spontaneous polyploidy is believed to be the specific factor of speciation. Besides, polyploidy can represent the primary differentiation between sibling species (e.g., between the diploid grey green treefrog *Hyla chrysoscelis* and the tetraploid *H. versicolor*). In addition, here it can only be reported that what seemed to be a unique feature of the order Urodela was the polyploidization of the whole family of Sirenidae as a mechanism for differentiating the various species (MORESCALCHI & OLMO, 1974; MORESCALCHI *et al* ., 1986). However, serious doubts that Sirenidae might constitute a family of polyploid salamanders have recently been cast by MOLER & KEZER (1993), due to the wider karyological results obtained in *Pseudobranchus*. With in mind the fine morphology of the specific C-banded karyotypes and the analysis of chromosome variation, MOLER & KEZER (1993) conclude their work suggesting that, if *Pseudobranchus* was derived by polyploidy, then subsequent karyological evolution has largely eliminated any trace of this unlikely mechanism of polyploidization. Polyploidy can also be provoked by means of cold or heat shocks or with the help of several other experimental methods. Studies on gene dosage are possible when hybridity and polyploidy are combined within an organism.

Area no. 7 - Karyotypes of several primitive amphibian families contain microchromosomes, the evolutionary fate of which was widely discussed by MORESCALCHI (1973), who suggested that karyotypes evolve from those comprising microchromosomes to those lacking microchromosomes and from asymmetrical and bimodal to symmetrical and unimodal ones. However, the evolutionary correlation between karyotype reduction as proposed by Morescalchi and morphological advancement is sometimes imperfect (see also: GREEN, 1991a). Therefore, the hypothesis of genome reduction preliminarly needs to establish what sort of genetic material is contained in microchomosomes and the nature and mode of their disappearance in evolution. Furthermore, a more detailed comparative analysis of structure and gene organization of both micro- and macrochromosomes in primitive and more derived families should be accomplished, as the results can be informative also to phylogenetic relationships.

Area no. 8 - The chromosome complement of several species of urodeles and anurans contains supernumerary chromosomes (or B-chromosomes), typical in the sense that they are variable in size, shape, number, and behavior during mitosis and meiosis. Their occurrence in amphibian karyotypes has particularly been studied by GREEN (1991b). However, molecular investigations on structure and organization of supernumerary chromosomes are still required to search for a cytological evidence of their origin as well as evolutionary and functional significance.

Area no. 9 - Spontaneous hybridization is known in some genera of urodeles (for instance: *Ambystoma*, *Plethodon*, and *Triturus*), but the propensity for natural interspecific hybridization is typical for anurans (for instance: *Bombina*, *Bufo*, *Hyla*, and *Rana*). The condition of natural hybridity can imply the occurrence of complex reproductive mechanisms at cytological level, requiring careful cytogenetic investigations. Reproduction can be furtherly affected when hybridity is associated to polyploidy. One can refer to MANCINO (1988, 1990) for a wider bibliography.

The case of "*Rana esculenta*" can represent an emblematic example showing the wealth of information obtained by traditional cytogenetics, and the further contribution that molecular cytogenetics is expected to give to the solution of unsolved reproductive and evolutionary problems in hybrids.

GALGANO (1931) had already grasped and proposed the hybrid condition of *R. esculenta* merely on the strength of an accurate cytogenetic description of gametogenesis of green frogs collected near Florence, long before the introduction of squashing, banding, and electrophoretic techniques. In those days, the systematic position of "*R. esculenta*" was a puzzling matter, although there were a few taxonomists, who were inclined to admit the hybrid condition of *R. esculenta*.

Galgano, in fact, observed in the green frogs studied by him the same serious meiotic disturbances, which occur in urodelan and anuran diploid and triploid interspecies hybrids, such as absence or partial pairing, low mean chiasma frequency, anomalous crossing over, atypical formation of chiasmata between non homologous partners, irregular cell divisions due to the presence of three- or multipolar spindles and the occurrence of lagging chromosomes, chromosome restitutions and non-disjunctions, formation of abnormally biheaded sperm and/or non-functioning gametes, ecc.

But Galgano's hypothesis, based on a cytogenetic observation performed by means of mere conventional tecniques, was evidently premature and therefore difficult to be accepted. Then, it was only in the 1960's that the hybrid nature of "*R. esculenta*" was proved on morphological and hybridological evidence by BERGER (1964 and following years) and on electrophoretic basis by several other authors (cf. BERGER, 1988 for a wider bibliography). "*Rana esculenta*" was therefore recognized as a widespread hybrid which arose (and arises) by hybridization between the Mendelian species *R. ridibunda* PALLAS, 1771 and *R. lessonae* CAMERANO 1882, two species that show at present also different ecological and ethological preferences and do not occur syntopically. The coexistence of the hybrid form "*R. esculenta*" and the pure parental species *R. lessonae* (L-E population system) is the most common geographic distribution. "*R. esculenta*" arose (and arises) by hybridization between the Mendelian species *R. ridibunda* and *R. lessonae*.

A further significant advancement in the knowledge of the reproductive biology of *R. esculenta* was obtained by means of a combination of genetic and cytogenetic results by several authors such as TUNNER (1974), GUNTHER (1975a), GRAF & MULLER (1979), UZZELL *et al* . (1980), TUNNER & HEPPICH (1981), TUNNER & HEPPICH-TUNNER (1991) who stated that the reproduction of most "*R. esculenta*" specimens and populations is typically hemiclonal, that is somatic cells conserve a hybrid constitution throughout the life cycle, whereas primordial germ-line cells, which too have a hybrid constitution, undergo hybridogenesis: hybridogenesis can be considered the cytological mechanism of hemiclonality: one parental genome (mostly that of *R. lessonae*) is selectively eliminated from the germ-line cells prior to meiosis taking place, but fertilization and development need sperm of *R. lessonae* or, plausibly, a second hybrid individual can occasionally participate in the cross in place of the parental species, as indicated by GUNTHER (1975b). Of course, elimination of one genome presumably occurring in germ-line cells must be followed by a compensatory duplication of the parental genome non-excluded which occurs either premeiotically or meiotically (that is, at meiotic pre-diplotene). Such hybridogenetic mechanism is in line with the results obtained by using DNA flow cytometry (VINOGRAD *et al* ., 1990), based on the assumption that the ridibunda genome contains 16% more DNA than the *lessonae* genome (MAZIN & BORKIN, 1979; BORKIN *et al* 1987).

Hybridogenesis is said to occur even in the case of gametogenesis of experimental triploid green water frogs (NISHIOKA & OHTANI, 1984; OHTANI, 1993). Triploid hybrids occur in high frequency (up to 83% in certain populations) in the northern part of the range of "*R. esculenta*" as shown by GUNTHER, 1970, 1975b): they can be either RRL or RLL and can be distinguished on the basis of gene-dosage effects visible in electrophoretic patterns of some enzymes and blood proteins. RRL and RLL triploid hybrids seem to coexist in Germany and Sweden; RRL alone seem to occur in Poland. All male triploids with an RLL constitution have been discovered in the Fontainebleau Forest, Seine-et-Marne, France; whereas hybrid triploids of either type seem to be absent in Austria, Yugoslavia, Italy.

NISHIOKA & OHTANI (1984) reported that oocytes I at lampbrush phase, from experimental allotriploid females consisting of two genomes of *R. brevipoda* and one of *R. lessonae*, had 13 "pure" bivalents, indicating that genome exclusion did occur in all female germ cells. OHTANI (1993) studied cytogenetically the male germ-line cells of experimental allotriploids that possess two sets of *R. nigromaculata* and one set of *R. lessonae*. The author noted that hybrid spermatogenesis presents a series of eliminations or a loss of single chromosomes or of small groups of deviant chromosomes of *R. lessonae*, concentrated together near the equatorial plate and remained stationary at anaphase. Therefore, only those diploid spermatogonia that had succeeded in synchronous elimination of all R. lessonae chromosomes (the so called "homogenizing" elimination according to VINOGRAD *et al* ., 1990) multiply vigorously, filling up the space occupied by degenerated spermatogonia, and developing into haploid *R. nigromaculata* spermatozoa through the normal process of meiosis. Generally speaking, the presence of triploid hybrids points out the formation of fertile diploid eggs, since capacity of fertilization by diploid spermatozoa seems to be largely affected.

However, there is the lingering suspicion that the term hybridogenesis is being often given to a cytological mechanism, which differs from the one regularizing gametogenesis of diploid hybrid green frogs, while it is shared by all allotriploid salamander and frog hybrids in the intrinsic attempt to produce "purified" gametes (cf. WHITE, 1946; BENAZZI & LEPORI, 1949; SPURWAY & CALLAN, 1960; MANCINO & SCALI, 1964, 1968; MANCINO *et al* ., 1978, 1979a and b). In short, both the somatic cells and the primitive germ-line cells are allotriploid. The former conserve the allotriplod constitution throughout the life cycle; the latter, during the first multiplicative phases of gonia, tend to eliminate the unique haploid set of the less represented parental species and to retain the double set of the more represented parental species. Then, subsequent meiosis takes place normally, as synapsis (= pairing) occurs between sister chromosomes forming bivalents sometimes indicated as pseudobivalents, and giving rise to haploid gametes. This same process was observed also by VINOGRAD *et al* . (1990), who maintained that the primary spermatogonia of triploid males have, as in diploid males, a somatic genotype until the exclusion of the minority haploid genome in the triploid set occurs at a certain stage of their development. As a result, diploid gonia with a "homogeneous" genotype of one parental species arise, and the process may be followed by normal meiosis without any compensatory duplication, taking place after the normal premeiotic synthesis of DNA ("meroclonal" inheritance in place of hybridogenesis). Also the all-male triploid RLL population "*R. esculenta*" of Fontainebleau Forest, studied by GRAF & POLLS PELAZ (1989), behaves in the same manner. In the course of gametogenesis, the hybrid individuals eliminate the unique ridibunda genome as to form diploid gametes LL. The persistence of triploid males in the Fontainebleau population is assured by crosses of RLL males with RL females, which apparently yield male offspring exclusively.

Despite the great contribution given by chromosome studies to the reproductive biology of *R. esculenta* and some other green water frog species hybrids, there are some cellular aspects which still wait for a definitive answer from both conventional and molecular cytogenetics, such as: the fundamental mechanism of chromosome elimination; the precise step of male and female gametogenesis when elimination occurs; whether the chromosomes of one parental species are eliminated at once or little by little with each division; in what way the different centromeres are involved in the differential exclusion phenomenon; the role of centromeric proteins in chromosome elimination; which are the factors regulating this genome exclusion or loss of one parental genome; the reasons why in both male and female germ-line cells of basically hybridogenetic hybrid specimens, there are individual cells which are not accounted for in the hybridogenetic mechanism and maintain a hybrid genome, encountering mitotic and meiotic difficulties; why *R. ridibunda* gives rise to hybridogenetic hybrids when mated to *R. lessonae*, while the occasional natural hybrids between *R. ridibunda* and the newly discovered Balkan species *R. shqiperica* and *R. epeirotica* are apparently resistant to hemiclonality induction and behave as typical hybrids; evolution of hybridogenesis within the framework of the two types of reproductions improperly said as "asexual" (either development of eggs not previously fertilized, or development of eggs after fertilization not followed by amphymixis of the two pronuclei) and "sexual" (development follows fertilization plus amphymixis).

CYTOGENETICS AND DEVELOPMENTAL BIOLOGY

After this quick survey of both the main results obtained in a few decades of cytogenetic studies in the field of chromosome evolution and some cytogenetic/evolutionary problems still unsolved in amphibians, now we can touch upon one trend of the present research, which shows the help given by cytogenetics to clear most of the links connecting genome activity in oogenesis to processes of development and differentiation, and consequently the possible modalities of interfering on the modes of evolution.

Much is known about transcription and transcripts during the lampbrush phase of oogenesis (cf. OLD *et al* ., 1977; DAVIDSON, 1986; MACGREGOR & SESSIONS, 1986a and b); instead, little is known so far on the fate and function of proteins of germinal vesicles synthesized during oogenesis and particularly on proteins bound to lampbrush chromosomes (ABBADIE *et al* ., 1987; DREYER *et al* ., 1981, 1982 and 1985; DREYER & MAUSER, 1983; DREYER, 1987; MILLER *et al* ., 1989; REDDY *et al* ., 1991; BELLINI *et al* ., 1993; HERBERTS *et al* 1993).

Therefore, the use of as many monoclonal antibodies as possible is of great advantage to discriminate among single transcription units and identify maternal proteins playing a role in precocious events of development. In other words, mAbs enable us to get more information on whether chromosomal proteins bound to the main transcription units distributed along lampbrush chromosomes exhaust their function with the end of oogenesis, or whether they survive in early development to influence post-fertilization and precocious developmental events.

This kind of research cannot be done without a solid cytogenetic background: for this reason, it takes into account particularly the genus *Triturus*, where cytogenetic and genetic, as well as ecological and ethological knowledge is mostly widespread (see MANCINO, 1988, 1990). Then, it can be shown an example involving the so-called spheres (= sphere organelles) present at precise sites on specific elements of the lampbrush karyotypes (cf. CALLAN & LLOYD, 1975). One can refer to BUCCI *et al* . (1993) for the detailed description of the results.

In summary, after discovering that B_{24} mAb specifically binds to spheres attached to lampbrush chromosomes, it was quite natural for cytogeneticists to wonder what was the genetic function of such cell organelles: thus, the fate of B_{24} protein was investigated in order to detect when it is synthesized, where it accumulates, and when it exhausts whether in oogenesis or during development. Of course, to answer this question, B_{24} protein had to be detected and localized in oocytes and embryos by means of immunoblotting and immunostaining.

On immunoblots, B_{24} mAb recognizes a polypeptide 104 KD long which is abundant in oocytes and in early embryos until mid-gastrula stage, when it starts to progressively decrease, disappearing after the tail bud stage; B_{24} antigen is not detectable any more in swimming larvae and in adult tissues (except of course, ovary, where B_{24} antigen reappears bound to lampbrush spheres).

By immunostaining of previtellogenic oocytes (with diameter less than 500 µm), germinal vesicle proves negative, while some fluorescence is found in cytoplasm, revealing that expression of B_{24} protein is already started, before the beginning of the lampbrush phase in oocytes. In the course of oogenesis, fluorescence in cytoplasm decreases, while it progressively increases within nuclei. Fluorescence becomes especially evident and exclusively located within the nucleus in larger yolked oocytes. Follicular cells are always negative.

During development, embryonic cell nuclei are immunostained starting from very early cleavage until gastrulation, and, at late gastrula, the nuclear fluorescence becomes restricted to endoderm cell nuclei. Fluorescence becomes progressively weaker until the tail bud stage. B_{24} antigen is not detectable any more on sections of larvae at and after hatching: thus, the results of immunoblotting and immunostaining are overlapping.

Several clones have been obtained by screening an ovary cDNA expression library of *N. viridescens* with B_{24} mAb. In brief the longest clone is 2587 bp long and has an ORF (= open reading frame) of 2234 bp ending with a stop codon at position 2234. The ORF is capable of encoding a polypeptyde of 744 aa with a calculated MW of 82 KD. A transcript of 3Kb was recognized by the clone on northern blot, only in the ovary; the band corresponding to the transcript is located between 18S and 28S rRNAs; the size of the transcript is large enough to encompass the ORF coding for a 104 KD protein, which is the MW of B_{24} protein (cf. Bucci *et al* ., 1993 for a more analytical description and documentation of the results).

The distribution of B_{24} mRNA within oocytes at different stages of growth was studied by *in situ* hybridization on ovary sections, using synthetic sense and antisense RNA probes: the results are the following: cytoplasm of previtellogenic oocytes (with diameter less than 500 µm) is intensely labelled by using antisense tritiated RNA probe, while germinal vesicles appear unlabelled. Both cytoplasm and nuclei of oocytes of the same size treated with sense tritiated RNA are unlabelled as do the surrounding follicular cells treated with both sense and antisense probes.

In later stages, grown and fully grown oocytes show a lower density of grains probably due to both a dilution effect caused by the increase of cellular volume and the expiration or gradual decrease of RNA synthesis on lampbrush chromosomes. Oocytes of whatever size hybridized with a sense RNA probe as a control, show no labelling after comparable exposure times.

In situ hybridization experiments have been carried out also on isolated lampbrush chromosomes of *Triturus carnifex* with the aim to detect and hybridize the active sites of transcription of B_{24} gene. By using antisense ^3H RNA probe to nascent transcripts, a loop of normal morphology and size, subterminally located on the long arm of chromosome I, appears labelled in small and medium size oocytes. Therefore, this loop may be considered the active transcription site of B_{24} gene, the activity of which presumably does not stop at once with the beginning of the lampbrush phase, but gradually decreases in the course of the growth of oocytes. No labelling of any loop was observed using sense ^3H RNA probe.

The determination of B_{24} clone sequence has provided some information concerning the conceptual protein it encodes.

A computer search analysis within the EMBL data library has revealed that B_{24} gene product is identical about 71.5% to mouse P_1 protein (THOMMES *et al* ., 1992); 53.2% to budding yeast (*Saccharomyces cerevisiae*) Mcm3 protein (GIBSON *et al* ., 1990; YAN *et al* ., 1991) and 48.1% to fission yeast (*Schizosaccharomyces pombe*) cdc 21 protein (COXON *et al* ., 1992).

P_1 protein may have a function in the replication of cellular DNA; Mcm3 protein may be involved in early steps of DNA replication and cdc 21 protein is believed to affect S phase and nuclear division.

The highest rate of identity is found between B_{24} gene and mouse P_1 gene. Therefore, B_{24} gene may be considered the amphibian homolog of mammalian P_1 gene, while B_{24} gene product may be an amphibian equivalent member of a recently discovered family of conserved nuclear proteins which have been implicated in early events of eukaryotic chromosome replication (DIFFLEY & STILLMANN, 1990; COXON *et al* ., 1992). Then, taking into account the present results and the observations by GALL & CALLAN (1989), WU *et al* . (1991, 1994), PHILLIPS *et al* . (1992), GALL (1992) and WU & GALL (1993), possible functions of B24 protein included in the sphere organelles can be suggested: preassembling, sorting of RNA processing components and/or storing snRNPs during oogenesis, and acting as a factor of initiation of DNA replication respectively during development.

CONCLUDING REMARKS

Amphibian cytogenetics still conserves an inherent attractiveness as it encompasses several levels of biological organization, ranging from the morphological to the molecular aspects of chromosome studies: in fact, it can use a variety of conventional and advanced technologies and develop the most suitable technology for cytogenetic investigations. Then, cytogenetics has greatly contributed, in a relatively short period, to the advancement of the knowledge of some basic aspects of evolution, such as several macroevolutionary cytological patterns, genome evolution, and karyophylogenetic relationships. In this framework, the study of lampbrush chromosomes has played a primary role in knowledging the fine structure and organization of eukaryotic chromosomes, and tracing the most correct way for evaluating the degree of chromosome and karyotype differentiation as well as conservation of chromosomal homologies among differently related species. Thus, it has lead to the views concerning the mode of evolutionary change in the molecular structure of chromosomes, such as that indicated as "repatterning" hypothesis (MANCINO *et al*., 1977), different from the "homosequenciality" hypothesis put forward by MACGREGOR & SHERWOOD (1979). The validity of the first one seems at present reinforced by the results of mapping by means of monoclonal antibodies (RAGGHIANTI *et al*., 1987, and unpublished), which show that interspecific differences in the chromosomal location of certain genetic units can be more easily explained by a redistribution of chromosomal elements within karyotypes, starting from ancient karyotypes, and determining evolutionary changes in sequence location that should be relatively conservative (that is, slow, unique, and irreversible in line with timing of amphibian evolution and in opposition to the rapid and reversible changes in the location of certain kinds of sequences, essentially determined by unequal crossing over, as supposed by the homosequenciality theory). The two hypotheses appear not mutually exclusive, according to the mediate interpretation by SESSIONS (1989), who admits the occurrence of: i) recently evolved sequences, which should be homogeneous in structure, localized in large clusters at or near the centromeres and telomeres, functionally inert and taxonomically restricted; and ii) ancient sequences that should be more complex, organized in small clusters in intercalary position, able to be transcribed, and likely to have a wider taxonomic occurrence.

Actually, lampbrush chromosomes have not yet entirely revealed the still obscure mechanisms of chromosome speciation and karyotype evolution. For this reason, they should be more deeply and widely investigated in as many species belonging to the three orders as possible, by means of suitable technology.

Moreover, lampbrush chromosomes, more than any other kind of chromosomes of vertebrates, offer the unique opportunity to follow the origin and the fate of the gene products of single genetic units by advanced cytological means and hereby detect the molecular basis of the genetic bridges connecting gene activity during oogenesis to development, and development to evolution. Thus, amphibian cytogenetics can throw a deep insight into the basic factors regulating amphibian and vertebrate life history.

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European newts: a model system for evolutionary studies

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Abstract: Phylogenetics is the backbone of evolutionary biology. Hence, a group with a well-established phylogeny may provide a sound basis for evolutionary studies. The European newt genus *Triturus* is one such group. New mitochondrial DNA sequence data support a phylogenetic hypothesis that had been constructed from allozyme and behavioural data. The re-established phylogeny is in turn exploited to address questions pertaining to methods of phylogeny reconstruction, the quality of data required for phylogenetic analysis, the study of character evolution, vicariance biogeography, species interactions and hybridization, and conservation biology.

Key words: Amphibia, Caudata, *Triturus*, model system, phylogenetics, evolution

INTRODUCTION

Today - and only today - I will paraphrase a famous statement by T. DOBZHANSKY (1973) as 'Nothing in biology makes sense, except in the light of phylogeny'. My aim is to demonstrate that, in the last decade or so, considerable progress has been made towards the understanding of amphibian evolutionary biology and that most progress is underpinned by a knowledge of the evolutionary relationships of species involved. For a taxonomist a phylogenetic reconstruction may be the finish, for an evolutionary biologist it is where the research becomes interesting. At this interface of systematics and evolutionary biology the genus of Old World newts, *Triturus*, has shown to be a good 'model system'. I will explore and exploit the phylogeny of this group.

In general terms, what phylogenies are available ? The following three types can be recognised: i) simulations, ii) known phylogenies, and iii) 'known' phylogenies.

Simulations are a popular approach for addressing basic and technical questions in phylogenetics (SOKAL, 1983; JIN & NEI, 1991; HEIJERMANN, 1992). The various models do have their merits but, being based upon assumptions about the evolutionary process, gains are limited and probably biased, or, as the modellers themselves put it: 'garbage in is garbage out'.

In a survey not claimed to be exhaustive three examples of known phylogenies were found - involving strains of laboratory animals, lines of cultivar plants, and viruses purposely propagated in a mutagenic environment (BAUM, 1984; ATCHLEY & FITCH, 1991; HILLIS *et al.*, 1992). These 'test tube phylogenies' have their application primarily in gaining a better understanding to the performance of various methods in phylogenetic analysis. Little is learned about evolution because no speciation is involved and selection is artificial (see also SOBER, 1993 and HILLIS *et al.*, 1993).

As we all know, there is nothing like the real thing. Unfortunately, phylogenetic reconstruction is a tedious task, the results of which we will never be sure are correct. The number of alternative phylogenetic arrangements is enormous, even when few taxa are involved (FELSENSTEIN, 1978). Referring to noise on the phylogenetic signal, M. LYNCH (1989) concludes that 'Phylogenetic trees fit to data from more than five species will almost always contain topological errors, even with very large data sets'. It has to be realised, however, that the 'noise' may be representing evolutionary adaptation, the very topic we are interested in. Much is to be gained from proper phylogenetic analysis. It will pay off to analyse the phylogeny for a few groups in the detail we can afford. This approach has been followed for the Old World newts, genus *Triturus*.

Phylogeny of the genus *Triturus*

The genus *Triturus* is widely distributed over the western Palaearctic (Fig. 1). Nine or twelve species are recognised (THORN, 1968; FROST, 1985), depending on the criteria used for species recognition when limited or insufficient data is available. I will refer to twelve species with four of them grouped together in the *T. cristatus* superspecies.

Several attempts are made to solve the *Triturus* phylogeny (BOLKAY, 1928; GONZALEZ & SANCHIZ, 1986; ARNTZEN & SPARREBOOM, 1987, 1989; RAFINSKI & ARNTZEN, 1987; GIACOMA AND BALLETTO, 1988; BUSACK *et al.*, 1989; MACGREGOR *et al.*, 1990, reviewed by HALLIDAY & ARANO, 1991), and one hypothesis is presented in Fig. 2. It is based on two data sets

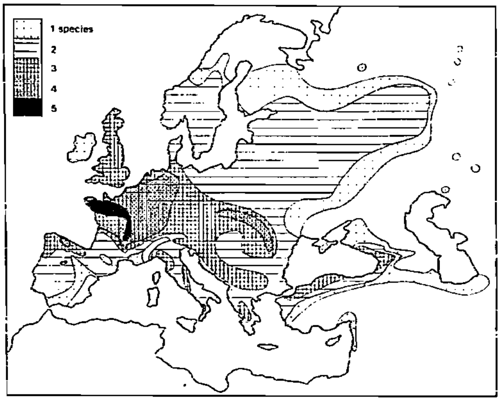

Figure 1: Density distribution map of *Triturus* species.

obtained through allozyme electrophoresis and the analysis of courtship behaviour (ARNTZEN & SPARREBOOM, 1989). Outstanding features of the hypothesis are that i) it is fully robust under the jack-knife test, ii) it is based on two clearly independent data sets that iii) provide phylogenetic resolution over different temporal scales. The allozyme data appear to refer to rapidly evolving characters and prove useful in describing the more recent evolutionary history of *Triturus*. The behavioural data on the other hand appear to be evolving relatively slowly and are thus most useful in defining the early speciation events of the genus. The reconstruction has been criticised by some (GREEN, 1989) on the basis that behavioural characters would be especially adaptive, and hence weak indicators of an evolutionary relationship. This may be true, but is not born out by measures on the information content and fit of the data which is similar for both data sets (ARNTZEN & SPARREBOOM, 1987). In general behavioural data are as good (or bad) indicators of phylogenetic relationships as morphological data and molecular (including allozyme) data (SANDERSON & DONOGHUE, 1989; DE QUEIROS & WIMBERGER, 1993).

Many of the branches of the *Triturus* phylogenetic tree are strongly supported by the data and among these the longest internal branch is the one leading to *cristatus* - *marmoratus*. The monophyly of this group is further supported by a powerful synapomorphy in the shape of a unique chromosome length polymorphism (Macgregor & Horner, 1980). This chromosome syndrome can be viewed as a genetic disease since it causes the death of half the offspring of *cristatus* (superspecies) and *marmoratus* (Rusconi, 1821; Horner & Macgregor, 1985). Apparently both species have been unable to remove this deleterious attribute over an estimated 10 Ma period of lineage independence (reviewed in Oosterbroek & Arntzen, 1992), notwithstanding high selection pressure.

Four sections of the phylogenetic tree have somewhat ambiguous support. First, the branching order (*helveticus* (*vulgaris*, *montandoni*)) is defined by allozyme data only. No qualitative differences are observed in courtship behaviour between the species. Second, the so-called 'flamenco' behaviour that was considered a derived character-state shared by *boscai* and *italicus* has recently also been observed in *marmoratus* and *helveticus* (M. FARIA & M. SPARREBOOM, pers. coms.). Moreover, a slight re-interpretation of some of the behavioural data would question the monophyly of the *boscai* - *italicus* clade (ARNTZEN & SPARREBOOM, 1989). Third, the allocation of *alpestris* to the subgenus *Triturus* can be challenged on similar grounds (ARNTZEN & SPARREBOOM, 1989). Fourth, the taxonomic position of *vittatus* as a sister-species to *cristatus* and *marmoratus* can be questioned.

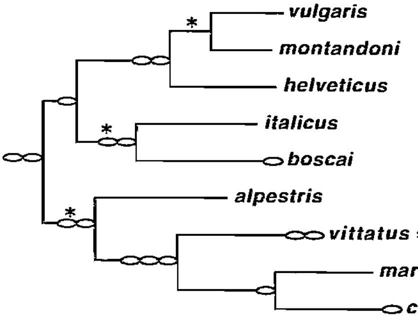

Figure 2: Hypothesis of phylogenetic relationships within the genus *Triturus* based upon simultaneous analysis of biochemical and behavioural data. Line transects refer to electrophoretically detectable biochemical evolution, oval symbols refer to evolutionary change in *Triturus* courtship behaviour. Asterisks refer to portions of the tree where support is ambiguous.

In the first ever classification of the genus (BOLKAY, 1928) *vittatus* is grouped with the small-bodied species (subgenus *Palaeotriton*) with which species it indeed shares many morphological similarities.

Testing of phylogenetic hypothesis

The question marks raised urges hypothesis testing through the analysis of new and independent data. To this end three sections of the mitochondrial (mt) DNA genome across nine *Triturus* species were sequenced using PCR-methodology (ZAJC, 1992, 1993). Primers were selected to amplify DNA representing genes known to experience slow (12S), intermediate (cytochrome-b) and fast (ATP-ase) mutation rates. Multiple outgroups were used. Taxon - probe combinations were chosen to acquire optimal phylogenetic resolution and to avoid unnecessary work. The most parsimonious solution supported by the data is represented by the sentence: (((((*montandoni*, *vulgaris*) *helveticus*) (*alpestris*, *cristatus*)) (*boscai*, *italicus*)) / (*vittatus*, *marmoratus*)), in which the slash represents the outgroup determined root of the tree. In three times out of four the proposed but questioned tree topology (Fig. 1) is confirmed by Zajc' DNA-sequence data: the *boscai* - *italicus* clade is monophyletic, *montandoni* shares most recent common ancestry with *vulgaris* and not with *helveticus*, and finally, *vittatus* belongs to the subgenus *Triturus* in as far as it is grouped together with *marmoratus*. Contrary to results obtained earlier, *alpestris* is grouped in the subgenus *Palaeotriton*. A surprising result is the grouping of *cristatus* with *alpestris*. This arrangement challenges the support for the monophyly of *cristatus* - *marmoratus*.

Beyond phylogenies

Phylogenies can be exploited in a variety of ways. The better the 'model system', the longer the list of applications and the more interesting and rewarding these are. Applications include the study of: i) the relative merits of methods of phylogenetic reconstruction, ii) the quality of data for phylogenetic analysis, iii) character evolution, iv) vicariance biogeography, v) species interactions, and finally, vi) recommendations for nature conservation can be drawn from phylogenies. These six applications will be considered in turn, featuring the Old World newts.

Methods of phylogenetic analysis.

A simple exercise is to compare phylogenies drawn from different methods of tree construction and character-state coding in their fit to the accepted phylogeny. Three different phylogenetic hypotheses have been put forward on the basis of a 19 loci allozyme data set (RAFINSKI & ARNTZEN, 1987). By using matrix comparisons (DIETZ, 1983) it can be shown that the UPGMA-method in this particular case produces a dendrogram with a better fit to the accepted tree than the distance- or character-Wagner methods. The UPGMA-method assumes evolutionary change to be linear with time. This assumption is not contradicted, giving some support to the application of the 'molecular-clock' model as a guideline for phylogenetic reconstruction from allozyme data.

Quality assessment of phylogenetic data.

The same approach for comparing methodologies can be applied to scrutinise data for phylogenetic reconstruction. Referring to the data of Fig. 2, the behavioural data show a fit to the accepted tree that is significantly better than that of the allozyme data. In hindsight it can be seen that the behavioural data contributed more to the phylogenetic resolution than the allozyme data.

Study of character evolution.

Established and 'known' phylogenies can be used to study character evolution through the 'comparative method' (FELSENSTEIN, 1985; HARVEY & PAGEL, 1991). The more similar the established phylogeny is to the (undetermined) real one, the more correct the conclusions will be.

Applying the comparative method the evolution of *Triturus* cytogenetic characters was analysed in the framework of a phylogeny (MACGREGOR *et al.*, 1990). A consensus phylogenetic hypothesis was first constructed from a large array of data, but not including cytogenetic characters. Some evolutionary trends could be observed, for example that changes in genome size, number of C-bands and amount of heterochromatin are evolutionary correlates of each other. The comparative method was also used to analyse evolution of the phenomenon of lek-breeding behaviour across *Triturus* species (GIACOMA & BALLETTO, 1988). However, this analysis is not straightforward because courtship data, likely to be correlated with lekking behaviour, were used to construct a phylogeny in the first place.

A third example of the study of character evolution refers to the phylogenetic tree derived from DNA sequence data (ZAJC, 1992 and pers. comm.). When contrasted with other information, including 12S mtDNA sequence data, it appears that cytochrome-b provides some misleading phylogenetic information. Following a cytochrome-b-based phylogeny for *Triturus* species we would infer that the chromosome-1 syndrome either would have evolved twice (on the lineages leading to *cristatus* and

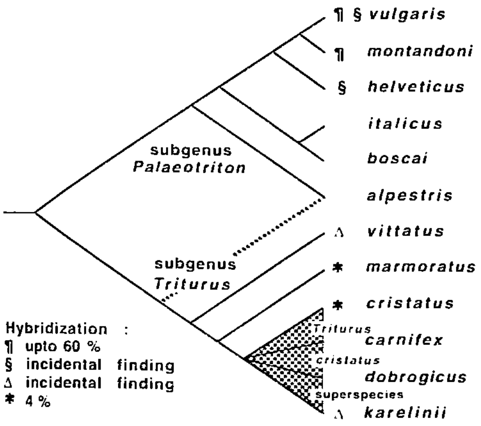

Figure 3: Consensus phylogenetic tree for the genus *Triturus*, based upon behavioural, allozyme and mitochondrial DNA sequence data (details see text). Phylogenetic relationships within the *cristatus* superspecies are unresolved. Observed levels of natural hybridisation are indicated by symbols (SCHMIDTLER & SCHMIDTLER, 1967; PECIO & RAFINSKI, 1985; GRIFFITHS *et al.*, 1987; ARNTZEN, 1991). The species *boscai* and *italicus* have allopatric distributions. Note that the same topology is derived on the basis only of the DNA sequence data plus the chromosome-1 syndrome synapomorphy for *cristatus* - *marmoratus*. Further note that the topology of the tree is identical to the one of Fig. 2, with the exception of *T. alpestris* for which species DNA sequence data suggest inclusion in the subgenus *Palaeotriton* (solid line), not *Triturus* (interrupted line).

marmoratus) or that it was lost three times (on the lineages leading to *alpestris*, *vittatus* and to the clade that includes *vulgaris*). The overall parsimony for this scenario is low. Moreover, the chromosome-1 syndrome is unique to *Triturus* and unlikely to have come into existence within the genus more than once. Also, it would appear that removal of the syndrome from the genome is impossible. The lesson that we learn is that molecular data, like other taxonomic data, do not necessarily lead to correct phylogenies. Had we not known some essentials of the classification of *Triturus*, the DNA-based phylogeny might uncritically have been accepted. By consequence, erroneous conclusions would have been drawn from the application of phylogeny based research methods. Now in turn, the phylogeny of *Triturus* can be of assistance to molecular biology in elucidating the aberrant pattern of cytochrome-b evolution.

Vicariance biogeography.

Well founded phylogenies are required in order to get away from the 'just so' stories in historical biogeography. To establish biogeographical patterns over time and space many ecologically diverse groups of organisms should be involved. An analytical study on the spatial and temporal pattern of radiation in the Palaearctic region has been carried out by matching phylogenies for a number of amphibian and insect taxa and by comparing the results to available paleogeographical data (OOSTERBROEK & ARNTZEN, 1992). Here it suffices to point out that the *Triturus* phylogeny has been a cornerstone to the generation of testable hypotheses in Palaearctic biogeography.

Species interactions.

Intraspecific hybridisation is frequent within the genus *Triturus* (reviewed in MACGREGOR *et al.*, 1990). Within the subgenus *Palaeotriton* closely related species such as *montandoni* and *vulgaris* do frequently hybridise in a narrow contact zone (PECIO & RAFINSKI, 1985). Species less closely related such as *vulgaris* and *helveticus* possess widely overlapping distributions and do not frequently hybridise (GRIFFITHS *et al.*, 1987). A similar relationship can be observed in the subgenus *Triturus* (ARNTZEN & WALLIS, 1991; SCHMIDTLER & SCHMIDTLER, 1967; see also Fig. 3). The link between extent of hybridisation and evolutionary

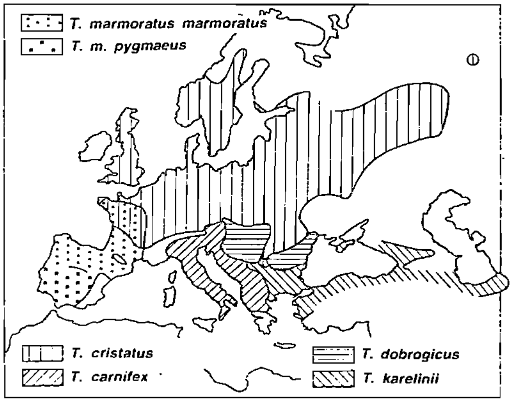

Figure 4: Distribution of five species of the *Triturus cristatus* species group. Note that all taxa within the *cristatus* superspecies (*cristatus*, *carnifex*, *dobrogicus* and *karelinii*) appear to have parapatric contact zones in all six possible pairwise combinations.

relatedness in itself is not surprising. What is remarkable is that the correlation which appears to exist between this set of characteristics and the distribution pattern displayed by the species: closely related species, capable of hybridisation, exclude each other geographically; species less related (and hybridising infrequently) may have widely overlapping distributions. Either way hybridisation is infrequent. This hypothesis deserves testing. The *cristatus* superspecies may be an excellent choice for further research - one reason is that the four taxa possess distributions that are all in contact with one another(Fig. 4). Contact zones representing all six possible pairwise combinations are currently being subjected to a detailed description. Before we can proceed, however, the phylogeny within the superspecies has to be solved. As yet conflicting results are obtained from the analysis of morphological, allozyme and mtDNA data (WALLIS & ARNTZEN, 1989; ARNTZEN & WALLIS, 1994; LITVINCHUK *et al.*, 1994).

Conservation biology.

Because resources are limiting the setting of priorities in protecting biological diversity cannot be avoided. In order to retain maximal organismal diversity, conservation guidelines might refer to the phylogenetic relationships of the taxa involved (VANE-WRIGHT *et al.*, 1991). The related problem of the conservation of genetic variation within a (super)species can also be approached through phylogenetic analysis. For example, a phylogeny has been constructed for 17 different mtDNA genomes found within four taxa of the *T. cristatus* superspecies (WALLIS & ARNTZEN, 1989). If the protection of, say, *cristatus* is ensured, one can ask what increase in diversity is gained by protecting other taxa within the superspecies. From a phylogenetic perspective the first choice would be *karelinii* from Turkey, since preserved phylogenetic diversity would increase most. Within species similar guidelines apply. *Triturus carnifex* shows significant geographical variation in mtDNA, and all other things being equal, conservation priority should go to genetically diverse populations. On the other hand, no variation is observed at all within *dobrogicus*, and thus with the current level of knowledge genetic data offer no clues as to which populations to give conservation priority. In conclusion, informed choices derived from phylogenetic criteria can markedly increase the organismal and genetic diversity that is safeguarded without increasing expenditure (FAITH, 1992).

Closing words

Phylogenies are the backbone of systematics, evolutionary biology and conservation genetics. Resolving phylogenies is a worthwhile enterprise. The accumulation of phylogenetic information from diverse sources will eventually lead to established phylogenies that go a long way in correctly representing the evolutionary history of the groups involved. Established phylogenies, in turn, can be utilised in a variety of ways to improve the phylogenetic methodologies as well as our understanding of the evolutionary process. The studies carried out on *Triturus* are a case in point.

A natural tendency appears to exist for researchers to avoid working on the same species and the same topics. The 'luxury' of a rich herpetofauna with up to 80 reptile and over 50 amphibian species in the same area, such as found in the Nearctic (KIESTER, 1971), allowing research projects to be non-overlapping, is not given to herpetologists working in the Palaearctic. Indeed, researchers representing a remarkable wide array of biological disciplines have made *Triturus* the object of their studies. Regular meetings and a collaborative spirit has led the foundations of some integrative evolutionary studies. From this point of view, the small number of herptile species in the Old World is a blessing in disguise.

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Protein electrophoretic data on taxonomic problems in East Mediterranean *Salamandra* (Urodela: Salamandridae)

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Abstract: Blood serum samples of *Salamandra* populations from Greece, Turkey, Israel, Algeria and Western Europe were compared by polyacrylamid gel electrophoresis. Polymorphism in the albumin and transferrin fractions was high but alleles were geographically restricted and therefore taxonomically informative. At the investigated loci, Asian *Salamandra*, and hence should be separated as a different species, *S. infraimmaculata*, with three ssp.: *S.i.infraimmaculata* in Israel, Lebanon, Syria and Southwestern Turkey, *S.i.semenovi* in eastern Turkey and the bordering regions of Iraq and Iran, *S.i.orientalis* in south and central Turkey. The Greek populations can be separated from *S.s.salamandra* as subespecies *S.s.wernwri*.

Key words: *Salamandra*. Turkey, taxonomy, blood serum proteins, electrophoresis.

INTRODUCTION

In his monographic treatment of the genus *Salamandra*, EISELT (1958, 1966) assigned all eastern Mediterranean Fire Salamanders to the nominate subspecies *S. salamandra salamandra* except *S. s. semenovi* Nesteroff, 1916, from NW Iraq and SE Turkey (Malatya, see map, fig. 1). SCHMIDTLER & SCHMIDTLER (1970), however, tentatively included the easternmost Turkish population (from Bitlis, fig. 1) in the nominate subspecies.

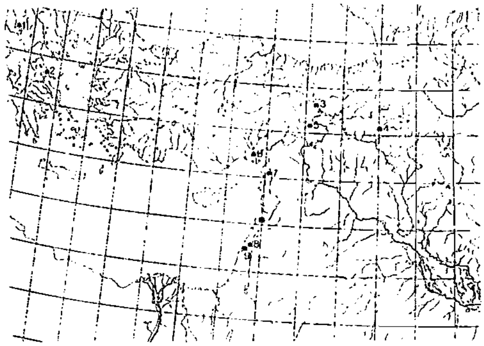

Figure 1: Localities of the investigated populations. 1: Ohrid, Macedonia (*S. s. salamandra*). 2: Pelion, Greece (Terra typica of *S. s. werneri* Gayda). 3: Kemaliye, Turkey. 4: Bitlis, Turkey. 5: Malatya, Turkey. 6: Adana, Turkey (Terra typica of *S. s. orientalis* Wolterstorff). 7: Antakya, Turkey. 8: Tel Dan, Israel. 9: West Galilee, Israel. Triangle: Terra typica of *S. s. semenovi* Nesterov. Square: Terra typica of *S. s. infraimmaculata* Mertens.

On the basis of different electrophoretic serum protein patterns, FACHBACH (1971) separated the population from Kemaliye/Erzincan (fig. 1) from *S. s. salamandra* and tentatively assigned it to *S. s. infraimmaculata* Martens, 1885, which had been described from the Lebanon. The latter subspecies was separated from *S. salamandra* by GASSER (1978a,b) because of its fundamental differences in serum protein patterns and regarded as a full species *S. infraimmaculata*.

DEGANI (1986) included the salamanders from Israel in *infraimmaculata*, which he regarded as subspecies of *salamandra*.

The Turkish herpetologists (ÖZ 1987, ÖZ & ARIKAN 1990, ARIKAN, ÖZETI & ÖZ 1990, BARAN & ÖZ, in press) mainly followed EISELT and SCHMIDTLER & SCHMIDTLER (l.c.) by leaving most Turkish populations with the nominate subspecies, but separating the Malatya population as *S. s. semenovi* and the Hatay population as *S. s. infraimmaculata* (tab. 1).

Population	Eiselt 1966	subsequent Authors*	this study
Pelion/ Taigetos	s.salamandra	s.salamandra s.werneri	s.werneri
Kemaliye/ Erzincan	-	s.infraimm. s.salamandra	i.semenovi
Bitlis	-	s.salamandra	i.semenovi
Malatya/ Aslantepe	s.semenovi	s.semenovi	i.orientalis
Adana/ Mersin	s.salamandra	s.salamandra	i.orientalis
Antakya/ Hatay	s.salamandra	s.infraimm.	i.infraimmaculata
Lebanon	s.salamandra	s.infraimm.	i.infraimmaculata
Israel	s.salamandra	s.infraimm.	i.infraimmaculata

* for citations see text

Table 1: Change of taxonomic opinion on the investigated populations.

In Greece, the subspecies *S. s. werneri* Gayda, 1941, was not recognized by EISELT (l.c.) nor by

GRILLITSCH & GRILLITSCH (1991), whereas BISCHOFF & BISCHOFF (1980) suggested its possible validity.

Although serum protein electrophoretic investigations proved to be good indicators of taxonomic relationships in *Salamandra* (FACHBACH & ALBERT 1971, GASSER 1978a,b, ARIKAN *et al.* 1990) previous studies suffered from insufficient availability of comparative material. We were now able to obtain live salamanders from all known Turkish populations[1], Greece and Israel, and *S. s. salamandra* from Lake Ohrid, Macedonia, as a reference. Moreover, we could compare serum protein patterns of the eastern *Salamandra* with nearly all of the western forms, which have been studied recently (JOGER & STEINFARTZ, in press).

MATERIAL AND METHODS

6 to 8 adult salamanders of each of the populations shown in fig. 1 were bled.

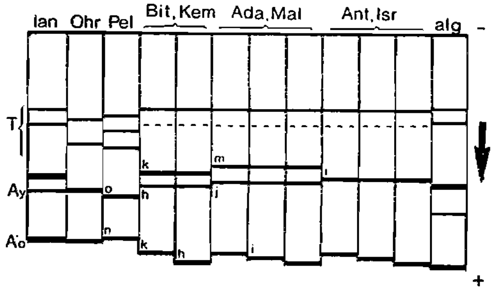

Figure 2: Observed electrophoretic patterns (cathodic fractions not shown). lan = *S. lanzai*; alg = *S. algira* (outgroup references). Other abbreviations correspond to localities of the investigated populations as shown in fig. 1.

After clotting, the blood was centrifuged and the serum was stored frozen. A total of 24 vertical, discontinuous PAG electrophoreses were executed as described previously (JOGER 1984), each with six individual probes. Serum samples of *S. s. terrestris* (Bordeaux, France), *S. s. crespoi* (Serra de Monchique, Portugal), *S. s. morenica* (Sierra Morena, Spain), *S. s. longirostris* (Sierra de Ronda, Spain), *S. s. almanzoris* (Sierra de Gredos, Spain), *S. algira* (Annaba, Algeria) and *S. lanzai* (Monvisa mts., Italy) served as markers for allocating band positions.

Interpretation of bands follows the principles outlined by JOGER & STEINFARTZ (in press).

RESULTS

An overview over the observed electrophoretic patterns is given in fig. 2. Alleles were named using and expanding the nomenclature of GASSER (1978) and JOGER & STEINFARTZ (in press). The cathodic fractions being highly variable individually, only the anodic albumins and the transferrins were used for comparison of populations. No differences between sexes were observed. As typical for many *Salamandra* (GASSER 1978a), there are three loci in the albumin fraction: pre-albumin (A'), first and second albumins (A). Populations from Israel, Antakya and the Balkans lack the second albumin (like *S. s. salamandra* and *S. s. terrestris*).

The Ohrid population is monomorphic with alleles typical for *S. s. salamandra*, whereas the Pelion population, albeit similar in general electrophoretic pattern, exhibits slightly different protein mobilities. Moreover it shows a unique three-band constellation in the transferrin region.

All Asian *Salamandra* have a characteristic transferrin of low mobility and a different system of prealbumins of much higher mobilities than those of eastern European salamanders. The populations from Adana, Malatya, Antakya and Israel have three prealbumins which occur in varying frequencies and combinations[2] (contrary to GASSER, 1978a, who found A'_i monomorphic in Lebanese *infraimmaculata*). There were no differences detectable between the two Israeli populations.

The populations from Bitlis and from Kemaliye lack A'_i, showing either A'_h or A'_k. Albumins are generally monomorphic in each population. Israel and Antakya have A_i as sole albumin; Adana and Malatya share both A_j and A_m, whereas Bitlis and Kemaliye have A_h and A_k in common (tab. 2).

DISCUSSION AND TAXONOMIC CONCLUSIONS

There are some parallel evolutionary trends in East Mediterranean as in West Mediterranean salamanders. In both regions, southern subspecies generally have faster migrating prealbumins but slower albumins as compared to the prealbumin allele A'_0 and the albumin allele A_y in Central and East European populations of *S. salamandra*, which are considered ancestral due to their presence in the relict species *S. lanzai* (JOGER & STEINFARTZ, in press, see also fig. 2).

Another similarity is the duplication of the albumin locus which is single in Central and

			PREALBUMINS fasted running ←					1st ALBUMINS				→	2nd ALBUMINS running slowest	
	Population	loc.n°	A'h	A'i	A'k	A'o	A'n	Ao	Ah	Av	Aj	Ai	Ak	Am
N	Ohrid	1				o				o				
|	Pelion	2					o	o						
|	Kemaliyc	3	o		o				o				o	
|	Bitlis	4	o		o				o				o	
|	Malatya	5	o	(o)	o						o			o
|	Adana	6	o	(o)	o						o			o
V	Antakya	7	(o)	o	o							o		
S	Israel	8,9	o	o	o							o		

Table 2: Distribution of alleles over the investigated populations. Alleles ordered by electrophoretic mobilities of protein morphs; populations in geographical order (North to South).

East European *S. salamandra*, but double on the Iberian Peninsula and in Turkey. The presence of two albumins is, however, likely to be a plesiomorphic character in *Salamandra*, as it is observed in species like *S. lanzai* and *S. algira* (Fig. 2). The absence of a second albumin is therefore interpreted as a derived feature linking the Hatay and Israeli populations. The occurence of the albumin A_i in Antakya, Israel and topotypical *infraimmaculata* (GASSER 1978a) is also regarded as synapomorphic.

Gasser already found electrophoretic differences between *infraimmaculata* and European *S. salamandra* large enough to justify a distinction on species level. Now we can include the Turkish populations, which are geographically closer to Europe (but still nearly 1,000 km apart from the nearest European population). Still there is no trace of any recent allele exchange between European and Asian *Salamandra*. The distribution gap is accompanied by a wide genetic gap. The inclusion of any Turkish population in *S. s. salamandra* is certainly not tenable. Even the Greek (Pelion) population appears sufficiently differentiated genetically from the nominate subspecies as to justify its subspecific status as *S. s. werneri*. The Asian populations are as different from *S. salamandra* as are species like *S. atra*, *S. lanzai* and *S. algira* (see JOGER & STEINFARTZ, in press). On the other hand, some genetic exchange between *infraimmaculata* and the Turkish populations (the geographically closer, the more common alleles) is obvious; therefore all Asian *Salamandra* should be included in the species *S. infraimmaculata*.

The Hatay population (Antakya) is very close serologically to Israeli *infraimmaculata* and could be included in the nominate subspecies *S. i. infraimmaculata*.

The populations from Bitlis and Kemaliye cannot be separated serologically from each other, but from all other populations. Their pattern of small, irregular blotches, as well as their northeastern distribution, suggest their identity with the subspecies *semenovi*; so they should be called *S. infraimmaculata semenovi*.

The Adana and Malatya populations are virtually identical serologically, only differing in allele frequencies of prealbumins (A'_h dominating in Adana, A'_k more frequent in Malatya) and in some morphological characters (smaller head and uniform black belly in Malatya, Baran & Öz, in press). They share with the Hatay and Israeli populations the occasional presence of the prealbumin A'_i, with the Bitlis and Kemaliye populations the presence of a second albumin, but both albumins are different in the two population groups. In colouration, the Adana-Mersin population has some resemblance with infraimmaculata, whereas the Malatya population shows tendencies of fragmentation of the yellow blotches in a similar way as *semenovi*. However, contrary to Eiselt and subsequent Turkish authors, the Malatya population is not identical with *semenovi* serologically and represents, together with the Adana-Mersin population, a different subspecies, which should bear the name *orientalis* Wolterstorff, 1932 (if not morphological differences warrant a subspecies status for the Malatya population alone).

In conclusion, the following taxonomic scheme appears most appropriate for the Asian *Salamandra*:

Species: *Salamandra infraimmaculata* (distinguished from *S. salamandra* by a totally different set of serum proteins)

- **Subspecies**: *S. i. infraimmaculata*: Israel, Lebanon, Syria, Hatay region, Turkey.
- **Subspecies**: *S. infraimmaculata semenovi*: NW Iraq, W.Iran[3], E Turkey (Bitlis, Kemaliye).
- **Subspecies**: *S. infraimmaculata orientalis*: Adana-Mersin and Malatya-Aslantepe regions, SE Turkey.

Asking for barriers that separate the Turkish subspecies from each other, we must consider that the Middle East Salamandra are confined to mountain areas. Therefore, the Euphrates valley might be an effective barrier isolating semenovi from the other subspecies, whereas the lowlands extending from the Gulf of Iskenderun up the Ceyhan and Aksu rivers or the Orontes valley separate the Adana-Malatya subspecies from the Hatay population.

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Footnotes:

1) The Turkish salamanders could be collected due to the kind help of M. Öz, whose cooperation is gratefully acknowledged.

2) In one specimen from Tel Dan, Israel, and another from Malatya, Turkey, both alleles A'_h and A'_k were present, showing a case of heterozygosity.

3) See Müller (1985).

Morphometric analysis of *Salamandra salamandra* in the Iberian Peninsula: Preliminary data

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Abstract: This paper is an attempt to estimate the significance of morphometric data in the taxonomical study of *Salamandra salamandra* by means of Principal Components Analysis (PCA). In order to characterise the morphometry 16 variables have been considered. The results show that morphometrical data alone cannot be used to define the present subespecific groups.

Key words: *Salamandra salamandra*, Morphometry, Taxonomy.

INTRODUCTION

The subespecific status of *Salamandra salamandra* in the Iberian Peninsula is very unclear because morphological, biological and biochemical criteria have been used in taxonomy independently . Nine subspecies have been described in different papers. However, it should be noted that the morphologic and morphometric criteria have been the most important characters used in the taxonomy of these species, and biochemical data were considered thereafter in order to confirm the morphological results.

The papers published that describe subspecies using morphometric or morphological characters (EISELT, 1958; FACHBACH 1976; MALKMUS, 1983, 1991; JOGER & STEINFARTZ, 1994) are too much descriptive and contain confusing taxonomic conclusions. In this paper we attempt to estimate the significance of morphometrical data in the taxonomic study of *Salamandra salamandra*.

MATERIAL AND METHODS

79 *Salamandra salamandra* specimens from the Iberian Peninsula were measured. Salamanders came from 3 places Museum collections: Museo Nacional de Ciencias Naturales (Madrid), Departamento de Zoología (Universidad de Salamanca) and Departament de Biología Animal (Universidad de Barcelona). The localities used in the morphometric study are: Caracollera (Ciudad Real), Laguna Grande de Gredos (Ávila), Benasque (Huesca), Oreja de Sajambre (León), Coballes (Asturias), Valle del Pas (Cantabria), Sierra Morena (Córdoba), Facinas y Benalup de Sidonia (Cádiz), Pinar de Lillo (León), Planoles (Girona), Montseny (Barcelona), Lagunas de Peñalara, Cáceres, Oviedo (Asturias), Lleida, Arribes de Duero, Meranges (Girona), La Selva (Girona), Salamanca, Zamora and Marruecos.

We attempted to obtain individuals from all the sites hrere subspecies had been reported. Despite our efforts the sample was not as large as we wanted but, as a preliminary result, we consider that there are enough morphometrical differences exist to describe a new ssp.

To characterise the specimen morphometry 16 variables have been considered: Length measures: (LT) Total length, (LCUA) Tail Length, and (LCB) Head Length. Head measures: (ACB) Head Width, (HCB) Head height, (DN) Distance between nares, (DUN) Distance from eyes to nares, (DUA) Distance from nose to anterior eye, (DUP) Distance from nose to posterior eye, (LCP) Distance from posterior head to nose. Parotid measures: (LP) Parotid Length, (AP) Parotid Width. Tail measures: (HCA) Base tail height, (ACA) Base tail width, (ACF) Final tail width, (HCF) Final Tail height.

Moreover, to locate the sites where the individuals came from geographically, we measure two geographical variables: Shortest distance to Gibraltar and Perpendicular distance to the Greenwich Meridian.

	Eigenval.	Propor.	Cumulative
PC 1	702.264	0.972514	0.97251
PC 2	11.295	0.015641	0.98816
PC 3	3.075	0.004258	0.99241
PC 4	1.651	0.002287	0.99470
PC 5	0.616	0.000853	0.99555

Table 1: Eigenvalues of the Covariance Matrix. Total Variance: 722.11239.

	C.P.1	C.P.2	C.P.3	C.P.4
LT	0.904	0.237	-0.335	-0.001
LCUA	0.369	-0.841	0.347	-0.105
LCB	0.079	0.134	0.334	0.502
ACB	0.096	0.117	0.491	0.151
HCB	0.044	0.133	0.217	-0.120
DN	0.022	-0.021	0.074	0.249
DUN	0.007	0.007	0.039	0.207
DUA	0.032	0.014	0.023	0.262
DUP	0.051	0.118	0.005	0.105
LP	0.071	0.123	0.202	0.017
AP	0.041	0.178	0.294	-0.366
HCA	0.051	0.193	0.223	-0.436
ACA	0.046	0.173	0.257	-0.347
ACF	0.017	0.081	0.233	-0.688
HCF	0.024	0.059	-0.018	-0.113
LCP	0.115	0.216	0.340	0.228

Table 2: Eigenvectors of the Covariance Matrix.

The main problem when attempting to describe ssp. using numerical methods on morphometrical variables appears when intrapopulation variability is greater than interpopulation variability, because the sample size has to be increased to show results. In our case this solution, was impossible. Since morphometric variables are strongly dependent on the size of the specimens and, at the same time, size depends on age and sex, if we drop the size-effect from our data we drastically reduce the intra population variability.

Principal Components Analysis (PCA) was used to do this, since it gives uncorrelated measures, principal components, with maximum variablity in each one. Moreover when PCA is applied to morphometric measures, the first component, which explains maximum of variability in the data, usually is the the specimen size. So taking into account the rest of significant components, which describe the specimen's shape, we obtained shape measures independent by of the size-effect. When these components were obtained, the relationship between the shape of specimens and their geographical location was studied by graphical methods (scatter plots).

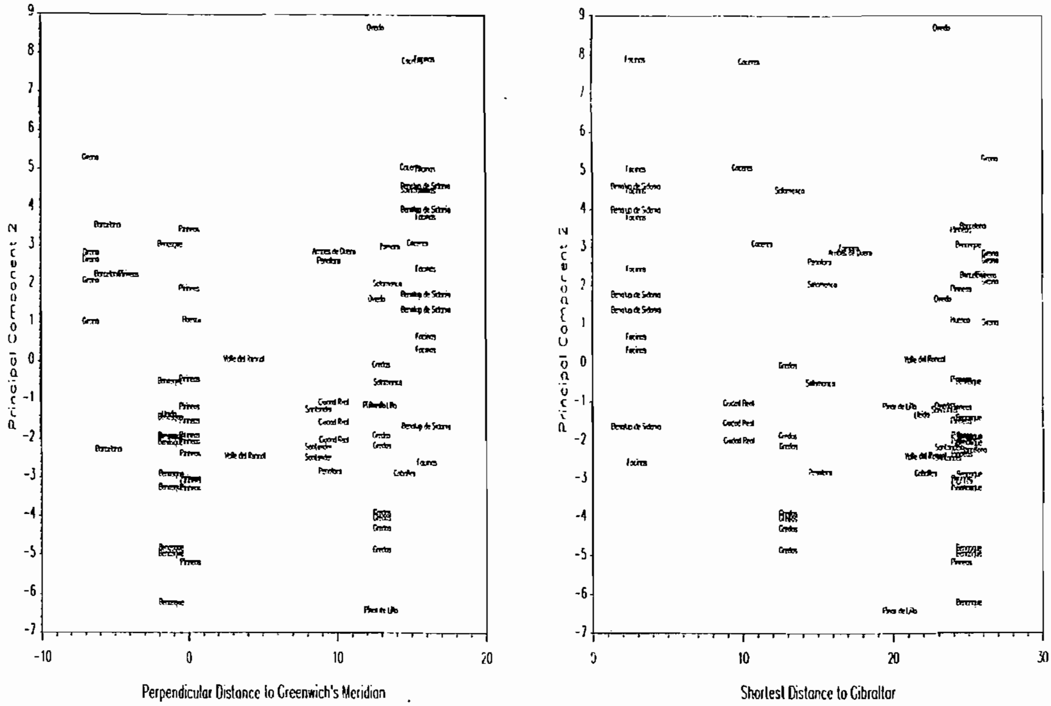

Figure 1: Relation between PC2 and Geographical location.

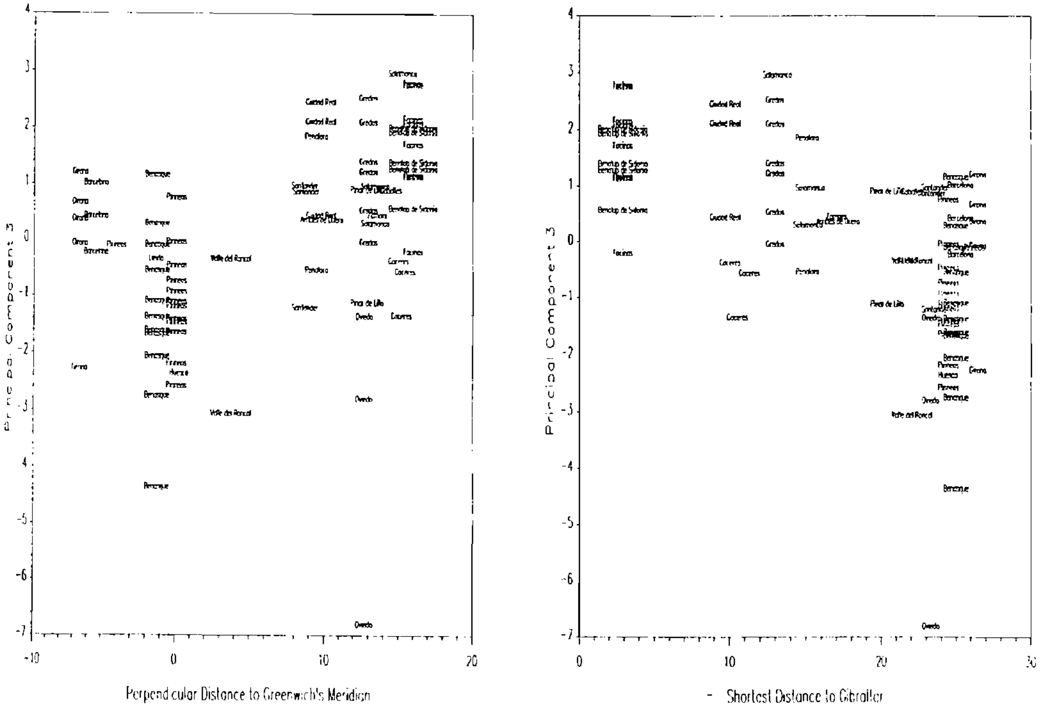

Figure 2: Relation between PC3 and Geographical location.

RESULTS AND DISCUSSION

The PCA explained 99.4% of the total variability with the first component (Table 1). The first component in the PCA was indeed interpretable as a measure of size looking at the eigenvectors (Table 2). The 2nd, 3rd, and 4th were interpreted as body elongated shape, general volumetric shape and head shape respectively from eigenvectors (table 2). Only these last three components were used in further analysis since the remaining one explains a very low proportion of the total variance (Table 1).

Figures 1 & 2 show the bivariate distribution of components 2 and the Geographical position. No patterns of relation are clearly identified in these plots. Figure 3 shows the bivariate distribution of Principal Components 2 and 3 for each population. Despite the reduction of intrapopulation varibility it seems to be as high as interpopulational variability, and no clear clusters of populations appear.

It is common in the Iberian Peninsula to consider individuals from different geographic areas as belonging to different ssp. Low morphological and morphometrical differences are considered the main criteria to describe new ssp even when the intrapopulation variability is, at least, as high as interpopulation variability.

Our results show that morphometrical data cannot be used alone to define the present subespecific groups. From the scatter plots we see how all the populations included are shown as the same population. No different morphometry patterns have been found throughout the studied populations.

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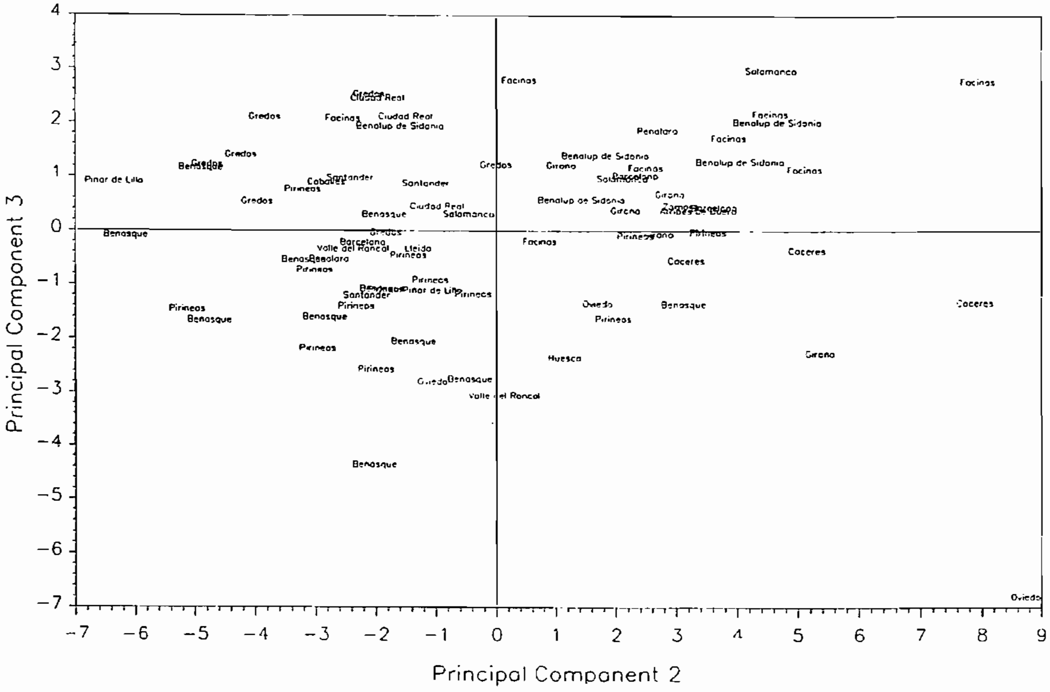

Figure 3.- Bivariate distribution of Principal Components 2 and 3 for each population..

Hybridogenetic processes involving *R perezi*: distribution of the P-RP system in Catalonia

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Abstract: The hybrid resulting from the hybridisation between the two sexual species *R. ridibunda* and *R. perezi* shows a hemiclonal reproduction mode where *R. perezi* is the parental excluded. The present study aims to analyse the presence of this hybridogenetic system in Catalonia, its Northeast distribution in the Iberian Peninsula. Karyotype analysis and morphometric characters did not prove useful in discriminating hybrid individuals from non-hybrids. However, the use of electrophoretic markers has enabled us to characterise hybrid and non-hybrid populations and determine the degree of genetic variability of the populations studied. The hybrids penetrate in Catalonia along two possible pathways following the course of the Rivers Segre, Ebro and Llobregat. The proportion of hybrids decreases towards the southern limit, a fact which together with the other distributional data supports the hypothesis that the R-P system is still in expansion in the Iberian Peninsula.

Key words: *Rana perezi*, *Rana* kl *RP*, Hybridogenesis, distribution, water frogs, Iberian Peninsula

INTRODUCTION

The hybrids belonging to the *Rana esculenta* group show a widespread distribution throughout Europe. They all reproduce hemiclonally, i.e. one of the parental genomes is excluded in the germ line prior to meiosis yielding haploid gametes with the genome of the non-excluded parental. In all cases known at present, *R. ridibunda* acts as one of the parentals, and most often the genome excluded corresponds to the other parental (GRAF & POLLS, 1989). One possible combination is that of the P-RP system where the parentals are *R. ridibunda* and *R. perezi* . The latter parental species is genetically distinct from *R. ridibunda* and its distribution stretches across the Iberian Peninsula through southern France and along the Rhône River beyond Lyon. It is widely distributed in Catalonia and is present in all water sources. However, it is plausible that the hybridogenetic P-RP system could also be present in that same area.

Apart from initial immunological studies indicating the presence of the RP hybrids in Zaragoza and the Basque country (UZZELL & TUNNER, 1983), no further data were available on the extension of the system in the Iberian Peninsula. Attending to the structure of the different systems within the R. esculenta complex throughout Europe, it can be expected that the RP hybridogenetic hybrids could have originated in France and extended into the Iberian Peninsula. According to the results in UZZELL & TUNNER (1983) it could be plausible that dispersal into Spain could have happened west of the Pyrenees. Another additional route may have been through the eastern Pyrenees along the major river basins. In an attempt to establish such paths of penetration we studied a range of populations from Catalonia.

MATERIAL AND METHODS

We collected a total of 107 adult frogs from Catalonia (Table 1). Samples of liver, heart, skeletal muscle and oocytes were obtained from anesthesized individuals and the tissues stored at -70° C. The supernatants of the homogenized tissues were later used for allozyme electropho-

Population	n	%
Delta Ebro	16	18.7
Solsonés	13	7.7
Cerdanya	3	100
Lleida	11	36.4
Garrigues	3	-
Tarragona	11	-
Delta Llobregat	12	8.3
Moianés	10	-
Pont de Suert	3	-
Pals	13	30.8
Portbou	7	-
Terra Alta	5	-

Table 1: Populations, sample size (n) and percentage of hybrids from Catalonia

resis. A total of 12 loci were analysed in all cases: Aspartate Aminotransferase (AAT, EC 2.6.1.1), Glycerol-3-Phosphate Dehydrogenase (G3PDH, EC 1.1.1.8), Glutamate dehydrogenase (GTDH, EC 1.4.1.2), Isocitrate dehydrogenase (IDH, EC 1.1.1.42), Lactate dehydrogenase (LDH EC 1.1.1.27), Malate dehydrogenase (MDH, EC 1.1.1.37), Malate dehydrogenase (NADP$^+$) (MDHP, EC 1.1.1.40), Mannose-6-Phosphate Isomerase (MPI, EC 5.3.1.8), Phosphoglucomutase (PGM, EC 5.4.2.2.), Superoxide Dismutase (SOD, EC 1.15.1.1). Electrophoretic buffers used were Tris-Citrate pH 6.0 (LDH , MDH, PGM, SOD) and Tris-Citrate pH 8.0 (AAT, G3PDH, GTDH, MDHP). Staining procedures are as those in SHAW & PRASAD (1970) and HARRIS & HOPKINSON (1976). Designation was numerical for loci (with the exception of LDH) according to increasing anodal migration and alphabetical for alleles according to decreasing migration. Allele frequencies and genetic variability coefficients were obtained using BIOSYS computer program (SWOFFORD, 1982).

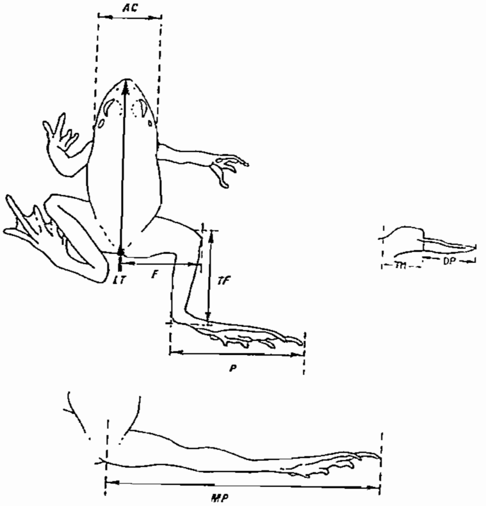

Figure 1: Variables measured in the water frogs

In order to find further means of differentiating between hybrids and non-hybrids both caryological and morphological analyses were carried out. The former consisted of the detection of C bands which could yield a differential heterochromatin pattern for hybridogenetic and non-hybridogenetic individuals. This was complemented by the analysis of the nuclear organiser regions (NOR). For both cytogenetic analyses the procedures followed were those described in HERRERO *et al.* (1990).

The purpose of the morphological analysis was to find possible ratios that could also help discerning R-P hemiclonal hybrids from *R. perezi* individuals, as similar ratios have been found to differentiate parentals and hybrids in other hybridogenetic water frog populations (POLLS, 1991). Measurements taken are shown in Figure 1. The parametric and non-parametric statistical analysis carried out by means of Statgraphics computer package consisted of a basic statistical description and a discriminant analysis.

RESULTS AND DISCUSSION

Locus Ldh-B, considered as diagnostic by previous authors (GRAF *et al*, 1977; GRAF & POLLS-PELAZ, 1989), was used to discern parentals from hybrids. *R. ridibunda* was always present with allele Ldh-B a (fast) while *R. perezi* was represented by two alleles: Ldh-B c and Ldh-B e (slow), the latter being the most common allele in hybrid and non-hybrid poulations. G3pdh was also diagnostic for *R. perezi* with allele G3pdh -b, while *R. ridibunda* showed allele G3pdh -a. This locus was particularly useful in those cases were heterozygotes were not easily attributable to a hybrid or a non-hybrid and no gonadal tissue was available. However, comparisons between soma and oocytes, to check for germ line exclusion, were possible in hybrids where we had access to primary oocytes. In those cases we confirmed that the *perezi* genome was excluded and that the *ridibunda* genome was the one expressed in the ova. The results from the screen with diagnostic loci Ldh-B and G3pdh indicate that six populations from the twelve surveyed have hybridogenetic individuals: Delta del Ebro, Solsonés, Cerdanya, Lleida, Delta del Llobregat and Pals, with Cerdanya showing the highest percentage of hybrids, followed by Lleida and Pals (Table 1).

All the remaining loci examined were polymorphic with the exception of Gtdh, Idh-2 and Sod. Percentage of polymorphism ranged from a minimum 22.2%, for the population of Port Bou, to 55.6% in Cerdanya, with an overall average of 35.16%. The maximum heterozygosity was observed in Pont de Suert (H= 0.259) followed by Lleida (H=0.239), the minimum values were those in Moianés (H= 0.081) (Table 2). As expected, the levels of polymorphism and heterozygosity were higher

	1	2	3	4	5	6	7	8	9	10	11	12
P	33.3	33.3	55.6	44.4	33.3	33.3	33.3	22.2	33.3	44.4	22.2	33.3
Ho	.115	.169	.213	.239	.222	.151	.215	.081	.259	.238	.120	.185
He	.153	1.71	.243	.245	.222	.139	.174	.123	.215	.237	.116	.230

Table 2: Genetic variability coefficients. P: Percentage of polymorphic loci found in the populations surveyed; Ho: Mean observed heterozygosity; He: Mean expected heterozygosity.

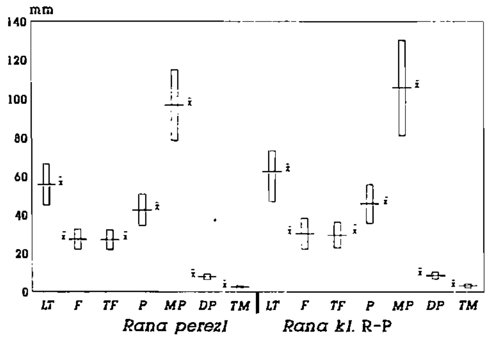

Figure 2: Mean and standard deviation of the biometrical measurements employed

for populations with presence of hybrids; however, non-hybrid populations also showed a notable degree of genetic variability.

The cytological and morphological analyses aimed at finding other sources of diagnosis of hemiclonal individuals did not yield satisfactory results. In the case of the Karyological survey the results were coincident with those found for *R. perezi* by HERRERO *et al* (1990). No variations in the C-band patterns were detected among the different populations analysed, which included hybridogenetic and non-hybridogenetic individuals.The study of the NOR gave similar results, being localized in the long arm of chromosome 10, as has been described for *R. ridibunda*, *R. lessonae* and *R.* kl. *esculenta*, all of which seems to confirm the highly conserved Karyological status of the water frogs.

Since the measurements indicated in Figure 1 were not discriminant by themselves (Figure 2), they were transformed into several ratios to gain more information: TF/LT; TF/TM; DP/TF; DP/P and DP/TM. The comparison of all these ratios by means of a t Student statistical test gave non-significant values ($p<0.05$), which indicates that they cannot be used to discern between hybridogenetic and non-hybridogenetic individuals of the P-RP system. A more precise study by means of a discriminant analysis only confirmed the previous results, showing that none of the ratios nor any of the possible combinations among them could discriminate between the parental *R.perezi* and the RP hemiclone.

In conclusion, the analysis using allozyme markers confirms that one of the possible paths of penetration into the Iberian Peninsula was through the lower areas of the eastern Pyrenees as suggested by the data of GRAF *et al.* (1977) and our own findings of hybridogenetic populations in the area of the Alt Empordà (NE Catalonia). We are also able to establish another possible way of dispersal into Catalonia through La Cerdanya (pop. 3). Both paths follow the course of the rivers Segre and Ebro in the first case and the coast in the second as can be clearly seen in Figure 3. The distribution of hybrids in the area of Catalonia and their scarcer presence towards the southern limit (River Ebro) seems to

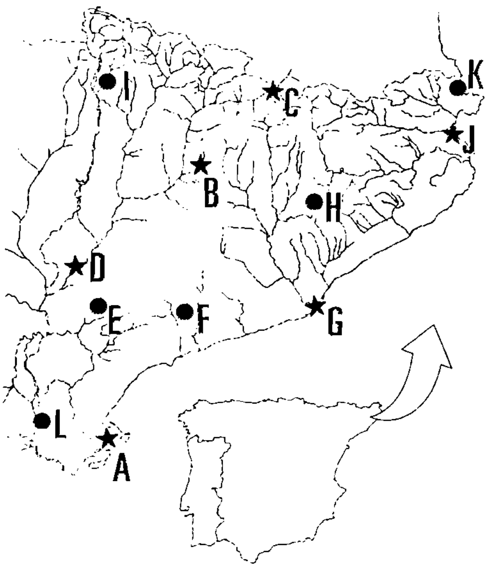

Figure 3: Distribution of the *RP klepton* in Catalonia. (star: hybrid population). A: Ebro Delta. B: Solsonés. C: Cerdanya. D: Lleida. E: Garrigues. F: Tarragona. G: Delta Llobregat. H: Moianés. I: Pont de Suert. J: Pals. K: Portbou. L: Terra Alta. Populations of Segre river: C.B.D .

indicate that the P-RP system would have initiated their dispersal into the Iberian Peninsula recently and would still be in expansion.

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Preliminary results of experimental androgenesis among hybridogenetic Water Frogs

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Abstract: Oocytes (genome R) from *Rana* kl. *esculenta* were subjected to U.V. irradiation, and thereafter inseminated with diploid sperm (genome LL) from natural allotriploid males (RLL). Some embryos developed until the free-swimming larval stage, at which point the experiment was stopped. The majority of such embryos were diploids of LL genome (the paternal chromosomic set). This was deduced from nucleolar counts, hemocytometry, karyotypes and electrophoresis of diagnostic isozymes. All available evidence suggested that the maternal chromosomic set was destroyed by the U.V. irradiation of the oocytes during the meiotic Metaphase II stage. Thus, the development of embryos of *Rana* without the maternal chromosomic set, constitutes a rare record of experimental androgenesis among vertebrates. The androgenetic embryos seemed to be damaged but it is uncertain if this was due to excessive U.V. irradiation during manipulation. Experiments on the viability of androgenetic frogs will be continued.

Key words: Androgenesis, development, diploid sperm, embryos, hybridogenesis, kleptons, *Rana* kl. *esculenta*, water frogs complex.

INTRODUCTION

Sexual reproduction implies the presence of both paternal and maternal chromosomic sets within the same egg (MAYNARD SMITH, 1978). In contrast, Apomixis (i.e. parthenogenesis, gynogenesis), considered an asexual mechanism of reproduction (Mogie, 1986), ordinarily implies only the presence of the maternal genome in the egg (the paternal genome being absent). In Vertebrates, natural occurrence of parthenogenesis and gynogenesis has been reported among several groups of amphibians and fishes (DAWLEY, 1989; POLLS PELAZ, 1990, 1991), but no natural androgenetic system has been described.

From now on androgenesis is defined as a hypothetical mechanism of reproduction involving the presence of only the paternal chromosomic set of the egg (the maternal chromosomic set being absent). Thus, if androgenesis only involves the paternal chromosomic set, all the offspring derived from such reproductive processes will be exclusively male.

The genetic complex of the European water frogs embraces a great diversity of natural populations presenting sexual parasitism of stable hybrid genealogies (the so-called kleptons, a new systematic-evolutionary category, see DUBOIS & GÜNTHER, 1982; POLLS PELAZ, 1990) on their associated "good" species (the sexual host). This mechanism of reproduction has been called hybridogenesis (SCHULTZ, 1969; TUNNER, 1974), and comprises at least 4 types of cytogenetic mechanisms present in the hybrids (kleptons) (Polls Pelaz, 1990, 1991). Hybridogenesis in water frogs implies a hemiclonal or clonal mode of reproduction (GRAF & POLLS PELAZ, 1989).

Nineteen percent of a hybridogenetic natural population in the Chanfroy pond in the Fontainebleau forest (France) was composed of allotriploid, clonal unisexual males (*Rana* kl. *esculenta*, genome RLL) (POLLS PELAZ, 1991). Spermatogenesis in such allotriploid males only produces diploid (LL) fertile sperm that contain dominant factors of male sexual determinism (POLLS PELAZ & GRAF, 1987, 1988 a). Gametogenesis in *esculenta* allotriploid frogs is ameiotic (see Polls Pelaz, 1992), but oogenesis in *Rana* kl. *esculenta* females (genome RL) involves a particular mode of meiosis (GRAF & MÜLLER, 1977; POLLS PELAZ, 1991), which produces *ridibunda* (R) haploid oocytes (without genetic recombination).

The presence of such diploid sperm (LL) and haploid (R) oocytes suggested the performance of a laboratory experiment of androgenesis in frogs.

MATERIAL AND METHODS

Frogs were collected from the enclosures of the *Station de Zoologie Expérimentale* at the University of Geneva, in whose laboratories the entire experiment was conducted (June, 1992). The genome and pedigree of parental *Rana* kl. *esculenta* females (RL) and males (RLL) was known by electrophoresis, morphology, biometry, hemocytometry and karyotypes. Such frogs were descended from selective crosses of frogs from the natural population of the Chanfroy pond in the Fontainebleau Forest (France).

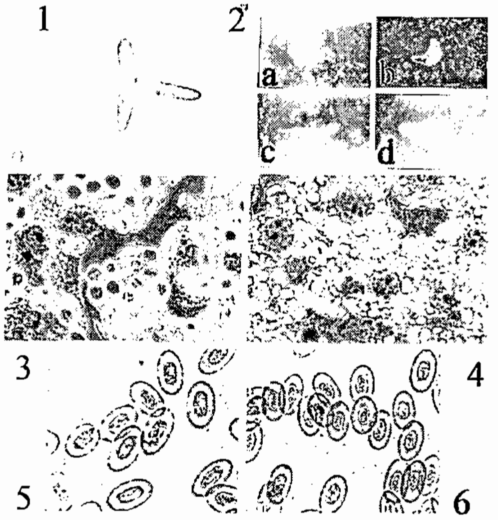

Figure 1: Double size of diploid (LL) sperm of *R.* kl. *esculenta* allotriploid males with respect to the haploid sperm. Figure 2a, 2b, 2c: Abnormalities observed in some LL androgenetic embryos. d: normal development of RLL embryos. Figure 3: 1-3 nucleoles observed in cells of RLL embryos at early stages of development. Figure 4: 1-2 nucleoles observed in cells of LL androgenetic embryos at the very same early stages of development. Figure 5: Hemocytometry in triploid tadpoles (22-25 μm of major axis length). Figure 6: Hemocytometry in diploid tadpoles (17-19 μm of major axis length).

After some days of being submitted to a temperature of 10°C in a cold chamber, female frogs were injected with a homogenate of hypophisis, and thereafter submitted to a temperature of 20°C. Ovulation takes place in a few hours. Oocytes were subjected to U.V. rays of 7,000 erg./cm^2 and 28,000 erg./cm^2 in dependence of the time of exposure to the U.V. irradiation (from 50 to 200 seconds). During the irradiation, oocytes were manipulated and oriented with the animal pole towards the source of U.V. rays. At such a time the oocytes are at the meiotic Metaphase II stage, thus facultating the destruction, by the U.V. rays, of the maternal chromosomic set, because at the MII stage the latter is located very near to the surface of the animal pole.

After irradiation, the oocytes were inseminated with diploid sperm (LL) from the testes of *esculenta* RLL males. Mobility and adequate density (13-27.10^6 spz/ml) of the sperm was verified. Homogenates of the testes were made in the liquid of Ringer (Na Cl 113 mM, Ca Cl$_2$ 0,7 mM, KCl 2mM, NaHCO$_3$ 2,4 mM, pH 7,8).

Insemination and rearing of embryos were also conducted using standard tecniques (POLLS PELAZ, 1991).The development of embryos was stopped at stage 25 in the table of GOSNER (1960). To verify the above results, a control experiment was conducted by inseminating non-irradiated oocytes (genome R) with diploid sperm (LL). Genetic dosage and identification of genotypes of the embryos was established by (1) counting by optical miscroscopy. (O.M., increase X 400) the number of nucleoles in squashes of the embryo at early stages of the embryonic development (OLERT, 1979) from gastrula to neurula, (2) by hemocytometry of tadpoles (Polls Pelaz & Graf, 1988 b) at the free-swimming larvae stage, as well as (3) by karyotypes and (4) by electrophoresis of diagnostic isozymes (see POLLS PELAZ, 1991, 1992).

RESULTS

All the embryos (n= 8) of the control (veryficatory) experiment displayed triploid RLL genotypes; this was established by the following: (1) counting 1-3 nucleoles in the squash of cells (around 30 cells in each embryo) (Figure 3); (2) hemocytometry of tadpole (n=4) red blood cells measuring 22-25 μm (12 cells in each tadpole) at the free-swimming larval stage; (3) karyotypes (n=4) with 39 mitotic chromosomes in mitotic metaphases; and (4) electrophoresis of LDH diagnostic isozyme patterns (n=4) displaying a heterozygotic pattern with double dosage of the L-band with respect to the R-band.

Seventy-eight percent (n=33) of the embryos whose oocytes had been subjected to U.V. radiation, displayed diploid LL dosage. This was established from the following: (1) counting 1-2 nucleoles (Figure 4) at an early stage of development (around 30 cells in each embryo); (2) hemocytometry of tadpole (n=5) red blood cells which measured 17-19 μm (12 cells in each tadpole); (3) karyotypes (n=5) with 26 mitotic chromosomes, and (4) diagnostic isozyme patterns of LDH (n=5) situated at the homozygote *lessonae* (L) band.

Thus, it can be concluded that some LL diploid androgenetic embryos reached the beginning of the larval period.

Diploid, androgenetic LL embryos -specially the long-term irradiated oocytes- showed (Figures. 2-a,b,c) a higher percentage of embryonic abnormalities than triploid controls (RLL) (Figure 2-d)

DISCUSSION

Clearly, not all the genetic material of the oocyte was destroyed by the U.V. source during the experiment. For instance, mit.DNA as well as different sources of RNA must have been preserved in the oocyte. The purpose of the U.V. irradiation was only to destroy the maternal chromosomic set. From this point of view, the experiment resulted in the successful development of some androgenetic embryos, and enables us to conclude that the maternal chromosomic set does not seem to be indispensable for the embryonic development of European water frogs. The results of androgenesis exposed upon constitute a rare record among vertebrates.

Further efforts will be focused to establish whether or not the embryonic abnormalities observed in the androgenetic frogs were due either to damage during manipulations or to the absence of the maternal chromosomic set. From this point of view the rearing of embryos until the adult stage constitute also a reasonable goal.

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Evolution of reproductive patterns in Gymnophiona Amphibia

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Abstract: In Gymnophiona, primitive species are oviparous. The metamorphosis of their free aquatic larvae is progressive. In more advanced oviparous species, it is quick. In viviparous Gymnophiona, the metamorphosis occurs into the uterus. Each female gives birth to completely metamorphosed animals. In the most advanced animals, viviparity has reached its highest degree. The gills of larvae are transformed into organs that are narrowly applied against the uterine wall giving a placenta-like structure.

Key words: Amphibia, Gymnophiona, metamorphosis, oviparity, viviparity.

INTRODUCTION

Gymnophiona order groups more than 170 species that are living in South and Central America, Asia and Africa (TAYLOR, 1968). These animals are terrestrial with burrowing habits but the most advanced are aquatic.

Several classifications of Gymnophiona have been proposed (TAYLOR, 1968, WAKE et CAMPBELL, 1983, DUELLMANN & TRUEB, 1986, LAURENT, 1986, LESCURE *et al*, 1986, NUSSBAUM, 1977, NUSSBAUM & WILKINSON, 1989). To summarize, gymnophiona order can be divided into primitive (i.e. *Ichthyophis*), intermediate (i.e. *Siphonops*, *Hypogeophis* or *Dermophis*) and evoluted forms (i.e. *Typhlonectes*).

The reproductive modes of these animals are still little known and only a few species have been studied (WAKE, 1977; EXBRAYAT, 1992). After the data given by the litterature, we can deduce the progression of reproductive patterns in correlation with the evolution of the group. It seems to exist a general trend to the reduction of metamorphosis duration, and a transition from oviparity to viviparity. The purpose of this communication is to present these trends.

METAMORPHOSIS

In Anura and Urodela, the metamorphosis has been well studied. In Gymnophiona, the data are very rare and only concern few organs.

1) The facts of metamorphosis (Fig. 1)

Gills. All Gymnophiona present only one generation of external gills. At metamorphosis, they disappear. This phenomenon has been described in both oviparous and viviparous species (SESHACHAR, 1942, WAKE, 1977, BRECKENRIDGE & JAYASINGHE, 1979, BRECKENRIDGE *et al*, 1987). In the viviparous *Typhlonectes*, the gills are transformed into vesiculous organs that are present until birth (DELSOL et al, 1981, 1986)

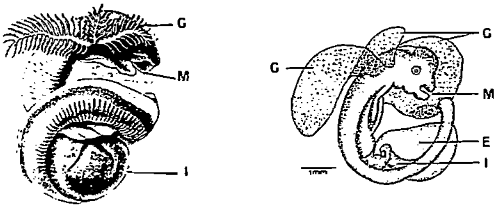

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Figure 1: Gymnophiona embryos. (1) *Syphonops annulatus* (after GOELDI, 1899); (2) *Typhlonectes compressicaudus*. E: ectotrophoblast; G: gill; I: intestine; M: mouth.

Locomotion organs. In the oviparous, *Ichthyophis glutinosus* a fin is observed on the tail during the free aquatic phase which lasts 10 months. It disappears when the animal becomes an adult (BRECKENRIDGE & JAYASINGHE, 1979, BRECKENRIDGE *et al*, 1987).

Sense organs. During metamorphosis, sense organs of Gymnophiona are transformed. The eyes regresse. A pair of tentacles in relation with the vomeronasal organ develops. In *Ichthyophis glutinosus*, these tentacles appear just before the terrestrial phase (BRECKENRIDGE & JAYASINGHE, 1979). In the viviparous *Dermophis*, *Gymnopis* and *Typhlonectes*, these organs begin their development at the metamorphosis stage (FOX, 1985, BILLO, 1986, BILLO & WAKE, 1987). In aquatic larvae, we can observe a lateral line organs that will disappear at the terrestrial phase (HETERINGHTON & WAKE, 1979, FRITZSCH & WAKE, 1986).

Skin. The structure of Gymnophionan larval skin looks like the Urodela one. The larval skin possesses Leydig cells that are secretory, in *Ichthyophis* (FOX, 1983, 1987, BRECKENRIDGE, 1983). In adult animals, the skin has the classical histological structure that is found in other Amphibians with, in addition, the presence of scales, in some species (LAWSON, 1963, FOX, 1983, 1986, 1987, ZYLBERBERG *et al*, 1980).

Skeleton. Some aspects of the ossification have been studied in *Dermophis mexicanus* (WAKE & HANKEN, 1982) and *Typhlonectes compressicaudus* (WAKE *et al*, 1985). Ossification increases at metamorphosis.

Digestive tract. In Urodela and Anura, the digestive tract presents some very important modifications that occur during metamorphosis. In Gymnophiona, the development of digestive tract has been studied in *Typhlonectes compressicaudus* (HRAOUI-BLOQUET & EXBRAYAT, 1992). After the reabsorption of the yolk, a first generation of enterocytes is observed which will be replaced later by a definitive one.

2)Place of the metamorphosis in Gymnophionan Amphibia (Fig. 2).

In the primitive species (i.e. *Ichthyophis glutinosus*), gills buds begin to develop 4 days after the fertilization. At the eclosion, the embryo possesses a pairs of triradiated gills, a small vitelline mass and a fin on the tail. Tentacles are not observed. Two days after the eclosion, gills disappear, vitellus is reabsorbed. After about 10 months, the fin disappears and the tentacles develop themselves. For the authors (BRECKENRIDGE & JAYASINGHE, 1979) this slow transformation is the metamorphosis.

In the advanced oviparous *Siphonops annulatus*, metamorphosis has not been observed. Yet, GOELDI (1899) observed that the embryos, still placed into a mucous envelope, possessed a pair of triradiated gills. The yolk was then reabsorbed. In *Hypogeophis rostratus*, the development is direct (MARCUS, 1909). The metamorphosis occurs before the eclosion and the new-born looks like a young adult.

In several viviparous species, the eclosion and the larval life occur into the uterus. The metamorphosis is essentially characterized by the degeneration of gills (WAKE, 1967, 1977, 1980)

In *Typhlonectes compressicaudus*, an advanced species different signs of metamorphosis have been observed: evolution of the skin, of the digestive tract, development of tentacles (EXBRAYAT, 1986, SAMMOURI *et al*, 1990, HRAOUIBLOQUET & EXBRAYAT, 1992). Yet, the gills are never triradiated and they persist even after the metamorphosis, until the birth of the animal. In this species, the metamorphosis seems to be quick and it occurs 3 to 4 months after the fertilization (EXBRAYAT, 1986).

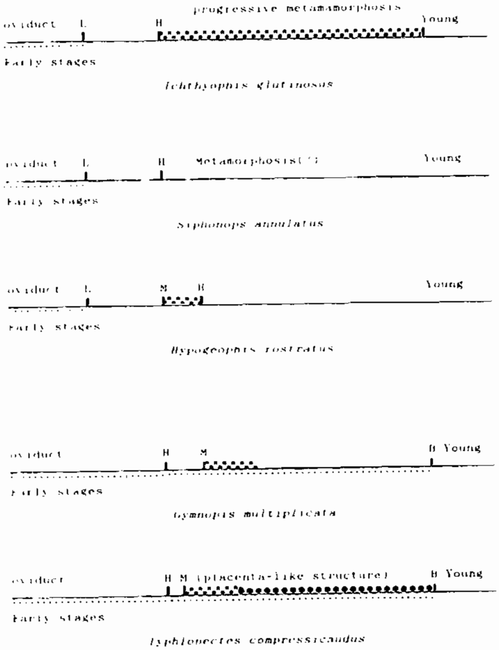

Figure 2: Different types of reproductive patterns in Gymnophiona: Part of development into female genital tract (...) Metamorphosis duration (■■■) Placenta-like structure (●●●). H: hatching; L: egg-laying; B: birth; M: metamorphosis.

OVIPARITY AND VIVIPARITY

To understand the evolution from oviparity to viviparity, some points have to be considered: eggs, female genital tract, specialization in embryos.

1)Eggs.

Eggs of oviparous species are united in clusters. Diameter of eggs is 4 to 10 mm (EXBRAYAT & DELSOL, 1988). These species lay 20 to 50 eggs, but there are some exceptions. *Idiocranium russelli* lay only 6 eggs (WAKE, 1977) and *Ichthyophis malabarensis* lay more than 100 ones (SESHACHAR *et al.*, 1982). In viviparous species, egg diametrer is generally 2 to 3 mm. Only 15 to 20 eggs are laid in the genital tract. At the end, only 6 to 8 embryos will be born.

2) Genital tract.

In oviparous species, each oviduct can be divided into 3 parts: an anterior pars convoluta, a median pars recta and a posterior pars utera that are secreting protein and mucous substances involved in the elaboration of the egg envelop (EXBRAYAT, 1989).

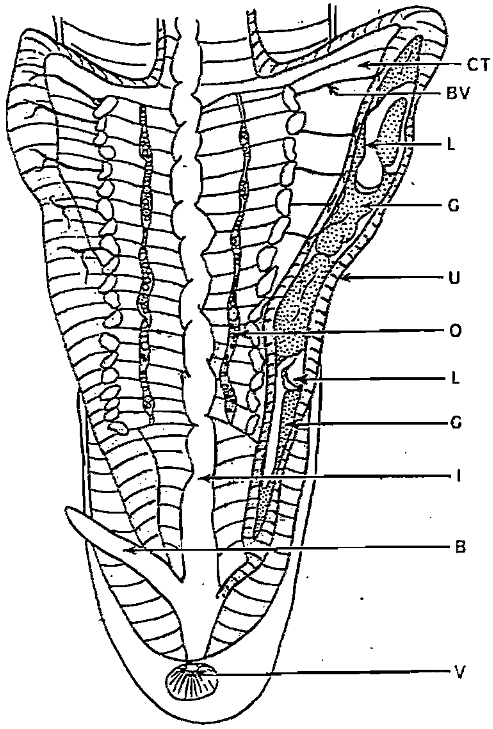

Figure 3: Intra-uterine larvae in *Typhlonectes compressicaudus* female.
B: bladder; BV: blood vessel; CT connective tissue; G: gill; L intra-uterine larva; I: intestine; O: ovary; U: uterus: V: vent.

In viviparous species, each oviduct is divided into two parts (Fig. 3). The anterior part is involved in the elaboration of the egg envelope. In the posterior uterus, embryos develop. During gestation, the uterine wall secretes different kinds of substances that are eaten by the embryos. In *Typhlonectes compressicaudus*, in addition, at the end of the gestation, the uterine wall is degraded and a new cellular structure is observed. This new epithelium will be used to contribute to the formation of a pseudoplacenta (EXBRAYAT, 1988).

3) Specializations in embryos

At the eclosion time, in oviparous species, each embryo possesses 3 pairs of gills (or only two, in *Gegenophis carnosus*), and a fin on the tail (SARASIN & SARASIN, 1887, 1890, BRECKENRIDGE & JAYASINGHE, 1979, BRECKENRIDGE *et al,* 1987, SESHACHAR 1942, SESHACHAR *et al,* 1982).

The gills of viviparous species are similar to the oviparous ones. In *Typhlonectes compressicaudus*, the gills are transformed into vesiculous structures and, at the end of the development, they are narrowly applied against the uterine wall. They fall down 24 or 48 hours after birth. The gill and the uterus constitute a placenta-like structure. In this same species, another specialization, the ectotrophoblast has been observed on the ventral ectoderm at the beginning of development (DELSOL *et al,* 1981, 1986)

In viviparous species, the fetuses possesse a special dentition that permit them to grasp the uterine wall increasing the uterine secretions (PARKER, 1956, PARKER & DUNN, 1964, WAKE, 1977, EXBRAYAT, 1986). At the time of birth, these teeth regresse. Finally, during the development, the first intrauterine source of nutriments is the yolk, then the animals eat secretions, epithelial cells, degenerated eggs and dead embryos.

In *Typhlonectes compressicaudus*, injection of tritiated thymidin in gestant females allowed us to show that there are transfers from mother to embryo (EXBRAYAT & HRAOUI-BLOQUET, 1992).).

DISCUSSION AND CONCLUSIONS

The Order of Gymnophiona is a very homogeneous group (see TAYLOR, 1968) in which we can observe a trend toward the evolution of reproductive patterns.

The metamorphosis is more or less quick, in correlation with the degree of evolution of the species into the group. In lesser advanced species, all oviparous, the aquatic larval life is long and metamorphosis slow. In more evoluted species, the metamorphosis is observed just before or after eclosion. In terrestrial and aquatic viviparous species, metamorphosis seems to be quick and it occurs after the eclosion but always before the birth.

The reproductive patterns show a trend from oviparity to viviparity with an evolution of the viviparity. In the more studied viviparous species (*Gymnopis, Dermophis*), the animals first reabsorbe yolk, then they eat the substances secreted by the uterine walls and also degenerative eggs and embryos. In the highest species (*Typhlonectes*), in addition, a placenta-like structure develops.

These facts can be correlated with the ecology of the animals. In the lesser advanced Gymnophiona (*Ichthyophis*), the long metamorphosis can be linked to the long aquatic larval phase. In the oviparous burrowing *Siphonops* or *Hypogeophis*, the metamorphosis is quick and occurs just after or before the eclosion. Consequently, the new-borns are quickly able to burrow. In other species, viviparity permits a better protection of the young animals that are not larval at birth. In the highest advanced species, viviparity attempted its more evoluted level characterized by a kind of placentation.

In spite of the lack of data, the order of Gymnophiona seems to be a good model to study the evolution of reproductive patterns in function of the environment.

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Results and perspectives in the study of scincomorph karyology

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Abstract: The study of the karyology of Scincomorphs may be a good model for investigating the role played by chromosomal and genomic variation in the different evolutionary steps. The karyology has been extensively studied only in some families, like Lacertidae, Teiidae and Xantusiidae, while our knowledge of the genome and of the chromosome set of other families, like Scincidae, is still greatly limited. The studies performed so far, have obtained very interesting information on three aspects: the differentiation and evolution of the sex chromosomes; the origin of the unisexual species; the systematics of lacertid lizards. In conclusion, the karyological researches seem to be a powerful instrument to investigate the phylogeny of scincomorphs and also some paricular aspects as the sex chromosome differentiation and the cytology of parthenogens. However it is very important to use an integrated experimental approach based on the most advanced karyological and molecular methods. Moreover, we think that in order to reach correct and valuable conclusions it is also important to compare the karyological data with other types of informtaions (morhpological, isoenzimatic and immunological).

Key words: Reptiles, Scincomorphs, Chromosomes, DNA, Evolution.

INTRODUCTION

The role of chromosomal variation in the evolutionary steps of a given group of living organisms is one of the most controversial issues in cytotaxonomy.

An important contribution to the solution of this problem can be provided by a comparison of the trend of karyological evolution among groups of phylogenetically related organisms, which, however, differ in their ways of life, adaptive strategies and evolutionary success. Scincomorphs are a good model for this kind of investigation. In fact, they are one of the species-richest infraorders within the Sauria, and include families clearly differing in number of species, distribution, ecology and evolutionary success.

Unfortunately, the karyology has not been studied in all the families sufficiently, and, in some cases these studies are only at a start. According to ESTES (1983), scincomorphs include three main lineages:

i) lacertoids, now represented by the two American families, teiids and gymnophtalmids, and by the Old-World lacertids, considered as the sister group of teiids.

ii) cordyloids including two small families with limited distribution: the xantusiids from North and Central America, and the cordylids from subsaharian Africa and Madagascar.

iii) scincoids, with the living scincids, which are the largest and most widespread family among saurians.

KARYOLOGICAL CHARACTERS

Teiidae and **Gymnophtalmidae**, which some classifications include in a single family, the Teiidae, were the first group to be quite extensively and systematically studied by GORMAN (1970) and COLE and co-workers (LOWE *et al.* 1970) by conventional techniques.

60 out of the total of the 198 living species (30%) have been karyotyped (Table 1). Most of them are teiids, the chromosome set of only two gymnophtalmids being known.

GORMAN (1970) recognized two karyologically distinct groups (Fig. 1): the *Dracaena* group, showing a karytotype with 12 biarmed macrochromosomes and 22 micro chromosomes, seldom showing chromosomal mutations; the *Ameiva* group, including also gymnophtalmids, that shows a larger number of chromosomes prevailingly uniarmed and a larger number of arms.

GORMAN (1970) suggested that the karyotypes of the *Ameiva* group might derive from those of the *Dracaena* group through the alternating of centric fissions and pericentric inversions. According to LOWE *et al.* (1970), instead, the karyotypes with uniarmed chromosomes might be primitive, and the 12+22 karyotype derived by progressive centric fusions.

The genus most extensively studied is *Cnemidophorus*, which includes bisexual and parthenogenetic species. In many cases, karyological studies have demonstrated the hybrid origin of parthenogenetic species (LOWE & WRIGHT, 1966; COLE, 1979) some of which show triploid karyotypes and intraspecific variation (PECCININI-SEALE & FROTA-PESSOA 1974).

Nucleolar organizer (NOR) localization is rather uniform, although intraspecific variability in the NOR number, with extra-sites, has been observed in *Ameiva ameiva* and *Cnemidophorus lemniscatus* (PECCININI-SEALE & FROTA-PESSOA 1974; PECCININI-SEALE & DE ALMEIDA 1986).

The scarce C-banding investigations are confined to *Ameiva ameiva* and three species of *Cnemidophorus*. C-bands are centromeric and telomeric in the former, extremely reduced in the latter (BICKHAM *et al.* 1976; PECCININI-SEALE & DE ALMEIDA 1986).

A single case of sex chromosomes has so far been observed in *C. tigris* (COLE *et al.* 1969). This species shows a male heterogamety of the XY type, and sex homologs differing in the position of the centromere. The Y- chromosome is supposed to be originated by pericentric inversion. In *A.ameiva*, PECCININI-SEALE & DE ALMEIDA (1986) observed a heterozygous C-band in a male meiosis bivalent which they suggest being related to an early stage of differentiation of probable sex chromosomes.

Lacertidae are probably the karyologically best studied family of saurians as regards both the number of species (82 out of 210, accounting for about 40%), and the variety of techniques used (Table 1).

When studied with conventional techniques, the lacertid karyotype appears very "monotonous", most species possessing 36 uniarmed macrochromosomes and 2 microchromosomes (Fig. 1). The few variations observed consist either in the centric fusion of macrochromosomes resulting in one or more pairs of biarmed chromosomes or in the number of microchromosomes (from 6 to 0) which appear to translocate at the level of the telomere of the macrochromosomes.

A completely different situation can be observed using banding techniques.

NOR localization would have cytotaxonomic significance (ODIERNA *et al.* 1987). It is always at a telomeric position but in one case (ODIERNA *et al.* 1987, 1990a, 1990b). Five different types of NOR-bearing chromosomes can be distinguished, which are classified as **m, S, MS, M** and **L**, according to their size (OLMO *et al.* 1990). NOR-localization analysis in 46 lacertid species shows that, dividing these species according to the various NOR types, all the groups obtained but one (**m**) include species sharing many karyological and non-karyological characters (OLMO *et al.* 1990). The first group, showing an MS-type NOR-bearing chromosome, includes *L.vivipara* and all the species ascribed to the so-called Ethiopian-Saharo-Eurasian clade *sensu* ARNOLD (1989). The second, with an M-type NOR bearing chromosome, includes *Podarcis, Algyroides, L.graeca* which have been assigned to the same lineage (ARNOLD 1989, MAYER & LUTZ 1989, CAPRIGLIONE *et al.* 1991), and *Psammodromus algirus* which instead would not be related to the other three (ARNOLD, 1989, MAYER & LUTZ 1989). A third group has an S-type NOR-bearing chromosome, and comprises all the species of the genus *Gallotia*. A fourth, very large, group, possessing an L-type NOR-bearing chromosome, includes all the species from *Lacerta* s.str., the *L.lepida-princeps* group and *Archaeolacerta*. The homogeneity of *Archaeolacerta*, on the one hand, and the affinity between *Lacerta* s.str. and *L.lepida-princeps*, on the other, are supported by several data (ARNOLD 1989). However, there is no undoubted evidence of close relationship between these two complexes.

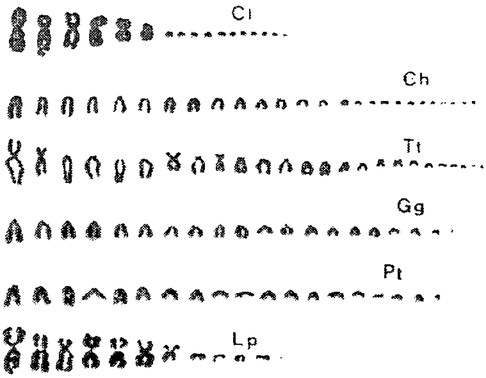

Figure 1: Haploid karyotypes of:(a) Cl= *Crocodilurus lacertinus*; (b) Ch= *Cnemidophorus hyperythrus*; (c) Tt= *Teyus teyu* (Teiidae); (d) Gg= *Gallotia galloti*; (e) Pt=*Podarcis tiliguerta* and (f) Lp= *Lacerta parva.*

G-banding studies, though being confined to few species, have also provided interesting cytosystematic information. A comparison among the G-banding pattern of several species has shown increasing differences in G-banding with the increase in the phyletic distance (OLMO *et al.* 1991; ODIERNA *et al.* 1993b). Most of these differences appear to be due to paracentric inversions and/or translocations of microchromosomes at the telomere of other chromosomes. Thus mutations altering G-banding, and hence the gene linkage of which the G-banding is the expression (QUMSIYEH & BAKER 1988; HOLMQUIST 1989), leaving unchanged the gross morphology of the chromosome, seem to have played an important role in the lacertids' karyological evolution.

FAMILY	TS	KS	%	BS	%
Cordylidae	54	14	26	2	4
Lacertidae	210	82	40	52	25
Scincidae	1029	104	10	36	4
Teiidae	198	60	30	4	2
Xantusiidae	14	10	71	0	0

Table 1: Summary of the scincomorphan species studied by karyological methods. TS= Total living species; KS= number of species studied by karyological methods and its percentage (%) of the total living species; BS= number of species studied using banding methods and its percetentage (%) of the total living species.

The distribution and amount of C-banded constitutive heterochromatin is one of the most variable characters of lacertid chromosomes. In these species heterochromatin appears mostly as centromeric and pericentromeric bands. Telomeric bands are rarer and usually less intense, whereas intercalary bands are virtually absent (OLMO *et al.* 1986b, 1988). C-bands brightly fluoresce when stained with DAPI, a fluorochrome specific for AT-rich DNA, and, as will be seen, contain highly repetitive DNA fractions (CAPRIGLIONE *et al.* 1989, 1991). Heterochromatin localization and amount is subject to rather wide inter- and intraspecific variation that does not appear to be reasonably related to the phylogenetic trend (OLMO *et al.* 1986b). In fact, differences in C-bands have been found in related and probably interfertile species, such as *L.trilineata* and *L.viridis*, and in specimens of *P.sicula* belonging to populations living in different areas of Italy (OLMO *et al.* 1986b; CARDONE *et al.* 1990; CARDONE, 1991).

A major role might have been played by heterochromatin in sex chromosome differentiation and evolution. Sex chromosomes have been found in almost all of the lacertid species studied with appropriate techniques. They are all of the ZW type, except in *L.vivipara* bearing sex chromosomes of the Z_1Z_2W type (OLMO *et al.* 1993).

Sex chromosome differentiation in lacertids would start with the storage of a specific DNA on either homolog accompanied by heterochromatinization (OLMO *et al.* 1987; ODIERNA *et al.* 1993a). However, this DNA differs from Bkm (CAPRIGLIONE *et al.* 1993), a sex-linked sequence found by JONES (1984) and SINGH *et al.* (1980) in snakes. A homomorphic and heterochromatic W, thus, would form (Fig. 2). It would have replication (VOLOBOUEV *et al.* 1970) and spiralization (CAPRIGLIONE *et al.* 1993) cycles different from that of the Z-chromosome and the autosomes, which would reduce or hamper the crossing-over between the two sex homologs. Subsequently, that W would undergo a progressive deletion transforming it into a microchromosome (OLMO *et al.* 1987; ODIERNA *et al.* 1993a). In *L.vivipara,* instead, the fusion of a primitive W with an autosome would give rise to the biarmed W-chromosome observed in this species (ODIERNA *et al.* 1993a). This process of sex-chromosome differentiation would have occurred repeatedly and independently in the various taxa of the family. In fact, W-chromosomes with different morphologies and degrees of differentiation have been observed in specimens belonging to the same species (OLMO *et al.* 1987; ODIERNA *et al.* 1993a).

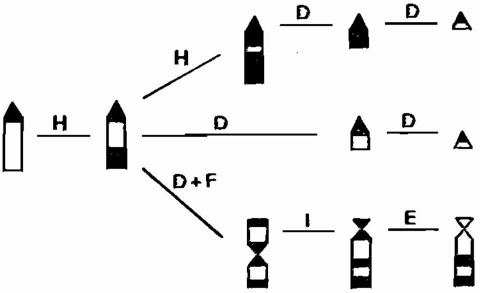

Figure 2: Hypothesis on the evolution of lacertid sex-chromosomes. H= heterochromatinization; D= deletion; F= fusion; I= inversion; E= euchromatinization.

About 70% of the living night lizards, belonging to the family **Xantusiidae**, have been karyotyped though only by conventional technique (BEZY 1972; BEZY *et al.* 1980, 1992) (Table 1). In this family, too, chromosome number and morphology show slight variations (Fig. 3). All of the species but three from the genus *Lepidophyma*, show 18 macro chromosomes and a number of micro chromosomes varying from 18 to 24. The first two macrochromosomes are remarkably larger than the others. The various species assigned to the genus *Xantusia* differ only in few pericentric inversions. Transition from *Xantusia* to *Lepidophyma* karyotype appears to have involved only the loss, or translocation, of a pair of microchromosomes. Greater differences can be observed within the latter genus, encompassing cases of pericentric inversion, centric fusion and a single case of reduction in microchromosome number. In several species of *Lepidophyma* there is a secondary constriction, perhaps related to the NOR, on the telomere of the chromosomes of the third pair. In *L.flavimaculatum obscurum*, there are unisexual diploid and triploid populations. No sex chromosomes have been found till now.

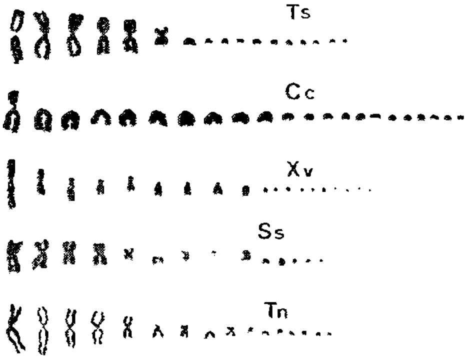

Figure 3: Haploid karyotypes of : (a) Ts= *Tetradactylus seps*; (b) Cc= *Cordylus cataphractus*; (Cordylidae); (c) Xv= *Xantusia vigilis* (Xantusiidae); (d) Ss= *Sphenops sphenopsiformis*; (e) Tn= *Tiliqua nigrolutea* (Scincidae).

Cordylidae, too, appear quite uniform karyologically. The karotype of 14 out of the 54 living species (more than 25%) has been determined in this family (OLMO & ODIERNA 1980; DE SMET, 1981) (Table 1). All the studied species from the subfamily gerrhosaurins and most of those from the subfamily cordylins possess 12 biarmed macrochromosomes and 22 microchromosomes (Fig. 3). Only two species of cordylins: *Cordylus giganteus* and *C.cataphractus*, possess different chromosome sets, probably derived from the 12+22 one by multiple centric fissions. In fact, the karyotypes of these species show one pair of biarmed and 10 pairs of uniarmed macrochromosomes and 22 microchromosomes. Intraspecific polymorphism in the number of uniarmed macrochromosomes is observed in *C.cataphractus*. In *C.giganteus* and *C.cataphractus*, a secondary pericentric constriction can be seen on the chromosomes of the second pair, perhaps related to the NOR. Recently ODIERNA (unpublished), using an AgNOR technique, has shown that, in *Zonosaurus laticaudatus* and *Z.karsteri* from Madagascar, the nucleolar organizer is located on a pair of microchromosomes. Studies carried out in *Z.laticaudatus* by ODIERNA (unpublished) with C-banding and Alu I restriction enzyme have shown a peculiar situation. C-banded heterochromatin could be visualized only on a pair of microchromosomes, probably the NOR-bearing ones. Conversely, treatment with Alu I, that in other lizards evidences the same heterochromatic areas as those evidenced by C-banding (ODIERNA *et al.* 1993a), produced clear pericentromeric bands on 8 out of the 12 macrochromosomes. Cases of sex chromosomes are not known.

Scincidae are the most speciose family of saurians and, unfortunately, one of the less studied karyologically, the karyotype of only 104 out of the over thousand species being known (Table 1).

Information on skink karyology is rather scarce and not always precise: for instance, the morphology of smaller macrochromosomes and of microchromosomes is often controversial and inadequately determined. Nevertheless, it is possible to draw some general indications. The diploid number ranges between 26 and 36, but most species possess 28, 30 or 32 chromosomes. The most widespread karyotype consists of 4 pairs of large biarmed macrochromosomes generally showing a rather conservative morphology, 4 or 5 pairs of medium sized chromosomes which appear to have a more variable morphology, and a rather variable number of microchromosomes which can hardly be distinguished from the smallest macrochromosomes (KING 1973, CAPUTO & ODIERNA 1990,1991; DONNELLAN 1991 a,b; CAPUTO *et al.* 1993a,b) (Fig. 3). The number of arms is quite variable, and can not be determined with certainty. The most frequent mutations are represented by pericentric inversions often involving medium-sized macrochromosomes (KING 1973); however, cases of loss of microchromosomes, as in *Eumeces* (BRANCH 1980), or of their translocation to medium-sized macrochromosomes or other microchromosomes, as in the genus *Tropidophorus* (OTA *et al.* 1991), have also been documented. Robertsonian mutation in macrochromosomes are rarer, and have so far been found mainly in *Eumeces* and *Scincus* (VEGNI-TALLURI 1968; BRANCH 1980; CAPUTO *et al.* 1993b).

In *Eumeces, E.algeriensis* and all the studied subspecies of *E.schneiderii* possess 32 chromosomes with several uniarmed macrochromosomes, whereas other species from the same genus possess 26 chromosomes, the same number of arms and less uniarmed macrochromosomes (VEGNI-TALLURI 1968; CAPUTO *et al.* 1993b). *Scincus scincus* and *S.hemprichii* show 32 and 36 chromosomes, respectively, with several uniarmed macrochromosomes (BRANCH 1980; CAPUTO *et al.* 1994). Rare cases of intraspecific variation in chromosome number have been found. NOR localization also varies, and does not appear to provide useful cytotaxonomic information. In fact, both differences in NOR number and localizations in related species and cases of heteromorphism in the same species can be found (DONNELLAN 1991a). However, in some cases, NOR localization has been fruitfully employed, together with other karyologically data, in order to demonstrate taxonomic relationships, as it is the case of *Chalcides* and *Sphenops* (CAPUTO *et al.* 1993c). An interesting sex-linked heteromorphism of the NOR has recently been reported by CAPUTO *et al.* (unpublished) in *S.scincus*.

The C-banding has been used in karyological studies of lygosomins from the *Egernia* group and from the genus *Lampropholis*, and scincins from the genus *Chalcides*. In lygosomins, heterochromatin is not very abundant, even though there are interspecific differences. C-bands are mostly centromeric and only sometimes telomeric (DONNELLAN 1991a,b). In a *Lampropholis* species, C-banded heterochromatin appears to be involved in Y-chromosome differentiation.

In the *Egernia* group inter- and intraspecific variations have been observed in the morphology of given chromosomes due to the addition of heterochromatin bringing about either a new heterochromatic short arm or the elongation of the long arm (DONNELLAN 1991a).

In *Chalcides*, variations in the localization, amount and composition of the C-bands have been observed, which seem to be related to cytosystematical relations. In *C.ocellatus* and related species, the C-bands are centromeric and mostly resistant to the treatment with the Alu-I restriction enzyme (CAPUTO & ODIERNA 1991; CAPUTO *et al.* 1993c). Vice versa, in *C.chalcides*, centromeric bands are greatly reduced or absent, whereas the paracentromeric bands can be readily visualized and are digested by Alu-I (CAPUTO *et al.* 1993c).

Sex chromosomes are rare and always of XY type except those found in *Scincella laterale* (DONNELLAN 1985). In this species sex chromosomes of the XY type are found in a population, and a sex chromosomes of the X_1X_2Y type in another (WRIGHT 1973). The mechanisms responsible for sex chromosome differentation in skinks appear to vary.

In the *Scincella* population with XY sex-chromosomes, the Y is smaller than the X and might have differentiated by deletion (WRIGHT 1973). In the other population with X_1X_2Y sex chromosomes, the Y would be the result of a centric fusion (WRIGHT 1973).

In *Lampropholis delicata*, the two sex chromosomes differ in the position of the centromere, which might be the result of a pericentric inversion (DONNELLAN 1991b); in another species from the same genus, instead, the Y would have differentiated by addition of heterochromatin (DONNELLAN 1991b).

Finally, another type of sex chromosomes would be that observed in *S.scincus* by CAPUTO *et al.* (1994), where the NOR is heteromorphic in males and homomorphic in females.

GENOME ORGANIZATION

Studies on genome organization are still scarce and are almost all concerned with cordylids and lacertids.

Genome sizes vary from a minimum of about 3 pg/N in teiids to a maximum of nearly 8 pg/N in cordylids (OLMO 1986).

The analysis of base composition has shown that, like in most poykilothermic vertebrates, scincomorph DNA is rich in adenine-thymine (AT) (OLMO 1981;OLMO *et al.* 1993). A more detailed study of genome composition has been made only in lacertids and cordylids. In both families, the single-copy DNA fraction, containing most structural genes, is almost the same in all the species studied, independently of variations in genome size; greater differences, instead, are found in highly repetitive DNA fractions, which are usually associated with constitutive heterochromatin (OLMO *et al.* 1986a, 1988).

Both in cordylids and lacertids several highly repetitive DNA fractions have been found. All those observed in the latter by CAPRIGLIONE *et al.* (1989, 1991) are rich in AT, whereas AT-rich and GC-rich highly repetitive DNA fractions have been observed in cordylids (OLMO *et al.* 1986a). Moreover, in lacertids, which show slight variations in genome size, variations in highly repetitive DNA amount do not seem to

Species	GS	HR	MR	SC	Het	GC	Lsat	Hsat
Cordylidae								
Cordylus cataphractus	7.9					3.6		
Cordylus cordylus	7.85					3.4	+	
Cordylus giganteus	7.8	1.8	2.5	3.5		3.4		
Cordylus polyzonus	7.7					3.3		
Cordylus rhodesianus	7.2							
Cordylus vittifer	6.9					2.5		
Cordylus warreni	7.2	1.1	2.8	3.3		3.1	+	+
Gerrhosaurus major	6.75					2.8		
Gerrhosaurus validus	6.2					2.5		
Plarysaurus guttatus	6.4					2.6		+
Platysaurus intermedius	6.2					2.5		
Pseudocordylus microlepidotus	6.3							
Tetradactylus seps	5.7							
Lacertidae								
Algyroides fitzingeri							+	
Algyroides moreoticus							+	
Algyroides nigropunctatus							+	
Acanthodactylus pardalis	2.9							
Gallotia galloti	4.7	0.5	1.1	3.1	0.2			
Ichnotropis paulensis	2.8							
Lacerta agilis	4.2							
Lacerta bedriagae							+	
Lacerta dugesii	4.9						+	
Lacerta graeca							+	
Lacerta saxicola							+	
Lacerta lepida	4.7	0.5	0.4	3.1	0.4	2.4		
Lacerta viridis	5.1	0.5	1.2	3.4	0.5	2.1	+	
Lacerta vivipara	2.2						+	
Latastia longicaudata	3.2							
Podarcis hispanica	2.4							
Podarcis melisellensis	5.3					2.4		
Podarcis muralis	4.7						+	
Podarcis pityusensis	5.4							
Podarcis sicula	4.4	1.0	0.4	3.0	1.1	2.6	+	
Podarcis taurica							+	
Podarcis tiliguerta	4.5					2.3	+	
Psammodromus algirus	5.9							
Takydromus sexlineatus	4.8					2.3		
Scincidae								
Chalcides mionecton	4.1							
Chalcides ocellatus	6.4					3.2		
Eumeces inexpectatus	5.3							
Mabuya macularia	4.2							
Sphenops sphenopsiformis	4.5							
Teiidae								
Ameiva ameiva	4.8							
Cnemidophorus lemniscatus	2.5							
Kentropix paulensis	2.3							
Pantodactylus quadrilineatus	2.9							
Tupinambis teguixin	3.8					1.6		

Table 2: Genome characteristics of various scincomorph species. GS= Genome size in picograms/nucleus; HR= Highly repetitive DNA content; MR= middle repetitive DNA content; SC= single copy DNA content; Het= Heterochromatin amount; GC= amount of DNA guanyne cytosine rich; LSat= Presence of higly repetitive DNA AT rich; HSat= Presence of highly repetitive DNA GC rich.

affect the total DNA amount; in cordylids, instead, showing greater differences in genome size, these differences depend almost completely on the different highly repetitive DNA amount (OLMO 1986, OLMO *et al.* 1988) (Table 2). It has suggested that this might depend on the occurrence, in the former, of constraints keeping genome size quite low, probably by limiting the repetitive DNA amplification or preventing its preservation and accumulation through deletion events. Such constraints would be less marked in cordylids (OLMO *et al.* 1993).

The most exhaustive molecular study has been made in lacertids, and particularly in *P.sicula*. CAPRIGLIONE *et al.* (1989, 1991) visualized some AT-rich highly repetitive DNAs which are localized in the C-bands. One of these DNAs is present only in species from the genus *Podarcis*, whereas other are found also in species ascribed to other genera, as *Algyroides* and *Lacerta*. These DNAs seem to be able to evolve quite freely, since their sequences diverge with the increase in the phyletic distance between the species examined (Fig. 4). Therefore, they are useful in the cytosystematical analysis, and have been employed to elucidate the phylogenetic relationships between the various species of lacertids (CAPRIGLIONE *et al.* 1991, and unpublished).

Other interesting molecular investigations involve mitochondrial DNA analysis; they, however, are concerned only with some unisexual species of *Cnemidophorus* (BROWN & WRIGHT 1979; MORITZ *et al.* 1989; DENSMORE *et al.* 1989a,b) and *Lacerta* (MORITZ *et al.* 1992).

COMMENTS ON SOME PARTICULAR ASPECTS

The data so far collected are too scarce to allow exhaustive and satisfactory conclusions. However, some interesting indications come out, prompting us to get a deeper insight into some particular aspects.

Different trends of karyological variations can be observed in the various families of scincomorphs. Cordylids and xantusiids, having a limited geographical distribution and occupying peculiar ecological niches, appear karyologically very conservative. Conversely, teiids and scincids, having a wide geographical disrtibution, show a greater chromosome variability, although the mutations involved are different in the two families. Teiids show greater variations in the diploid number, due to centric fusions and pericentric inversions involving all chromosomes (GORMAN, 1970). In skinks, the diploid number are less variable, the most frequent variations being due to pericentric inversions involving mostly medium-sized chromosomes. The morphology of the eight largest chromosomes appears constant (KING 1973).

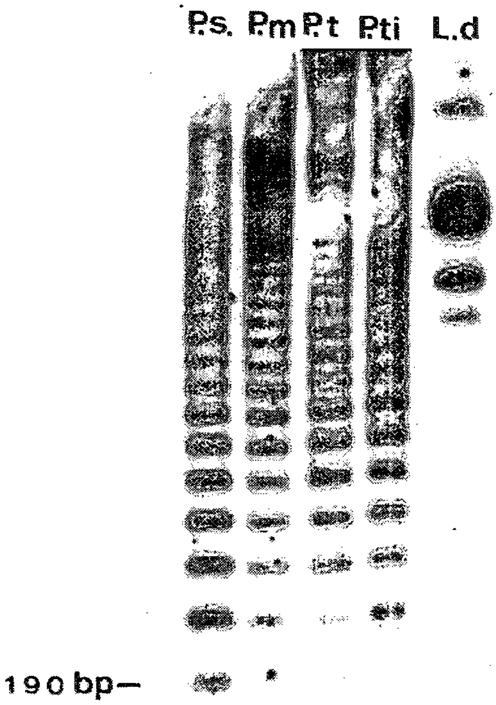

Figure 4: Southern blot hybridization of a satellite DNA from *P.sicula* on the genomic DNA of various lacertid species. Ps= *P.sicula;* Pm= *P.muralis;* Pt= *P.taurica*; Pti= *P.tiliguerta;* Ld= *L.dugesii*

Finally, a peculiar situation is observed in lacertids. Though being a widespread family they show a very conservative karyotype, in which, with few exceptions, the most frequent variations involve heterochromatin amount and distribution, NOR number and localization (OLMO *et al.* 1993b). As already mentioned, variation in heterochromatin would have no effect on the reproductive isolation of different populations. Differences in NOR are supposed to be more important phylogenetically (ODIERNA *et al.* 1987).

These different trends in the karyological evolution are hard to explain, also because our present knowledge is not complete and might reflect the inadequacy of the methods used than a real situation. In this regard, it is noteworthy that the chromosomes of lacertids which seem to be morphologically very uniform, after a G-banding staining show a greater variability (ODIERNA *et al.* 1993b). Moreover a C-banding study has revealed a new mutation which might have played a role in the karyological evolution of scincids: i.e. addition of a new short arm or elongation of an existing one by the amplification of portions of constitutive heterochromatin (DONNELLAN 1991 a,b)

Figure 5: C-banded chromosomes of *P.sicula*. Note that the heterochromatin is mainly centromeric.

BICKHAM & BAKER (1979) suggested that the different degree and type of chromosome mutation depend on the evolutionary stage attained by a given group of organisms. This hypothesis could explain some of the differences observed in scincomorphs, but not the situation of lacertids. In fact, it cannot be hold that teiids, showing a larger chromosome variability with Robertsonian variations and pericentric inversions, are still at an active stage of their radiation, whereas lacertids, which are more stable karyologically, have reached the final stage of their evolution.

Another hypothesis relates the different degree of chromosome variation to the way of life and the degree of eusociality (BUSH 1981). However, no investigations have been performed so far on the possible karyotypic differences in species or populations showing different ways of life.

A third hypothesis ascribes the differences in the type and frequency of chromosome variations to the intrinsic tendency of some chromosomes toward a mutation rather than others (WHITE's karyotype orthoselection 1973). This hypothesis seems to be supported by various recent studies (MAYR *et al.* 1984, ELDER & HSU 1988, MEYNE *et al.* 1990, LUKE *et al.* 1992) and agrees well with the observation that, in each scincomorph family, mutations are more frequent in certain chromosomes, while other chromosomes remain unchanged.

A remarkable extension of research by chromosome banding and molecular methods is required to verify the validity of the orthoselection hypothesis. However, interesting data were collected on lacertids, having a very conservative karyotype consisting of nearly uniarmed chromosomes. In this family, heterochromatin is usually more abundant than in other families, and is nearly always centromeric, in addition it contains AT-rich DNA fractions, as previously mentioned (CAPRIGLIONE *et al.* 1989, 1991) (Fig. 5).

Figure 6: C-bande chromosomes of *L. aurelioi*. Note that the bands are intense and centromeric in the uniarmed chromosomes, while they are pericentric and very light in the biarmed chromosomes.

Moreover in the biarmed chromosomes, when present, the centromeric heterochromatin is very low or absent (OLMO *et al.* 1986b) (Fig. 6). Within other families, the few species investigated by C banding, possessing a more variable karyotype with several biarmed chromosomes, show a lower amount of heterochromatin with a rather heterogeneous composition (OLMO *et al.* 1990; ODIERNA *et al.* unpublished). This agrees well with MAYR's *et al.* (1984) hypothesis that the presence of a large amount of AT-rich DNA on the centromere would limit centric fusion events giving rise to biarmed chromosomes starting from uniarmed ones.

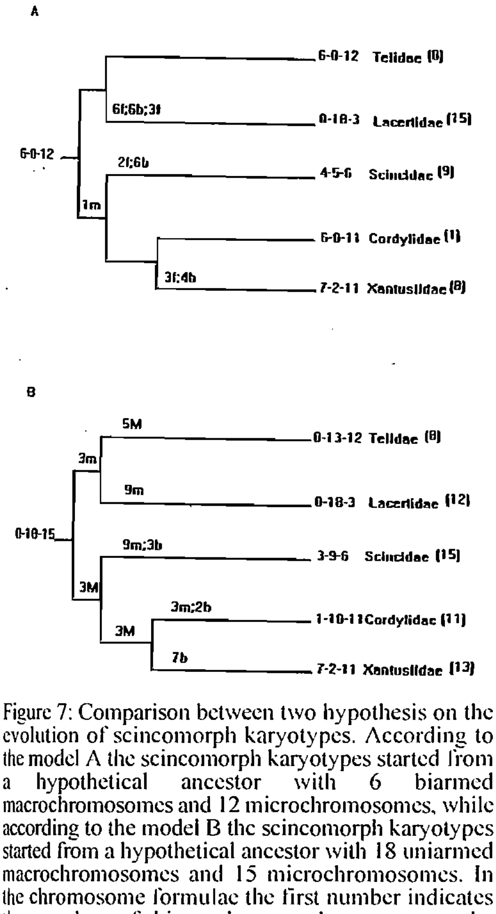

Figure 7: Comparison between two hypothesis on the evolution of scincomorph karyotypes. According to the model A the scincomorph karyotypes started from a hypothetical ancestor with 6 biarmed macrochromosomes and 12 microchromosomes, while according to the model B the scincomorph karyotypes started from a hypothetical ancestor with 18 uniarmed macrochromosomes and 15 microchromosomes. In the chromosome formulae the first number indicates the number of biarmed macrochromosomes, the second indicates the number of uniarmed macrochromosomes, the third indicates the number of microchromosomes. The number along the lines indicates the number of mutations: b= formation of a biarmed chromosomes; f= centric fusions; M= loss of macrochromosomes; m= loss of microchromosomes. The number in brackets indicate the minimal number of mutations requested for explaining the origin of the extant karyotypes from the ancestor.

No clear and univocal conclusions can be drawn on the role played by chromosomalvariations in the evolutionary steps of scincomorphs. In fact it has been observed that certain karyological characters, like the NOR localization or the distribution and amount of heterochromatin, seem to have a cytotaxonomic significance in certain taxa, while show greater inter- and intraspecific variability apparently unrelated with the taxonomic relationships in others. Moreover it has been observed that in some scincomorphan families, like teiids, the divergence between subfamilies and genera were accompanied by strong karyotypic mutations, while in other families, like cordylids and xantusiids, the same divergence was characterized by very few or no chromosomal variations. Other interesting information on the possible involvement of chromosome variations in the speciation process is given by the investigation carried out by CAPUTO and coworkers (CAPUTO & ODIERNA 1991; CAPUTO 1993; CAPUTO *et al.* 1993 b,c) by karyological, morphological and isoenzymatic methods in several species and subspecies from the genus *Chalcides*, living in parapatry or in areas showing narrow overlapping zones. These species and subspecies might have differentiated by allopatry starting from small-sized founder populations and clearly differ in the morphology of some chromosomes (CAPUTO 1993). CAPUTO *et al.* (1993c) suggest that these chromosome variations originated as a by-product of the geographic isolation according to the model of secondary chromosome allopatry proposed by KING (1981), and that they might contribute to strengthen postmating isolation mechanisms.

All these observations suggest that the chromosome variations in scincomorphs may not be the primary cause of the main evolutionary events. They are probably the consequence of vicariance or allopatry processes and may play a role in reinforcing postmating isolation mechanisms.

The identification of the so-called ancestral karyotype in saurians is an issue debated by cytotaxonomists. Two hypotheses have long been challenging each other. Some authors (GORMAN 1973; PAULL *et al.* 1976) suggest that the karyotypes of all saurians would be derived from that with 12 biarmed macrochromosomes and 24 microchromosomes similar to the observed in many families of the order; others (LOWE *et al.* 1970; KING 1981) believe that the saurian ancestral karyotype was formed of all uniarmed chromosomes.

In scincomorphs there is no decisive evidence in support of either hypothesis , however, in my opinion, the hypothesis of a 12+24 karyotype is more acceptable. In fact, a similar karyotype is found in teiids and cordylids (OLMO 1986); in addition, the similarity among the various uniarmed karyotypes may be only apparent;

finally, the origin of the present karyotypes starting from a karyotype of all uniarmed chromosomes would require more mutations than that from a 12+24 ancestors (Fig. 7).

The use of increasingly up-to-date and high resolution cytological and molecular techniques has proved effective to study the presence and evolution of sex chromosomes, the origin of parthenogenesis and the lacertid taxonomy.

So far sex chromosomes have been found mainly in the families investigated by banding and molecular techniques, since several cases of sex chromosomes have been observed in which the two homologs have the same size and shape, differing, however, in the amount and/or localization of constitutive heterochromatin. Sex chromosomes have not been found in cordylids and xantusiids, in teiids only two cases, both of the XY type, have been observed. In skinks, 30 cases, all showing male heterogamety, have been detected; in lacertids sex chromosomes have been found in all the species studied by C- or G-banding (nearly 36). This family always displays female heterogamety, usually of the ZW type,more rarely of the Z_1Z_2W type.

The data so far collected in lacertids and scincids suggest that sex-chromosome differentiation has taken place several times within the same family or even the same genus and might have involved different cytological mechanisms, at least in lygosomins (OLMO *et al.* 1987; DONNELLAN, 1991a, b; ODIERNA *et al.* 1993a). In both families geographical variations have been observed in the type or level of sex chromosome differentiation, (KUPRIYANOVA & RUDI 1988; DONNELLAN 1991b; ODIERNA *et al.* 1993a), suggesting that the latter is associated with speciation processes (DONNELLAN 1991b). The most exhaustive study of sex chromosomes has been carried out in lacertids the origin and evolution of which have been outlined in a rather complete and acceptable way. The results of this analysis convincingly support JONES & SINGH's (SINGH *et al.* 1980; JONES 1984) hypothesis that sex chromosome differentiation might depend on the heterochromatinization of one homolog due to the accumulation of a specific HR DNA which would precede any morphological modification. In fact, in many species of this family, essentially the most generalized ones, the W-chromosome is heterochromatic and shows delayed replication and spiralization cycles compared to the Z chromosome and the autosomes. This condition is supposed to suppress the crossingover in the chromosome segment bearing one or more sex-determining genes, even if no variations occur in sex chromosome morphology (JONES 1984). The sex chromosomes of skinks (DONNELLAN 1985, 1991b) and one case recently observed in teiids (PECCININI-SEALE & DE ALMEIDA 1986) indicate that the above mentioned mechanism of sex-chromosome differentiation is the most widespread, though not the only one, present among scincomorphs.

The study of sex chromosomes in lacertids has clearly stressed the need to use various and up-to-date methods. In fact, in *P.sicula*, were sex chromosomes had not been found either with conventional methods or with C-banding clear ZW heterogamety was revealed using G-banding (ODIERNA *et al.* 1993a).

The study of the origin and evolution of parthenogenetic species has also been enhanced by karyological investigations. Unisexual forms are found in lacertids, teiids, and one parthenogenetic form also in xantusiids:*Lepidophyma obscurum* (BEZY 1972). Most of these unisexual lizards are diploid, but triploid species or populations are also known (DAREVSKY *et al.* 1985).

The hybrid origin of parthenogenetic scincomorphs has been convincingly demonstrated by conventional karyological studies, that have sometimes allowed the identification of parent species, as in several species of *Cnemidophorus* (LOWE & WRIGHT 1966; BICKHAM *et al.* 1976; COLE 1979) and in some species of *Lacerta* (DAREVSKY *et al.* 1985, 1986; KUPRIYANOVA 1989). More precise information has been obtained studying mitochondrial DNA that, as is well known, is inherited only from the mother.

Investigations conducted on several species of *Cnemidophorus* and *Lacerta* have confirmed the hybrid origin of the parthenogenetic species and have allowed to identify the maternal species. In addition they have also provided interesting information on the number of hybridization events characterizing the origin of some unisexual species, the age and the evolutionary history of unisexual lineages, and the mechanisms leading to triploidy (BROWN & WRIGHT 1979; DENSMORE *et al.* 1989 a,b; MORITZ *et al.* 1989, 1992).

A study on parthenogenetic lacertids by MORITZ *et al.* (1992) suggests that unisexual scincomorphs are generally of recent origin and that the nature of founder event in the origin of these parthenogens is variable.

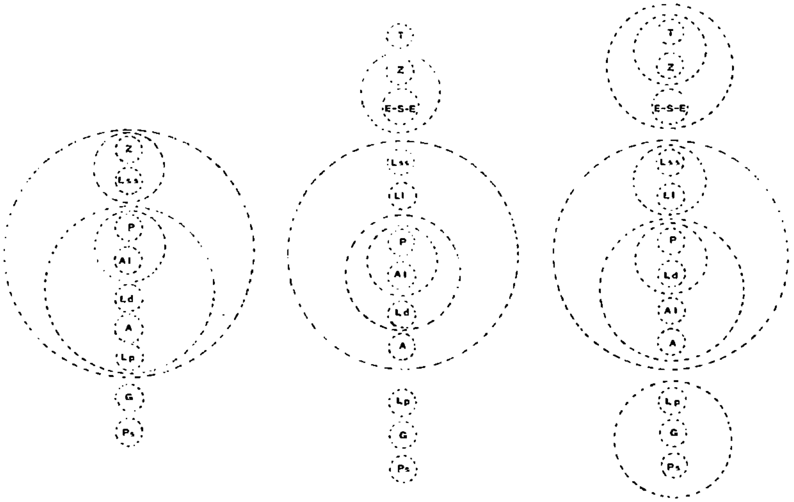

Figure 8: Hypothesis on lacertid systematics based on the karyological data compared with hypotheses based on the analysis of Mayer and coworkers (MAYER & LUTZ 1989) (on the left) and of ARNOLD (1989) (on the right). A= *Archaeolacerta* ; Al= *Algyroides*; E-S-E= Ethyopian-Saharo-Eurasian clade sensu Arnold; G= *Gallotia*; Ld= *L.dugesii*; Ll= *Lacerta lepida* and its allied; Lp= *L.parva*; Lss= *Lacerta* s.str.; P= *Podarcis*; Ps= *Psammodromus*; T= *Takydromus*; Z= *Zootoca*.

Surprisingly, chromosome banding techniques are not commonly used, though in some parthenogenetic species of gekkonids they have provided as clear and conclusive information as those obtained by mitochondrial DNA studies (MORITZ 1984; MORITZ & KING 1985).

The usefulness of karyological investigations for systematic and phylogenetic purposes is particularly evident in lacertids. As has already been mentioned, this family is the most studied karyologically using the widest range of molecular and chromosome-banding techniques. Not only have these investigations provided a great deal of information on the diploid number and the karyotype morphology of many species, but also on other cytological and molecular characters, such as NOR localization, the amount distribution and composition of constitutive heterochromatin, the presence of some DNA fractions, their distribution in the various species and the evolution of their sequence (OLMO *et al.* 1990, 1991, 1993). Some of these characters appear to vary randomly independently of the taxonomic relationships; some, instead, show a distribution and a trend of variation which agree well with the phylogenetic relationships between the species studied. In this regard, of particular relevance are NOR localization, evolution of the G-banding pattern, and the presence and evolution of particular highly repeated DNA fractions located on the centromere.

Based on the critical analysis of these and some other karyological characters, we put forward a hypothesis on lacertid systematics (summarized in fig.8), comparing it with other hypotheses based on morphological, isoenzymatic and immunological data (OLMO *et al.* 1991, 1993). As can be see, our hypothesis agrees well with those suggested by other authors, in particular that of ARNOLD (1989). The present cytosystematic study of lacertids also stresses the need to use various karyological and molecular methods and to integrate the different data obtained in order to avoid drawing partial or wrong conclusions.

CONCLUSIONS AND PERSPECTIVES

Though studies of scincomorph karyology are far fom being exhaustive, it is already possible to put forward some suggestions on the trends to be followed by future research.

Karyological studies appear a very effective potential tool for investigating the systematics and phylogeny of the scincomorphan families, ad some particular problems, such as the origin of parthenogenesis or sex-chromosome differentiation and evolution. However, in order to get reliable and conclusive information, it is not sufficient to define chromosome number and morphology, but it is essential to employ various high-resolution techniques also providing information on the compositional and molecular characteristics of the genome and the chromosome set.

A comparison between investigations conducted in lacertids and in xantusiids can be explanatory.

Though in the former only 40% of the species have been investigated, the data collected are such that it has been possible to formulate plausible hypothesis on sex chromosome evolution, the hybrid origin of parthenogenesis and the phyletic relationships between some of the main taxa of the family. Conversely, though about 70% of the living species of xantusiids have been karyotyped by conventional methods, it is unknown even whether there are sex chromosomes or not, and the information obtained is not sufficient to sketch out any taxonomic hypothesis.

Another important issue is the different trend and degree observed in the chromosome variability of the various scincomorphan families. This degree is not always related to the size or distribution of a family, and might depend on several causes. An important role might be played by intrinsic factors of the genome, such as the presence of given DNA sequences allowing or favouring some chromosome mutations, hampering others. Moreover, some families seem to be subjected to constraints aiming at preserving stability in genome size and composition and in chromosome-set morphology. In other families, instead, these constraints would be much less strict.

Much work can and has to be done, but the following takes priority:

-To study more in depth the karyology of some families so far scarcely investigated, such scincids, and in particular species living in certain geographical areas, such Africa and Asia, that have so far been neglected completely, but are well-known to have a key-role in understanding the evolutionary events of many scincomorphs.

-To more frequently employ banding and molecular methods, since the trend and the mechanisms of karyological evolution can be understood only by a deep knowledge of the composition and organization of the chromosomes and the genome.

-To compare the karyological data with morphological, isoenzymatic, immunological etc. ones. In fact, the karyological data alone can be insufficient to reconstruct taxonomic and phylogenetic relationships in a convincing and reliable way.

In our opinion, the comparison and critical analysis of different types of data is the most correct, if not the exclusive, way of approaching a reliable phylogenetic reconstruction, considering also that different characters (morphological, karyological etc.) do not always evolve at the same rate and are not always subject to the same selective pressure.

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Satellite DNA and phylogeny of Lacertid Lizards

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Abstract: Satellite DNA located on heterochromatic areas are of particular interest, since they may be used as a probe to estimate phyletic distances between species. In this review, four different satellite DNA families so far isolated from the lacertid genome are described. Though conservativeness and divergence rate are not the same for all satellite DNAs, they appear as a useful tool for phylogenetic and taxonomic investigations. In fact, the results obtained agree quite well with those from morphological and immunological studies.

Key words: Satellite DNA, Reptiles, Phylogeny.

INTRODUCTION

The occurrence and evolution of highly repeated DNAs, also called satellite DNAs, is being increasingly studied as a means of investigating phylogeny and taxonomy. The significance of this kind of investigations is supported by the numerous observations showing that these DNAs are free to evolve, and their divergence is directly proportional to the increase in the phyletic distance between species (MIKLOS, 1985; LIMA DE FARIA *et al.*, 1984).

However, the rate of variation and the turnover of satellite DNAs are not always the same. In fact, some of them can be found in a single species or in closely related species (BARSACCHI, 1991; MACGREGOR, 1991; CAPRIGLIONE *et al.*, 1989), others show a wider distribution in species belonging to different genera or even different families (CAPRIGLIONE *et al.*, 1993; MACGREGOR, 1991; BARSACCHI, 1991).

I have been carrying out an investigation on the characterization of the lacertid genome for some years. It has provided interesting results, and is a good example of how studies of satellite DNAs can provide significant information on the existing phylogenetic relationships at various taxonomic ranks.

SATELLITE DNAS IN LACERTIDS

Four different satellite DNAs have so far been isolated from lacertid genomes. They show quite a wide distribution.

Two of these sequences have been isolated from the genome of *Podarcis sicula*. One, called pLCS, has been isolated by the TaqI restriction enzyme; it is rich in adenine-thymine and is localized at the level of centromeric heterochromatin (CAPRIGLIONE *et al.*, 1989, 91). It is found with few variations in all of the species examined belonging to the genus *Podarcis* (Tab.1), in two species of *Algyroides* (*fitzingeri* and *moreoticus*), in *Lacerta graeca* and *dugesii*. pLCS sequences of *Algyroides* and *L.graeca* show little divergence from those of *P.sicula*, unlike that of *L.dugesii*, which diverges much more. Finally, no pLCS sequence has been found in species ascribed to other genera of the family, such as *Zootoca*, *Lacerta s.str.* and *Archaeolacerta*.

This distribution and evolution of pLCS indicates that *Podarcis* is quite a homogeneous natural group, and that *Algyroides*, *L.graeca* and, to a lesser extent, *L.dugesii* are related to *Podarcis*. This is in good agreement with similar results obtained from morphological, immunological, isoenzymatic and karyological studies (LUTZ & MAYER, 1985; ARNOLD, 1973, 89; ODIERNA *et al.*, 1987; OLMO *et al.*, 1989).

The other sequence, called pLHS, has been isolated by the HindIII restriction enzyme, and is found almost exclusively in the genus Podarcis. This DNA, too, is rich in adenine-thymine, and its sequence differs from that of pLCS only by 20%. It is, however, localized at pericentromeric and telomeric levels. This has suggested that pLHS is derived from the differentiation, translocation and subsequent reamplification of some pairs of pLCS by a mechanism also observed in other organisms (FLAVELL *et al.*, 1980; DOVER, 1982; MIKLOS, 1985; BOSTOCK, 1986).

The occurrence of this DNA only in *Podarcis* suggests that it might have a more recent origin. It would have appeared only after the divergence of this genus from other lacertids belonging to the same lineage.

Though being restricted to *Podarcis*, pLHS, however, shows a more rapid evolutionary rate. In fact, pLCS displays very little or no variation in all the *Podarcis* species; pLHS, instead, is practically the same in *P.sicula* and *P.muralis*, but shows marked differences in *P.taurica* and *P.tiliguerta*. This observation is also interesting from the systematic standpoint, since it confirms that *P.sicula* and *P.muralis* are closely related, and the two other species belong to lineages other than *P.sicula*, as indicated by immunological and genetic studies (LUTZ & MAYER, 1985).

	Clone	**pGPS**	**pLCS**	**pLHS**	**pSHS**
	Mon.Size	160	180	140	130
Species	**Chr.Loc.**	p	c	p.t.	?
Podarcis sicula		++	X	X	-
Podarcis muralis		++	+++	+++	?
Podarcis tiliguerta		++	+++	++	?
Podarcis taurica		+	+++	++	?
Podarcis hispanica		+	?	?	-
Algyroides fitzingeri		?	+++	-	-
Algyroides moreoticus		++	++	?	-
Lacerta graeca		X	+++	?	?
Lacerta dugesii		?	+	-	?
Lacerta lepida		?	-	-	-
Lacerta viridis		++	-	-	-
Lacerta vivipara		++	?	-	-
Archaeolacerta bedriagae		++	-	-	-
Archaeolacerta saxicola		-	?	-	X
Psammodromus hispanicus		?	?	-	-

Table 1: Presence and distribution of highly repetitive DNAs in lacertid lizards.**Mon.Size** = size of the monomeric unit in base pairs.**Chr.Loc.**= chromosome localization: **p** = pericentromeric, **c** = centromeric, **t** = telomeric. **?** = unknown. **X** = species from which the highly repetitive DNA has been isolated. **+** = presence of the satellite DNA, the number of + indicates the level of hybridization and then the affinity between the species examined. **-** absence of the satellite DNA. **?** = the presence of the satellite DNA was not tested.

A third, highly repeated DNA, called pSHS, has been isolated from *Lacerta saxicola* with HindIII. It does not show any similarity to pLHS, but, like the latter, has a limited distribution. In fact, it hybridizes only to the homologous DNA, whereas it fails to hybridize either to that of species belonging to other genera or to that of species ascribed to the genus *Archaeolacerta*, in which some investigators also include *L.saxicola* (ARNOLD, 1973, 89).

A different behaviour is displayed by the fourth satellite sequence (pGPS), which has recently been isolated from *L.graeca* with the PstI restriction enzyme. In fact, this DNA has quite a wide distribution, being found with some differences both in species related to *L.graeca*, such as several *Podarcis* and *Algyroides*, and in species that are not considered related to it, such as *L.viridis*, *L.bedriagae* and *L.vivipara*. Moreover, pGPS is not present in *L.saxicola*, which appears to have a much more differentiated DNA than many other lacertids so far investigated.

Its presence in different genera suggests that pGPS might be a very ancient sequence appearing before the divergence of the main lacertid taxa.

COMMENTS AND CONCLUSIONS

As already mentioned, the study of satellite DNAs has provided interesting contributions to the knowledge of lacertid phylogeny and taxonomy.

The most exhaustive and convincing results concern *Podarcis*, which is the genus most investigated. In fact, the analysis of satellite DNAs has confirmed that this genus is quite a homogeneous natural group; in addition, it has clearly shown its relation with other taxa of the family (*Algyroides*, *L.graeca*, *L.dugesii*), and has contributed to elucidating the relationships existing between some species of the genus, such as *P.sicula*, *P.muralis*, *P.taurica* and *P.tiliguerta*.

The contribution of these studies to other genera of the family is more limited. However, they provide interesting data deserving further investigation, such as the evidence that *Archaeolacerta* is probably a heterogeneous group and *L.saxicola* genome is greatly differentiated compared to that of all the other species studied.

The reliability of the results should also be emphasized. In fact, they agree with morphological, immunological and karyological results.

In conclusion, the study of satellite DNAs appears a useful tool for phylogenetic and taxonomic investigations. However, some remarks should be made.

The effectiveness and reliability of the study of satellite DNAs is more marked when related species are compared; the results of comparisons between more distant species, instead, are sometimes questionable. This depends on the rather complex trend that the evolution of a certain highly repeated sequence can show. Some of them are preserved for a long period, and their sequence can undergo gradual differentiation with time. Some, instead, can be reduced or disappear by deletion processes, or give rise to new sequences by divergence and reamplification of some of their copies, and this is the case of pLCS and pLHS (CAPRIGLIONE *et al.*,1994). Moreover, the rate of divergence is not the same for all satellite DNAs.

Therefore, a joint analysis of more than a sequence should be made, and satellite DNAs from different species should be compared. In this regard, it is significant that, as already mentioned, the most satisfactory results concern *Podarcis* and its allied, in which we observed three different DNA sequences.

Finally, the results of studies of satellite DNAs should be compared with data from other kinds of investigation.

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Podarcis muralis and *Lacerta oxycephala* (Reptilia, Lacertidae) on the islands of Skadar Lake: Distribution and genetic relationships of populations

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Abstract: Region of Skadar Lake (Montenegro) presents part of the southern border of species range for two lacertid lizards: *Podarcis muralis* (Common Wall Lizard) and *Lacerta oxycephala* (Sharp-snouted Rock Lizard). Beside coastal area, they also inhabit 27 small islands which form south-western Skadar Lake archipelago. Insular populations seem to be allopatric on all islands except one. In the scope of complex investigations we have performed an starch-gel electrophoretic study on both species. A total of 24 gene loci was analyzed for 11 populations of *P. muralis* and for 8 populations of *L. oxycephala*. These data were used to estimate the genetic variability level within populations as well as degree of genetic differentiation among populations. The extent of population genetic structuring in both species is discussed, taking into consideration the possible influence of isolation effects.

Key words: *Podarcis muralis*. *Lacerta oxycephala*, Island populations, Genetic relationships, Gene flow.

INTRODUCTION

Skadar Lake and its coastal area lie in the Mediterranean climatic zone, 20 km from Adriatic Sea, to which it is connected by the Bojana River (Figure 1.). The lake is surrounded by mountains belonging mostly to Dinaric Alps. Climatic influence from lowland is also greatly felt on mountain slopes above Skadar Lake. The whole area is characterized with apparent richness of herpetofauna.

Among six members of family Lacertidae which are known as inhabitants of Skadar Lake islands and surrounding area (CRNOBRNJA-ISAILOVIC *et al.*, 1994), *P. muralis* and *L. oxycephala* appeared to be the most abundant (DZUKIC, 1977). Beside coastal zone, where they seem to occupy different habitats, those two lizard species are also distributed throughout southwestern Skadar Lake archipelago (Figure 1.). The small karstic islands, lying along the southwestern bank at the base of mountains Rumija and Sutorman, represent the highest parts of their north - eastern hills (BESIC, 1980). The origin of this archipelago is a result of continuous dropping of terrain in the broad area of mountain Rumija and Skadar Lake.

Insular populations seem to be allopatric on 25 islands except the most southern one. Common Wall Lizard was noticed only on 10 islands, instead of 16 inhabited by Sharp Snouted Rock Lizard. Among continental populations, it is noticeable that *L. oxycephala* occupy rocky south-western coastal area at the feet of mountains Rumija and Sutorman, expelling *P. muralis* on the upper altitudes. The Common Wall Lizard is more widespread along lowland northern and north-eastern bank (CRNOBRNJA *et al.*, in prep.) and deeper into the continental area to the North.

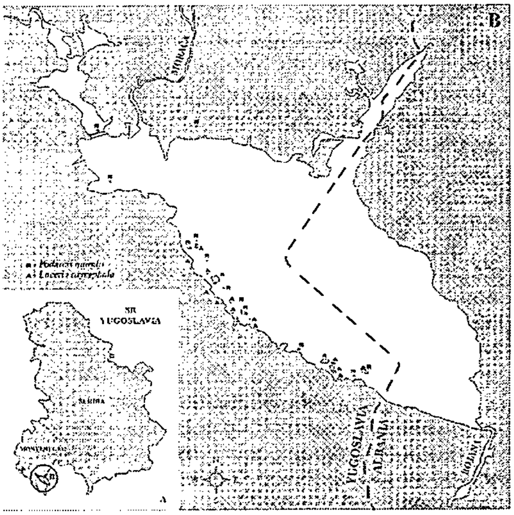

Figure 1. Distribution of *Podarcis muralis* and *Lacerta oxycephala* populations on Skadar Lake islands.

Region of Skadar Lake presents part of the southern border of species range for those two lizards (ARNOLD & BURTON, 1978; DZUKIC, 1991; BRUNO, 1991). *P. muralis* has wide distribution in Albania, where subspecies *muralis* and *albanica* seem to occur (BRUNO, 1991). Also, populations from Skadar Lake are very variable in size, color and pattern, even on the intrapopulation level. *L. oxycephala*, which

Pop. no.	Population name	Area (sq.km) for islands	Elevation (m.) for islands	N	H	P*	n_a
	Podarcis muralis						
1.	Malo Starčevo	0.009	20	30	.101	.333	1.6
2.	Krš od Starčeva	0.001	<10	30	.052	.250	1.3
3.	Kaurica	0.003	13	30	.123	.417	1.7
4.	Malo Besko	0.017	23	30	.164	.500	1.7
5.	Moračnik	0.112	44	22	.084	.292	1.6
6.	Gradac	0.010	21	30	.068	.250	1.4
7.	Gorica Škurt	0.004	14	30	.116	.375	1.5
8.	Široka Gorica	0.012	16	30	.115	.375	1.5
9.	Lesendro	0.007	15	20	.109	.375	1.5
10.	Plavnica	*	*	30	.107	.417	1.8
11.	Čarapica Brest	*	*	11	.126	.375	1.5
	Lacerta oxycephala						
1.	Starčevo Velje	0.069	30	9	.060	.208	1.2
2.	Golubovo	0.015	20	30	.084	.333	1.7
3.	Beška Velja	0.159	38	30	.089	.292	1.6
4.	Zalug	0.008	18	30	.068	.250	1.6
5.	Gorica Smoje	0.008	23.5	29	.064	.250	1.5
6.	Moračnik Mali	0.007	15	10	.058	.167	1.2
7.	Vranica	0.027	33	21	.072	.292	1.4
8.	Dužica	0.138	47	17	.062	.167	1.3

Table 1: Sampling localities and genetic variability measures. N=number of individuals per sample, H=mean observed heterozigosity per locus, P=proportion of polimorphic loci, n_a=mean number of alleles per locus. *A locus is considered polymorphic if the frequency of the most common allele does not exceed 0.95

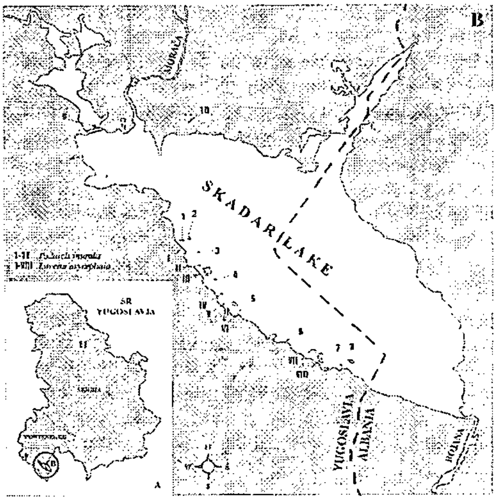

Figure 2. Location of all populations sampled in this study.

is one of endemic lizard species for southern part of Balkan Peninsula (DZUKIC, 1991), is not observed in Albania southern from Tarabosh hill (BRUNO, 1991), near Skadar Lake.

In the scope of complex investigations we have performed an electrophoretic study on both species, trying to understand better their distribution and genetic relationships of populations. Samples collected from 8 island and 3 mainland populations of Common Wall Lizard were included in the study, together with 8 island samples of Sharp-snouted Rock Lizard (Table 1. and Figure 2). Basic data for *P. muralis* samples are presented in CRNOBRNJA *et al.* (1994), while for *L. oxycephala* they will be printed elsewhere (CRNOBRNJA-ISAILOVIC *et al.*, in prep.).

MATERIAL AND METHODS

Study site

Skadar Lake is subtropical body of water lying in a tectonic basin of karst topography. Although its precise origin is not known, Skadar Lake was probably formed during the Pleistocene, as a rest of an ancient sea bay (LUKOVIC, 1935) which persisted until the end of Miocene and beginning of Pliocene epoch. Later geotectonic movements caused the formation of fresh-water body. (BESIC, 1980). Probably not all former hills became islands at the same time. According to the bathymetric chart (LASCA et al, 1981), islands Duzica and Vranica originated first. Malo Besko and Moracnik aroused in the second group,followed then by Starcevo Velje, Krs od Starceva,

Protein*	locus	Enzyme commission number*	Electrophoretic conditions**	species examined
1. Acid phosphatase	Acp-1	3.1.3.2	2	*mur/oxy*
2. Alcohol dehydrogenase	Adh-2	1.1.1.1	6	*mur*
3. Adenylate kinase	Ak-1	2.7.4.3	2	*mur/oxy*
4. Adenylate kinase	Ak-2	2.7.4.3	2	*mur/oxy*
5. Adenylate kinase	Ak-3	2.7.4.3	2	*mur/oxy*
6. Aldehyde oxydase	Ao	1.2.3.1	3	*mur/oxy*
7. Aspartate amino transferase	Got-(-1)	2.6.1.1	1	*oxy*
8. Aspartate amino transferase	Got-(-2)	2.6.1.1	1	*mur/oxy*
9. Esterase	Est-1	3.1.1.1	1	*mur/oxy*
10. Esterase	Est-2	3.1.1.1	1	*mur/oxy*
11. Glucose-6-phosphate dehydrogenase	G6pd	1.1.1.49	6	*mur*
12. Glucose-6-phosphate isomerase	Gpi-(-1)	5.3.1.9	4	*mur/oxy*
13. Glutamate dehydrogenase	Gdh	1.4.1.3	6	*mur/oxy*
14. Glyceraldehyde-3-phosphate dehydrogenase	Gapd	1.2.1.12	2	*oxy*
15. α-Glycerophosphate dehydrogenase	αGpd-2	1.1.1.8	6	*mur*
16. Isocitrate dehydrogenase	Idh-1	1.1.1.42	2	*mur/oxy*
17. Isocitrate dehydrogenase	Idh-2	1.1.1.42	2	*mur/oxy*
18. Isocitrate dehydrogenase	Idh-(-1)	1.1.1.42	2	*oxy*
19. L-lactate dehydrogenase	Ldh 1.	1.1.27	5	*mur/oxy*
20. Malate dehydrogenase	Mdh-1	1.1.1.37	4	*mur/oxy*
21. Malate dehydrogenase	Mdh-2	1.1.1.37	4	*mur/oxy*
22. Malic enzyme	Me-1	1.1.1.40	2	*mur/oxy*
23. Malic enzyme	Me-2	1.1.1.40	2	*mur/oxy*
24. Octanol dehydrogenase	Odh-3	1.1.1.73	5	*mur/oxy*
25. Phospho-gluco-mutase	Pgm-(-1)	2.7.5.1	5	*oxy*
26. Protein	Pt	-	1	*mur/oxy*
27. Sorbitol dehydrogenase	Sordh-2	1.1.1.14	6	*mur*
28. Xanthine dehydrogenase	Xdh	1.2.1.37	3	*mur/oxy*

Table 2: Protein loci and electrophoretic conditions. *mur* - enzyme loci examined in *P. muralis* samples, *oxy* - enzyme loci examined in *L. oxycephala* samples.
*Nomenclature Commitee of the INTERNATIONAL UNION OF BIOCHEMISTRY (1984).
**(1)Lithium hydroxide; (2)Tris-citrate pH 8; (3)Tris-versene-borate; (4)Phosphate-citrate; (5)Tris-maleate pH 7.4; (6)Tris-boric acid for dehydrogenase pH 9.

Golubovo, Kaurica, Gorica Skurt and Siroka Gorica. Malo Starcevo and Beska Velja are of later origin. Finally, the youngest islands in the archipelago could be Gorica Smoje, Gorica Zalug, Moracnik Mali and Gradac,with average depth around them being between 2 and 3 meters.

Electrophoresis

Allozyme variation was assessed in 293 *P. muralis* and 176 *L. oxycephala* specimens collected on various islands. Three mainland populations of *P. muralis* also were examined (Table 1.). The liver, heart and kidneys of each specimen were collectively homogenized in deyonisated water and the supernatant was frozen at -80°C. Techniques of horizontal starch-gel electrophoresis and protein staining techniques are mainly the same as it was described in SELANDER *et al.* (1971) and AYALA *et al* (1972). Among 24 protein loci examined, 20 were found common for both species (Table 2).

Statistics

The statistical procedures were previously described in CRNOBRNJA *et al.* (1994). Additionally, we used adequate routines of BIOSYS-1.7 (SWOFFORD & SELANDER, 1989) to estimate Nei and Rogers coefficients of genetic similarity and distance among two species.

RESULTS

Within species variability

Basic results for *P. muralis* samples were presented in CRNOBRNJA et al (1994). *L. oxycephala* samples, collected from 8 islands, are characterized with variable extent of polymorphism (Table 1.), from .167 to .333. Average heterozygosity per locus varied from .058 to .089. The lowest level of genetic variability was observed for smallest island (Table 1.) but, generally, there were no significant correlation between island size and heterozygosity (SPEARMAN's rank-correlation).

	islands		shore		all	
	H	P	H	P	H	P
SKADAR LAKE						
P. muralis	.103	.349	.108	.396	.104	.317
	(.052-.164)	(.250-.500)	(.107-109)	(.375-.417)	(.052-.164)	(.250-.500)
L. oxycephala	.070	.245	-	-	.070	.245
	(.058-.089)	(.167-.333)	-	-	(.058-.089)	(.167-.333)
ADRIATICSEA						
(GORMAN et al. 1975)						
P. melisellensis	.039	.172	-	-	.039	.172
	(.000-.077)	(.000-.320)	-	-	(.000-.077)	(.000-.320)
P. sicula	.044	.168	.090	.363	.064	.251
	(.029-.057)	(.140-.210)	(.059-.128)	(.270-.450)	(.029-.128)	(.140-.450)
L. oxycephala (pooled sample)	.053	.210	-	-	.053	.210

Table 3: Genetic variability levels in island and mainland populations of lizard species examined in this study and in GORMAN *et al.* (1975).

samples	rare alleles	unique alleles	samples	rare alleles	unique alleles
Podarcis muralis			*Lacerta oxycephala*		
ISLAND:			ISLAND:		
1. Malo Starcevo	4	-	1. Starcevo Velje	-	-
2. Krs od Starceva	1	-	2. Golubovo	6	2
3. Kaurica	1	-	3. Beska Velja	3	1
4. Malo Besko	2	-	4. Zalug	5	-
5. Moracnik	7	1	5. Gorica Smoje	5	-
6. Gradac	3	-	6. Moracnik mali	-	-
7. Gorica Skurt	1	-	7. Vranica	3	-
8. Siroka Gorica	2	-	8. Duzica	1	-
SHORE:					
9. Lesendro	1	-			
10. Plavnica	5	2			
CONTINENT:					
11. Carapica Brest	1	-			

Table 4: Distribution of rare alleles ($p<0.05$) and unique alleles (found in only one population) in analyzed population samples of two lizard species.

Mean genetic variability measures are higher for *P. muralis* samples belonging to both insular and mainland subsets than for *L. oxycephala* samples or for insular populations of *P. melisellensis* and *P. sicula*, analysed by GORMAN et al (1975)(Table 3.). Unique alleles were found in two island populations, Golubovo (Gdh^{98} , p=.025) and Beska Velja (Est-1^{99}, p=.054; Est-1^{101}, p=.518). Rare alleles were observed in almost all samples (Table 4.), varying in number from 0 to 6.

NEI's (1978) unbiased genetic distance coefficients for *L. oxycephala* showed range of values from .007 to .083. ROGER's (1972) coefficient of genetic similarity also was computed, because of comparison with various authors (Table 5.). UPGMA clustering technique expressed the same degree of unexpectedness as in *P. muralis* samples (CRNOBRNJA et al, 1994): For both coefficients of genetic differentiation, the clusters showed degree of closeness somewhat inconsistent with geographic proximity of populations (Figure 3.). Samples from islands Starcevo Velje, M. Mali and G. Smoje appeared to be the most tightly linked. Together with G. Zalug and Beska Velja, they formed cluster apparently distinguishable from Vranica and Duzica. The other two always formed separate cluster, but genetically less close. The position of sample from Golubovo was subjected to a change, depending of coefficient used : from member of the northern subgroup of examined islands to position of genetically the most divergent population sample.

Distribution of allele frequencies differed significantly among populations for all loci in both species, indicating significant genetic

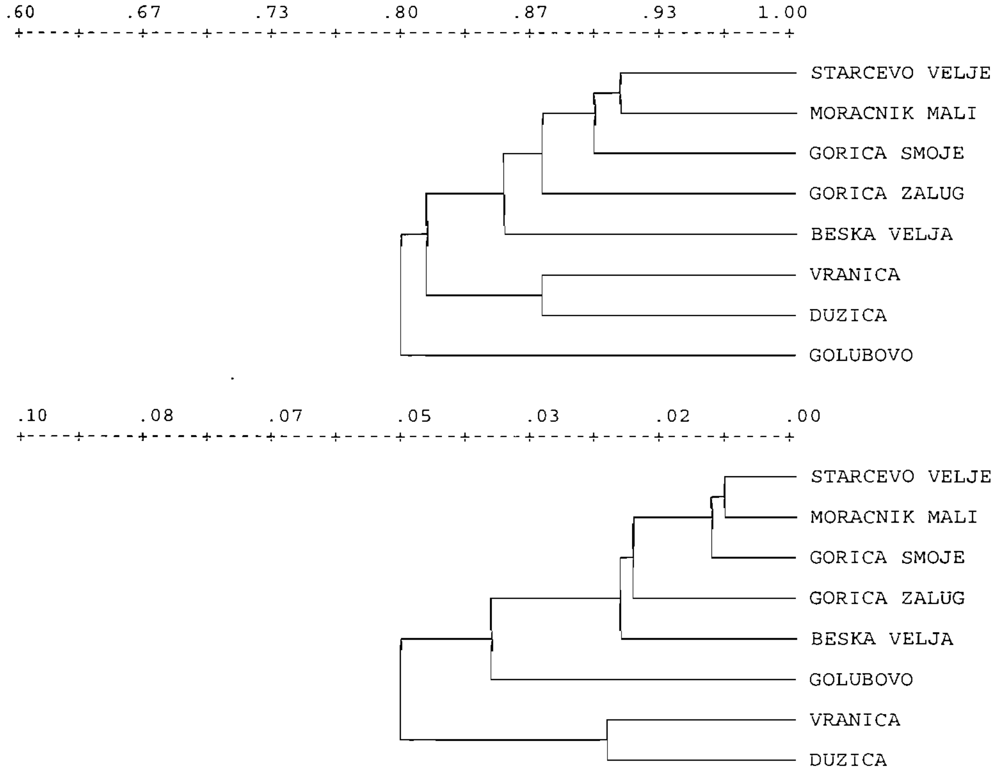

Figure 3. UPGMA cluster analyses for *L. oxycephala* samples: ROGER's (1972) genetic similarity measures (above) and NEI's (1978) unbiased genetic distance measures (below).

Species	No. of pops.	1		2	
1. *P. muralis*	11	.051 (.009-.110)	.881 (.794-.953)		
2. *L. oxycephala*	8	.339 (.279-.403)	.677 (.622-.726)	.037 (.007-.083)	.914 (.871-.957)

Table 5: NEI (1978) unbiased genetic distance (left) and ROGERS (1972) genetic similarity (right) average values for sub- and conspecific population samples of *P. muralis* and *L. oxycephala* samples with ranges in parentheses.

SPECIES	No. of pops.	1		2	
1. *P. melisellensis*	12	.036 (.000-.116)	.938 (.864-.998)		
2. *P. sicula*	7	.445 (.336-.573)	.622 (.547-.691)	.036 (.000-.098)	.919 (.852-.991)
3. *L. oxycephala*	1	.940 (.825-1.001)	.380 (.357-.425)	.880 (.836-.978)	.407 (.381-.420)

Table 6. NEI (1978) unbiased genetic distance (left) and ROGERS (1972) genetic similarity (right) average values for sub- and conspecific population samples of *P. melisellensis*, *P. sicula* and *L. oxycephala* (by GORMAN et al. 1975).

Locus		island populations				coastal populations						
	No. of alleles	Chi-square	D.F.	P	No. of alleles	Chi-square	D.F.	P	No. of alleles	Chi-square	D.F.	P
EST-1	3	262.070	14	***	3	.334	2	n.s.	3	342.727	20	***
EST-2	3	105.134	14	***	2	3.404	1	n.s.	3	234.446	20	***
ADK-3	3	95.376	14	***	3	2.795	2	n.s.	3	121.458	20	***
ACP-1	2	21.255	7	**			2			32.993	10	***
IDH-1	2	170.42	17	***	2	4.665	1	*	2	177.450	10	***
IDH-2	2	95.946	7	***	2	45.007	1	***	2	154.367	10	***
ME -1	3	286.162	14	***	3	12.760	2	**	3	329.380	20	***
ME -2	3	42.342	14	***	3	6.243	2	*	3	329.380	20	***
G6PDH	3	152.087	14	***	3	7.002	2	*	5	308.892	40	***
GDH	3	43.659	14	***	3	4.255	2	n.s.	4	89.943	30	***
MDH-1	3	122.577	14	***	3	1.259	2	n.s.	3	174.840	20	***
XDH	3	77.507	14	***	3	36.232	2	***	3	249.736	20	***
(Totals)		1474.538	147	***		123.955	19	***		2311.084	240	***

Table 7. Contingency chi-square analysis at all loci among *P. muralis* insular populations. n.s.=non-significant, *P<.05, **P<.01, ***P<.001

Locus	No. of alleles	Chi-square	D.F.	P
EST-1	3	167.619	14	***
EST-2	3	32.452	14	**
ADK-2	3	85.564	14	***
ADK-3	4	203.581	21	***
ACP-1	2	28.008	7	***
G3PDH	3	91.112	14	***
ME-2	2	45.830	7	***
LDH	2	301.293	7	***
GDH	5	124.734	28	***
MDH-1	4	144.986	21	***
XDH	3	54.216	14	***
(Totals)		1279.394	161	***

Table 8. Contingency chi-square analysis at all loci among *L. oxycephala* insular populations. **P<.01, ***P<.001

heterogeneity among populations. In *P. muralis*, where different subsets of samples can be distinguished, the heterogeneity remained significant for island populations alone (Table 7.). But, three mainland populations showed no significant difference at 45% of polymorphic loci, indicating greater divergence among island populations. In *L. oxycephala*, where only island population samples were included into the study, significant heterogeneity of distribution was noticed for alleles at all polymorphic loci (Table 8.).

The use of F-statistics (WRIGHT 1965, 1978) showed that, contrary to *P. muralis*, in island populations of Sharp-snouted Rock Lizard intrapopulation component (F_{IS}=.258) has slightly stronger influence on total genetic differentiation than spatial subdivision alone (F_{ST})=.241, Table 9). Anyway, the total fixation index (F_{IT}= .363) appeared to be more outstanding in *L. oxycephala*.

Among species variability

Mean genetic divergence level, express by NEI's (1978) coefficient of genetic distance, between *P. muralis* and *L. oxycephala* populations from Lake Skadar amounted .339 (Table 5.). For comparison, the average genetic distance between Sharp-snouted Rock Lizard from Adriatic islands and *Podarcis sicula* was .940. Genetic distance between *L. oxycephala* and other member of the same genus, *P. melisellensis*, was somewhat lower , of about .880 (Table 6.). According to the results, the divergence level between two lizard species from Lake Skadar is closer to the that obtained for two *Podarcis* species from Adriatic Sea: Mean genetic distance between *P. sicula* and *P. melisellensis* amounted .445 (data used from GORMAN et al, 1975).

UPGMA cluster (Figures 4. and 5.) for both lizard species sampled in this study was constructed by using 20 common gene loci instead of 24 which were scored per species. Composition of the sample of loci analyzed influenced on pattern of links within the species, but main subgroups remained the same.

DISCUSSION

The studies of genetic relationships in insular populations of lizards were very popular at one time (WEBSTER et al, 1973; GORMAN et al, 1975; GORMAN AND KIM, 1976). All of them had in common the establishing of differences in genetic structure between insular and continental populations. General conclusions aroused from those studies were that amounts of genetic variation should be greater in mainland than in island populations, and on large than on small

	Mean			
	F(ST)	F(IS)	F(IT)	$2N_em$*
Podarcis muralis				
Island populations	.229	.132	.331	1.68
All populations	.247	.154	.363	1.52
Lacerta oxycephala				
Island populations	.241	.258	.437	1.58
Podarcis melisellensis (by GORMAN *et al.*, 1975)				
Island populations	.472	(-)	(-)	.56
Podarcis sicula (by GORMAN et al, 1975)				
Island populations	.126	(-)	(-)	3.46
All populations	.377	(-)	(-)	.83

Table 9. Mean F-statistics and gene flow level among populations of lacertide lizard species examined under the present study and by other authors. * Estimated upon WRIGHT's (1943) equation. (-) means no data.

islands (GORMAN et al, 1975). It also became evident that many demographic, historical and geographical specificities can influence on final genetic variation pattern. In that way, both species under our study expressed high levels of genetic variability, somewhat inconsistent with sourcies of analyzed populations (Table 1.). Expected heterozygote deficiencies in insular populations were not always noticeable, especially in the case of *P. muralis*. The degree of polymorphic loci and the mean number of alleles observed also varied independently of island size. Comparing our results with those from GORMAN et al (1975) study, we found that both populations of *P. muralis* and *L. oxycephala* from Lake Skadar are genetically more variable than island and mainland populations of *P. melisellensis* and *P. sicula* from Adriatic Sea (Table 3.). The all examined populations of last two species belong to different subspecies and those populations were found to be highly polymorphic for some morphological features - the same facts as they would be in the case of *P. muralis* populations from Lake Skadar (CRNOBRNJA et al, 1994). One important difference was that origin and isolation of islands could not be equalized in these two cases. The islands in Adriatic Sea were in contact some 10 000 to 15 000 years ago. According to the bathymetric chart, islands of Skadar Lake must be of later origin. It is known that average water level of the Lake aroused 4 meters during the last five centuries and that annual fluctuations amount from 2 to 5 meters (STANKOVIC, 1976). Under such circumstances it is expectable that isolation effects cannot be treated on the same way in those two area. Also, *L. oxycephala* populations from Lake Skadar showed the similar and even grater level of genetic variability and polymorphism than some populations of polytypic *P. sicula* and *P. melisellensis* from Adriatic Sea (Table 3.). The Sharp Snouted Rock lizard was previously designated as monotypic species (MERTENS AND WERMUTH, 1960), without inter-island variation or intrapopulation polymorphism(GORMAN et al, 1975). Recent works indicated that some microdifferentiation could exist (DZUKIC, 1991).

Degree of genetic subdivision, estimated for *P. muralis* and *L. oxycephala* populations under the study, exceeded values known for other lizard species (MCKINNEY et al, 1972, SITES & GREENBAUM, 1983; SARRE et al, 1990). Within the family, the values of F_{ST} were not so high as among the *P. melisellensis* populations inhabiting Adriatic islands, but are evidently higher than value estimated among *P. sicula* insular populations (Table 9.). Italian Wall Lizard show the lowest F_{ST} value among all four comparable lacertide species.

Degree of genetic differentiation of populations depends of the level of gene flow between them. When average number of migrants per generation (N_em) is less than 0.5, local demes are largely unconnected in the dynamics of gene alleles under any model of gene flow (NAGYLAKI, 1983). According to WRIGHT (1940), that is the case of real "island" model of population differentiation. The model includes strong isolation among demes and apparent fixation or elimination of alleles within them. Populations of lizards from Skadar Lake were characterized with N_em between 0.5 and 1.0, which means significant independence of local demes,but does not exclude ability of gene exchange among them. Number of rare alleles observed in island populations of both *P. muralis* and *L. oxycephala* and high proportion of polymorphic loci could indicate some sort of within-species hybrid zone of very complex behavior (BARTON AND HEWITT, 1985). The presence of rare alleles (Table 4.) could mean the existence of gene flow among demes (SLATKIN, 1985).

Genetic divergence levels among population pairs of Common Wall Lizard and Sharp-Snout Rock Lizard didn't exceed the species level, compared to the results obtained from GORMAN et al (1975) (Tables 5. and 6.). According to our field impressions, subspeciation processes should be investigated on the level of external morphology,. Two facts remained unclear: the

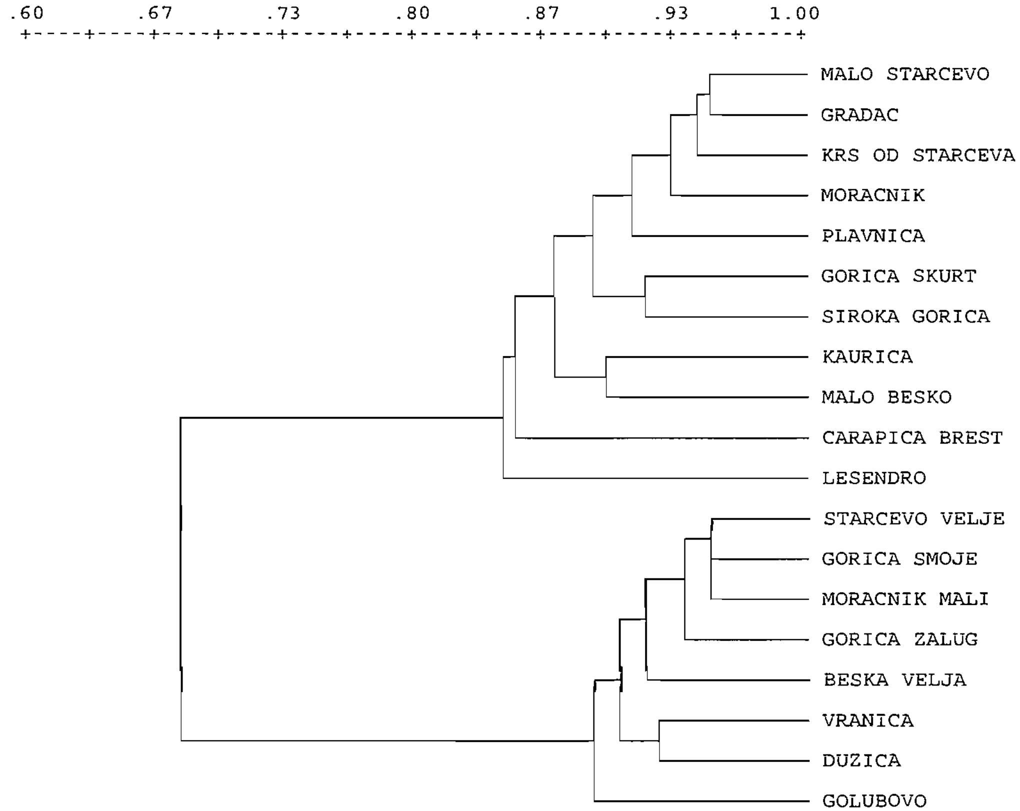

Figure 4. UPGMA cluster analyses for both lizard species sampled in this study: ROGER's (1972) genetic similarity measures.

incompatibility of genetic divergence coefficients' values with spatial distance between certain population pairs and relatively low level of genetic divergence between species belonging to the different genera.

CONCLUSIONS

The results of starch-gel electrophoresis performed on population samples of *P. muralis* and *Lacerta oxycephala* from the Lake Skadar showed maintenance of high levels of genetic variability and absence of strong isolation effects whose would be expected in island populations.Mean genetic variability level for *P. muralis* amounted .104 while for *L. oxycephala* was lower (H=.070). Gene flow level exceeded 1.0 for both species. The inconsistency of spatial proximity with genetic divergence level was noticed, together with relatively low genetic distance value between two species belonging to the different genera (D=.339).

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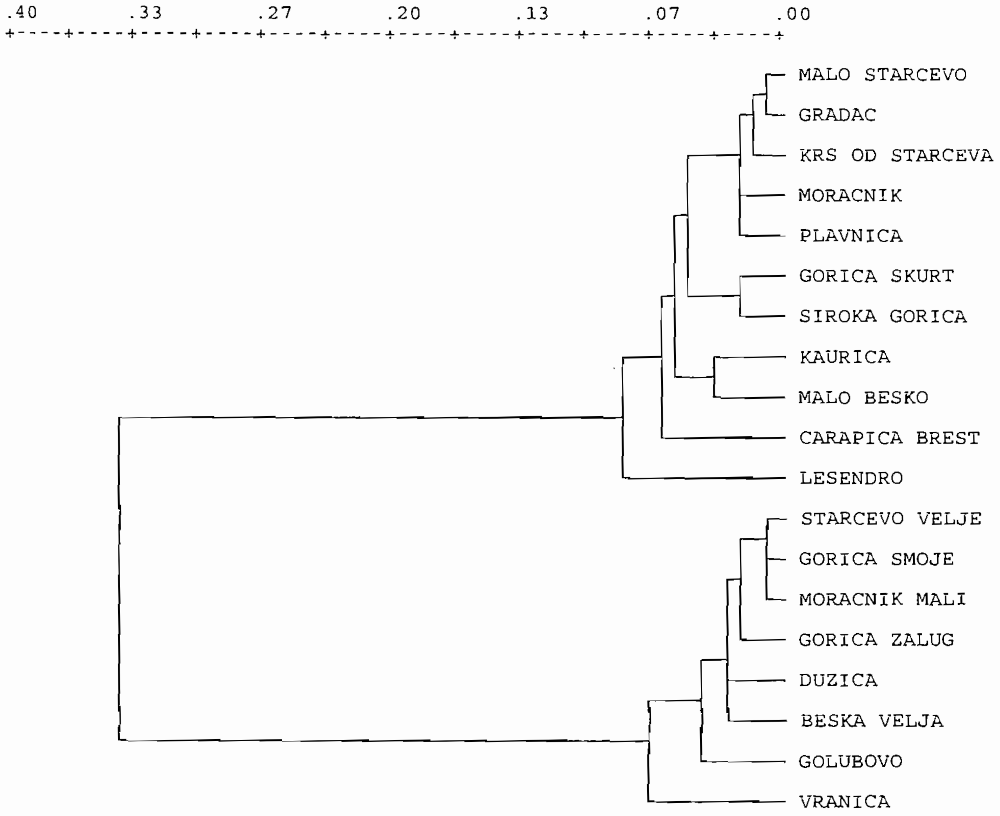

Figure 5. UPGMA cluster analyses for both lizard species sampled in this study: NEI's (1978) unbiased genetic distance measures.

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Origin, colonization, adaptive radiation, intrainsular evolution and species substitution processes in the fossil and living lizards of the Canary Islands.

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Abstract: The Canary Island lizards constitute a monophyletic group which separated from the rest of the family shortly after the first islands of the archipelago emerged. Five living and at least one recently extinct species belong to the genus *Gallotia*. In addition, two of the living species, *Gallotia simonyi* and *Gallotia stehlini* have become extinct on Gomera and Tenerife, respectively. Juveniles of all species present tricuspid teeth. This character is preserved in the adults with changes to one degree or another in *G. galloti*, *G. caesaris*, *G. simonyi* and *G. goliath*. In *G. atlantica* there are only two cuspids and *G.stehlini* has 4 or more. It has been confirmed that in current species there is a direct relationship between the number of cuspids in the adults and the extent to which they are herbivorous. The number and arrangement of teeth on the pterigoides also presents some differences in terms of species: only one row in *G. atlantica*, *G. galloti*, *G. caesaris*, *G. stehlini*, *G. goliath* from La Gomera and the smaller *G. simonyi*. *Gallotia goliath* on other islands and the larger *G. simonyi* specimens present a lot of teeth in two rows. A model is suggested according to which the phenomenon of giantism in these lizards is related to favourable environments of recent volcanic origin, while the extinction of large species is associated with selective predation on the larger breeders by Man and by domestic animals.

Key words: Canary Islands, Lacertidae, lizard, evolution, giantism, extinction.

INTRODUCTION

The seven islands that make up the Canary Islands archipelago are situated from East to West with the nearest to the African coast, Fuerteventura, at less than 100 kms, and the furthest, La Palma, over 450 kms away (Figure 1). Distances between islands are variable, but they are relatively near to each other.

The winds on this archipelago generally come from the sea, with the northwesterly winds that make up the so-called Trade Winds predominating and blowing strongest in the summer. A southeasterly or easterly wind is also relatively frequent, bringing large quantities of fine dust from the Sahara, particularly to the South- and East-facing slopes of the islands (FERNANDO PULLÉ, 1976). The system of prevailing winds on the Canary Islands gives rise to a phenomenon crucial to an understanding of the biological processes: the formation of a cloud layer. This is caused by the wind from the sea, which, as is it rises along the altitudinal gradient of the most mountainous islands, determines the differential climatic features of any one island.

As a result of this phenomenon, in the Canary Islands and in other Atlantic archipelagos, relict examples of ecosystems that were once numerous throughout the Mediterranean region have been conserved: the Laurisilva forests (See AXELROD, 1975). There were many of these forests and their specialized transitional ecotones on the islands with more pronounced relief (all except Lanzarote and Fuerteventura) until the arrival of people approximately 2,000 years ago. On each island the disposition of these forests was determined by the direction of the trade wind, the geomorphological features and the relief in such a way that on islands like Tenerife they were arranged in long narrow strips, and, on other more or less circular islands, the upland parts were capped with continuous cover. (See Figures in LÓPEZ JURADO & MATEO, 1992).

However, in the scientific literature those islands that present the largest area of larisilva are usually wrongly identified as wet when, in fact, the high relative humidity is only maintained in the interior of the forest. Below the forest the environment is always dry (See, for example, LÓPEZ JURADO, 1992).

The Canary Islands are of different geological ages. Their volcanic origin caused them to take shape graduallly in such a way that the most easterly islands, Lanzarote and Fuerteventura, are the oldest. The most westerly, La Palma and El Hierro, are the youngest (See ABDEL-MONEM *et al.*, 1971; ABDEL-MONEM, 1972, SCHMINCKE, 1982, MAYER & BISCHOFF, 1991).

All the eastern islands and islets (Fuerteventura, Lanzarote, Lobos, Montaña Clara, Alegranza and Graciosa) were apparently (See the diagram suggested by MAYER & BISCHOFF, 1991) once joined and formed one

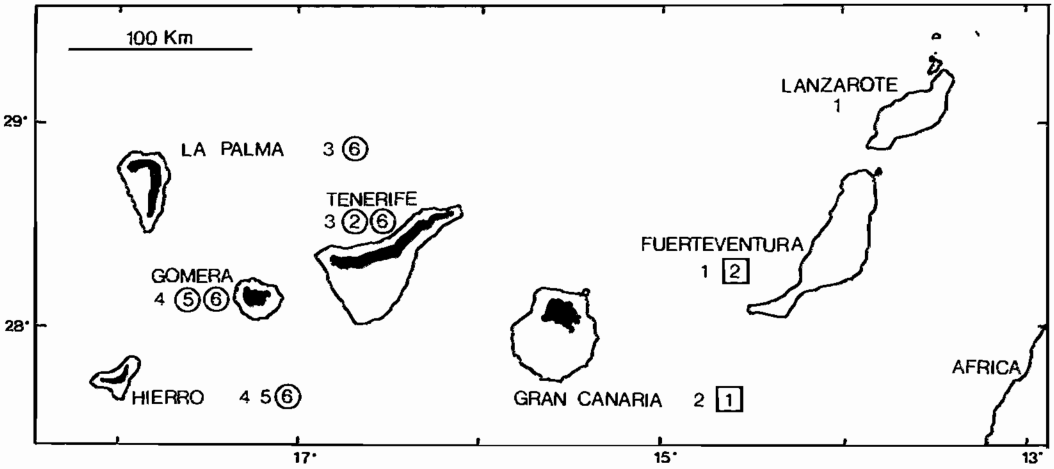

Figure 1: The Canary Island Archipelago. The areas that are potentially covered in laurisilva are shown in black. The numbers that appear with the name of each island are the species of the genus *Gallotia* that are present or have been present on each one of them. 1. *Gallotia atlantica* 2. *G. stehlini* 3. *G. galloti* 4. *G. caesaris* 5. *G. simonyi* 6. *G. goliath*. The circled numbers are the species currently extinct on an island. The numbers in a square represent recently intoroduced species.

large island quite close to the African coast.

Subsequently, Gran Canaria, Gomera and the Anaga peninsula (now the island of Tenerife) formed. An island later emerged in the area that is today occupied by the Teno peninsula. Anaga and Teno were subsequently joined following the eruption and formation of Teide.

El Hierro and La Palma were the last two islands to emerge. All these events began about 33 million years ago and finished about 2 million years ago (Abdel-Momem, *et al.* 1971; Abdel-Monem, 1972, Schmincke, 1982; Mayer & Bischoff, 1991).

However, the volcanic nature of the islands means that this type of activity has never really finished. In fact, the lava flows are continually destroying and creating land on all the islands.

The fields of recent lava constitute what is known in the Canary islands as "malpaís": rocky areas clearly separated from the adjacent ecosystems. These "malpaises" may appear in any part of the islands and the speed of their meteorization and biological colonization depends fundamentally on geographic position in relation to the prevailing winds and on altitude (See López Jurado & Delgado Luzardo, 1989).

On islands with a predominantly dry environment and low altitude above sea level such as Lanzarote and Fuerteventura, the "malpaises" constitute differentially wet environments. In fact, thanks to its porosity, the volcanic rock has the peculiar characterisitc of trapping the humidity of the winds from the sea. At the same time, the rock cover on these lava areas efficiently prevents evaporation (López Jurado & Delago Luzardo, 1989). In these conditions, when the weather has started to generate soil, the "malpaises" contain the greatest density of invertebrate and land vertebrate species (reptiles and small mammals) on the whole island (López Jurado & Delgado Luzardo, 1989; Lopez Jurado & Mateo, 1992). At the same time, within a limited space such as the island, the "malpaises" represent a new "island" to colonize, and, as we will see later, during this process the Canary Island lizards experience evolutionary phenomena that are unique in the world (See López Jurado & Mateo, 1992).

ORIGIN OF THE CANARY ISLAND LIZARDS

In the past some authors recommended classifying the Canary Island lizards with other, very different, lizard species such as *Lacerta* *lepida*, *Psammodromus algirus* or *Lacerta jayakari*, basing their argument on characteristics such as body shape, behaviour or sounds (Peters, 1961; Böhme, 1971; Böhme & Bischoff, 1976). However, advances in knowledge have demonstrated that it is impossible to prove a close relationship between any of the living species of lacertidae and the Canary Island lizards (See Lutz, Bischoff & Mayer, 1986, own data).

When it became known that the karyotype of the Canary Island lizards was clearly separate from other known lizards and that it was identical for the 3 most common Canary Island lizard species, this established the bases for the definitive separation of the genus *Gallotia* from any direct link with current lacertidae (Cano *et al*, 1984; López Jurado *et al*, 1986).

The latest immunological and molecular research carried out with these species suggests that the Canary Island lizards have been isolated from other continental lizards for between 27 and 35 million years, in other words, since the end of the Oligocene (Lutz, Bischoff & Mayer, 1986; Mayer & Bischoff, 1991; own data), coinciding with the origin of the Canary Island archipelago (See Abdel-Monem *et al.*, 1971 and 1972, Mitchell Thome, 1976, Schmincke, 1982). At that time, many species of the family, especially the bigger ones and the more specialized, disappeared due to the profound change in climate (Rage, 1986; Auge, 1989), while other more generalist lizards underwent a great radiation (Busack & Maxson, 1987).

All the fossil lizards found in European and North African deposits from the beginning of the Miocene were small and presented not very specialized dentition, with many almost undifferentiated teeth and generally three symmetrically spaced cuspids, as occurs today in several species of the genus *Gallotia* (See Cooper, 1963, Hutterer, 1985). *Miolacerta tenius* from Central Europe, *Lacerta filholi* from the South of France or *Lacerta* sp. from Bni Mellal follow this description (Rocek 1984; Auge, 1989; Rage, 1976). Consequently, although the similarity in dental features speculatively suggests that the origin of the Canary Islands lizards lies in one of the small lizard species that lived in southwest Europe or northwest Africa during this period.

Of all the species of the genus *Gallotia* described to date, four present tricuspid teeth (*G. galloti*, *G. caesaris*, *G. simonyi* and *G. goliath*). In one the adults present bicuspid teeth (*G. atlantica*) and another presents four or more cuspids (*G. stehlini*) (Figure 2). Our analysis of the Canary Island lizards will begin with a study of dental morphology in order to understand the colonization and evolution processes.

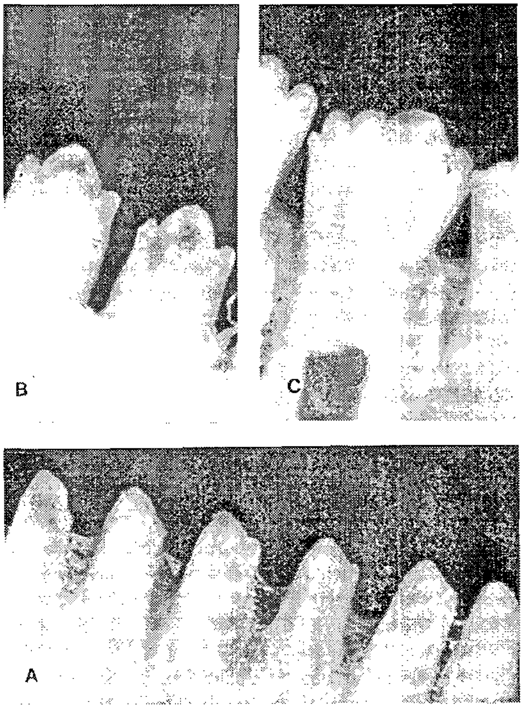

Figure 2: The dental morphology of species of the genus *Gallotia*. A. bicuspid teeth in *Gallotia atlantica* B. tricuspid teeth in *G. galloti* C. polycuspid teeth in *G. stehlini*.

CHARACTERIZATION OF EXTINCT FORMS ACCORDING TO BODY SIZE AND DENTAL MORPHOLOGY

To date all the lizards studied have had symmetrical tricuspid teeth at birth. The central cuspid is somewhat bigger than the lateral ones (Cooper, 1963, own data). As they develop they change towards bicuspid shapes typical of the teeth of almost all current lizards (*Lacerta*, *Podarcis*, *Psammodromus*, *Acanthodactylus*, *Eremias*, etc.), with some variations in the larger species such as *Lacerta lepida*, *Lacerta pater* or *Lacerta siculimelitensis* (See Böhme & Zammit Maempel, 1982; Estes & Williams, 1984) and in some Galliota species at very different levels.

In the skeletons of both fossil and living Canary Island lizards we found variations that allow us to distinguish taxa on the basis of body size, morphology of dental cuspids and the toothed region of the pterigoides.

On the basis of this, other authors described 3 possible forms of Canary Island lizards: *Gallotia goliath*, *Gallotia maxima* and *Gallotia* aff. *stehlini* (See MARTENS, 1942; BRAVO, 1953; LÓPEZ JURADO, 1985; own data). Besides this, two living species (*Gallotia simonyi* and *Gallotia stehlini*) are now considered extinct on different islands to those that they now inhabit (See HUTTERER 1985, own data). All the lizards that were already extinct had in common their large size.

However, the value of some conclusions, and even of some of the species and subspecies descriptions, may be considered rather doubtful since they were arrived at with little evidence and do not include a study of ontogenic variations or any other type of variation in the population. For example, it would be necessary to check that the occurrence of two large species such as *G. goliath* and *G. simonyi* on one island is not really a case of two different size classes of a single species. Until a more thorough study is carried out, we will assume, on the basis of data in the literature and on our own unpublished data, that *Galliota goliath* was present on Tenerife (MERTENS, 1942, BRAVO, 1953), Gomera (HUTTERER, 1985), Hierro (IZQUIERDO *et al*, 1989) and La Palma (MERTENS, 1942, own data), and that *Gallotia simonyi* is apparently found as a fossil on Gomera (HUTTERER, 1985) and, of course, living on El Hierro.

Gallotia stehlini is one of the two living species whose distribution area has decreased. We found a fossil example of this species that nowadays lives only on Gran Canaria (we will not include the accidental introduction on Fuerteventura - See NARANJO *et al*, 1991). We found a fossil example from the Pleistocene on the Anago peninsula (Tenerife). It is a contemporary of most of the known fossils of *Gallotia goliath*, and was with *Canariomys bravoi* in the same deposit with no trace of *G. goliath*. As with the living form from Gran Canaria its teeth present 4 or more cuspids formed by divisions in the three original cuspids.

On Gran Canaria *Gallotia stehlini* became smaller at the time people arrived on the island, changing from about 35 cm SVL 2,000 years ago to 28 cm, which is what some current specimens measure (MATEO & LÓPEZ JURADO, 1992). However, the remaining bone characteristics that were analyzed had not undergone any changes since then.

The other living species is *Gallotia simonyi*. This lizard recently disappeared from a small islet off the coast of El Hierro and today only survives on an almost inaccessible cliff on that island. Fossil and subfossil bones attributed to this species have also been found on La Gomera (HUTTERER, 1985). Their teeth are tricuspid and perfectly symmetrical with the central cuspid only a little bigger than the lateral ones.

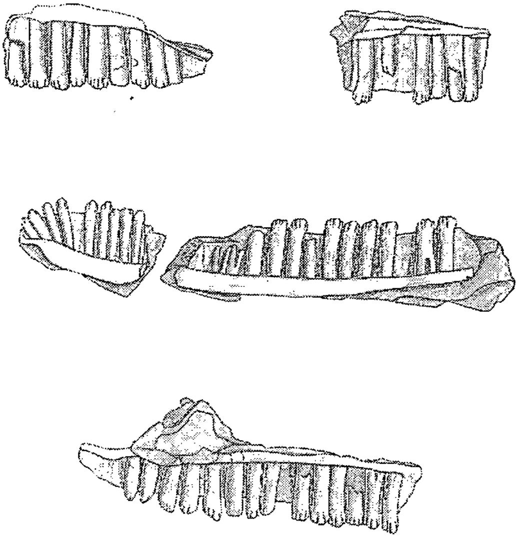

Figure 3: Fossil remains of *Gallotia* aff. *stehlini* from Taganan (North of Tenerife).

The largest Canary Island fossil lizard (*Gallotia goliath*) sometimes measures over 50 cm from head to tail and numerous remains have been found of specimens whose estimated size was between 40-45 cm head-tail (Figure 4). Their teeth always present 3 cuspids, but important differences in the robustness of the dental samples have been found. In fact, the following mandibula types are easily recognizable in the La Gomera deposits: relatively robust in the North of Tenerife and another more delicate type in the South of the same island (Figure 5). La Palma and El Hierro are different and should be considered separately.

Apart form robustness, these forms show certain differences in dental cuspids. The most robust present a very developed central cuspid to the detriment of the two lateral ones, which are sometimes vestigial. On the other hand, in the most delicate, all three cuspids are well-developed although the central one is always the most developed. This character is much more noticeable in the fossil lizard population in the South of Tenerife. A large *simonyi* would be easy to identify among them.

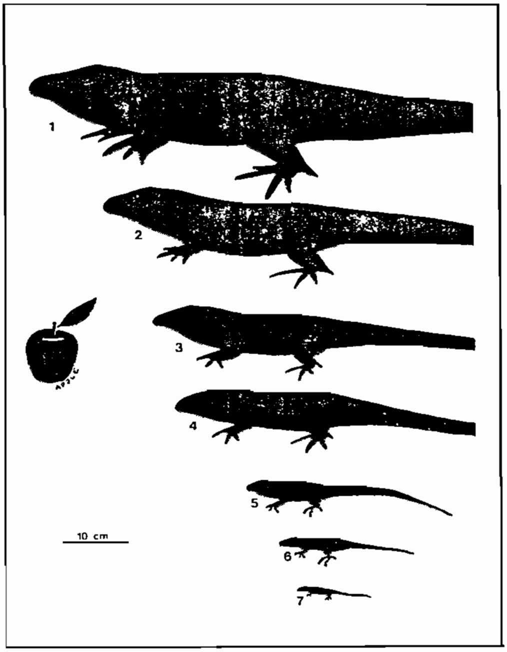

Figure 4: Relative size of the species of the genus *Gallotia*. 1. large specimen of the species *Gallotia goliath* 2. large *Gallotia stehlini* specimen prior to the arrival of people. 3. the biggest current *G. stehlini* speicmen 4. maximum size of *Gallotia simonyi* at present 5. adult male *Gallotia galloti* 6. maximum size of *Gallotia caesaris* 7. maximum size of *Gallotia atlantica* from the islet of Montaña Clara.

Therefore, we found that, except for *Gallotia stehlini*, the dental morphology of the remaining forms of extinct or almost extinct Canary Island lizards are almost identical to that of the species considered as their possible ancestors. Following this criterion, the "*simonyi-goliath*" group of forms (living in the most recently formed islands) would have conserved some of the original characteristics of the colonizing species.

In the description of *Gallotia goliath*, MERTENS (1942) stated that in this species the parietal foramen was closed and that it presented about 18 teeth in each pterigoides arranged in two convergent rows. In his diagnosis of *Gallotia maxima*, BRAVO (1953) maintained that in relation to *G. goliath* this last species was characterized by being bigger and by having up to 55 irregularly arranged pterigoides teeth and an open parietal foramen. However, the fact is that, apart from the types used in these two descriptions, until now on Tenerife only the remains of lizards with open parietal foramen have been found as in *Gallotia maxima* and with pterigoidal teeth similar in number and arrangement to *Gallotia goliath*.

The interpretation of the pterigoides of the form described by Bravo may be made if we study the biggest current living specimen of all the lizards: *G. stehlini* on Gran Canaria. In most of the individuals of this species the pterigoides teeth are arranged in one row (Figure6). However, there is a little studied phenomenon that consists of excessive and rapid growth in some males. These individuals become very big and present a large number of teeth on the pterigoides arranged around an irregularly shaped mound in exactly the same way as occurs in the holotype of *Gallotia maxima*.

All the above, and the fact that the parietal of the type of *Gallotia goliath* was broken at the level of the foramen suggests that *Gallotia maxima* and *Gallotia goliath* would, in fact, be the same species and that *maxima* should be considered as synonymous with *goliath*. This observation had already been made by GASC (1971) who added that *G. goliath* could be a giant form of *G. simonyi* judging by the similar morphology and by the large quantity of spongy tissue in the bones of the larger specimens. CASTILLA *et al* (1994) recently corroborated this hypothesis when they described the finding of mummified remains of *G. goliath*.

Gallotia goliath individuals in the North of Tenerife and El Hierro generally present two rows of pterigoides teeth, one usually being twice as long as the other although, in some cases, both are equal. In the South of Tenerife the morphology and arrangement of the pterigoides teeth is more varied as, in addition to the cases described, some smaller individuals have only one row.

On La Palma they all present two short rows of teeth on each pterigoides (Figure 6), including in the smaller individuals. On the other hand, on La Gomera almost all the pterigoides found presented a single row, including those whose estimated head-tail length exceeded 45 cm. Some specimens on the latter island had a second row made up of a few teeth as in the larger *Gallotia simonyi* specimens from the Caserio de Guinea (lizards eaten by the El Hierro aborigines).

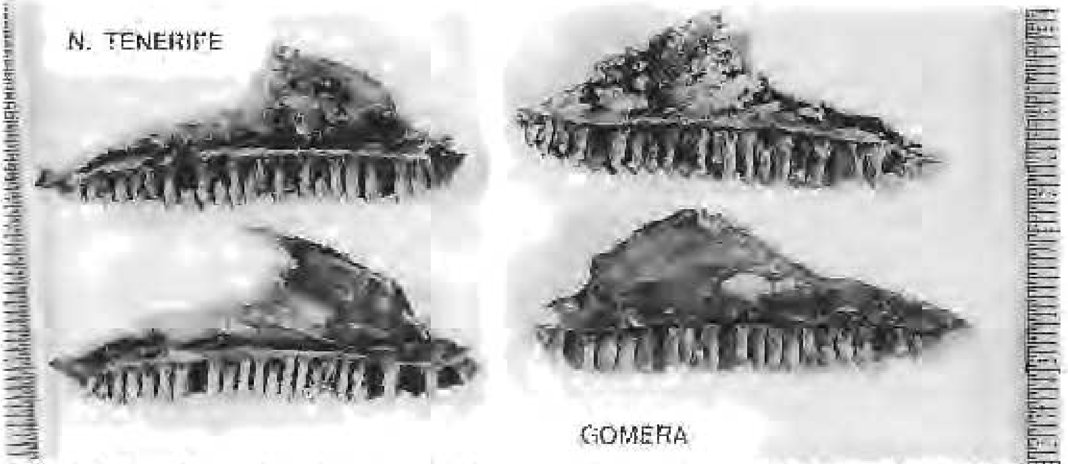

Figure 5: On the right, maxiliars of *Gallotia goliath* from La Gomera (robust); on the left, maxiliares of the same species from deposits in the Cueva de la Arena (North of Tenerife).

THE COLONIZATION, ADAPTIVE RADIATION AND INTRAINSULAR EVOLUTION PROCESSES.

Some authors have hypothesized about island colonization processes in the Canary Islands. For example, BISCHOFF & MAYER (1991), using immunological data, suggest that the large eastern island (Lanzarote and Fuerteventura and islets) must have been the first to be colonized from the continent by a pre-*Gallotia*. Subsequently and as a result of this, Gran Canaria would have been colonized by a pre-*stehlini*, the primitive island of Anaga (North of Tenerife) by a common precursor of *Gallotia galloti* and of *G. simonyi*. After this, the lizards of Anaga colonized the island of Teno (southwest of Tenerife) and with the later union of Teno and Anaga into the large island of Tenerife the conditions were right for both forms to mix, and they are now differentiated in the subspecies of North and South Tenerife (*G. g. galloti* and *G. g. eisentrauti*).

These hypotheses are merely speculations based more or less on some kind of evidence, but, even so, they do not explain the origin of *Gallotia goliath* in the strict sense or the *goliath-simonyi-galloti* relationship in the wider sense. Perhaps an approximation to what occurred at that time should consist of exposing the facts that exist today as a result of the colonization of the young volcanic ecosystems or "malpaises" on islands that are not very complex ecologically such as Lanzarote and Fuerteventura.

In the Canary Islands in the last few thousand years vulcanism has caused eruptions of different magnitudes on almost all the islands. From an ecological point of view, the characteristics of these recently created neo-systems known as "malpaises" vary according to geographical location due to the effects of altitude and island geomorphology. The degree and speed of colonization fundamentally depend on climate, type of volcanic soil and time, in that order of importance.

The most easterly islands of the Canary Island archipelago are not very high above sea level. Fuerteventura, in particular, is 807 m and Lanzarote only 670 m above sea level. The slight elevation and proximity to the Sahara desert coast (about 100 kms away) explains why rain is very scarce, and also why the moisture-laden trade winds do not release it on these islands, unlike on the rest of the islands. As a result, on Lanzarote and Fuerteventura an arid ecosystem almost always prevails with very little diversity in both plant and animal populations. In this situation, the biomass is concentrated in the existing "malpaises" and maximum levels of biological diversity and population density are reached there (LOPEZ JURADO & MATEO, 1992).

The *Gallotia atlantica* populations which colonize the young volcanic ecosystems (malpaises) undergo a rapid increase in body measurements and weight as well as a change in other phenotype characteristics (CASTROVIEJO *et al.* 1985; LOPEZ JURADO & MATEO, 1992).

Average body measurements vary from about 55 mm for males from Montaña Clara and Lobos to almost 90 mm for males from the La Corona "malpais" on Lanzarote with weights of between

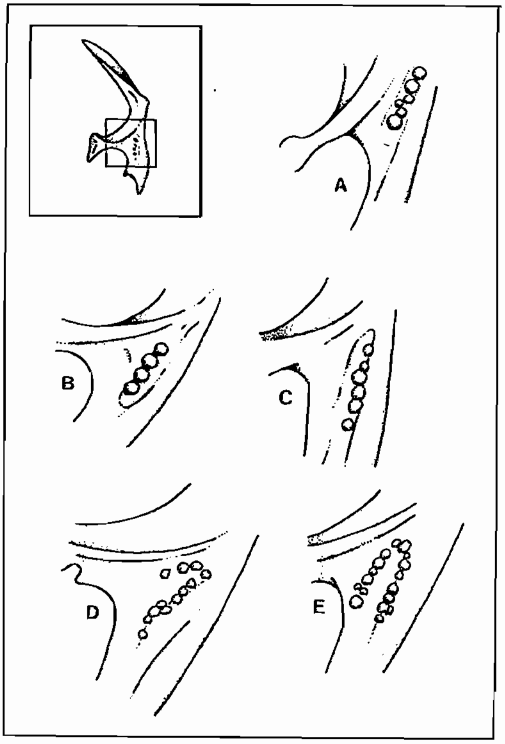

Figure 6: Arrangement of the teeth on the Pterigoides bone (inside the square). A. *Gallotia atlantica* from Fuerteventura, just one row. B. *Gallotia galloti* from Tenerife, just one row (*Gallotia caesaris* presents a similar arrangement).

7 and 35 grammes.

In addition, on Lanzarote and Fuerteventura several phenotypes associated with this species exist. The main difference between them is related to body size and the size, position and colour of the lateral spots (LÓPEZ JURADO, MATEO & GENIEZ, 1995). One of them consists of two rows of small round light green patches that generally extend from the scapular region to the inguinal. It is present both in juveniles and in males and females of all the populations on Lanzarote, Fuerteventura and the islets.

There is a second phenotype in which these green circular marks become bigger until sometimes they form a large green mark which extends along both flanks and even reaches the stomach, dorsolateral parts, limbs and lips. The third presents, in a similar way to the first, two rows of small blue spots. The fourth consists of a much more irregular series of spots (sometimes just one in the scapular region) often joined up in a large dorso-lateral patch. The spots or patches are blue.

The most important thing is that the second and fourth phenotypes are only present in the "giant" males of the *G. atlantica* populations living in young volcanic ecosystems (to the northeast and west) of Lanzarote. It has not been observed in any recent hatchling, juvenile, subadult or female; only in some (not all) adult males of the two abovementioned populations. It is, therefore, a character linked to sex and size.

In fact, the taxa described (See CASTROVIEJO *et al*, 1985) constitute more or less the extremes of a clinal variation with a strong adaptive component for the lizard populations on Lanzarote island. The data from our own observations allows us to confirm that the maximum values for size are reached in young volcanic ecosystems and the lowest in areas that are geologically much older with few resources. Moreover, in volcanic areas, recent hatchlings are always bigger (LÓPEZ JURADO & MATEO, 1992).

Between both ecosystems, which can be considered extremes, there exist intermediate situations that are always linked to specific microclimatic conditions in such a way that nowadays in the *Gallotia atlantica* populations on Lanzarote one can see a true adaptive radiation as regards average lizard size, which is manifested throughout the old and recent volcanic ecosystems and through large expanses of sand and clay terrain dispersed over the whole island.

In this respect, the high correlation existing between *G. goliath* fossil remains and their physical location in young volcanic terrain should not be forgotten.

The clinal characteristics of the distribution of lizard populations on Lanzarote are in stark contrast to the monotony on an ecologically similar island such as Fuerteventura (CASTROVIEJO *et al*, 1985; BISCHOFF, 1985). Apparently, no great clinal variation as regards the characters described above (size, weight and phenotype) exists there despite the fact that different microclimatic situations, including young volcanic ecosystems, also exist.

What then causes the differences in patterns of morphological variation to be so pronounced in similar ecological environments? A close analysis of both islands indicates that in biological terms the only important difference consists of the absence on Lanzarote and adjacent islets of the skink *Chalcides simonyi*.

This omnivorous lizard can weigh 65 grammes and is especially numerous in the young volcanic ecosystems of Fuerteventura.

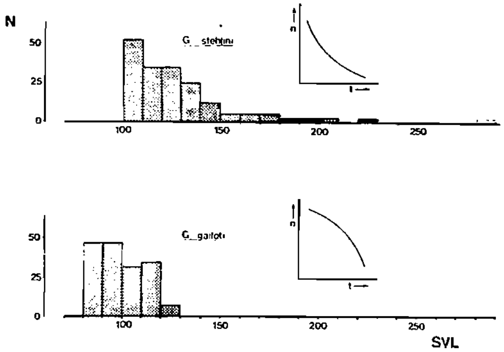

Figure 7: Size structure (frequency histograms) of the *Gallotia stehlini* adults in the Barranco del Taurito population (South of Gran Canaria) (above), and of *Gallotia galloti* adults on San Andrés (North of Tenerife).

On the eastern islands there is also a native species of insectivorous mammal (*Crocidura canariensis*) with very high population densities in the "malpaises" where there are more arthropods available. Outside of the "malpaises" it is only present on the islets of Montaña Clara and Lobos where the density of invertebrate prey is very low. On the former, the shrews feed on *Gallotia atlantica* and Tarentola angustimentalis which they paralyze beforehand with their poisonous glands (LÓPEZ JURADO & MATEO, 1995) (Figure7). On this islet adult *G. atlantica* are by far the smallest sizes for the species (See CASTROVIEJO *et al*, 1985 & LÓPEZ JURADO & MATEO, 1992).

In this way, competition and predation appear to be important regulatory elements in lizard growth on these islands.

In short, it can be said that the adaptive radiation of the Lanzarote lizards is, without doubt, a direct result of the appearance of volcanic phenomena, and responds to subtle and different environmental stimulae. It manifests itself due to the peculiarties of the most extreme ecosystems and fundamentally the phenomena of predation and competition at work there.

Consequently, the neo-volcanic character of the "malpaises" and their geological recentness define the young nature of the lizard populations there, which must of necessity be descendents of the populations located on the periphery. This indicates that the selection pressures undergone by these animals underwent a very rapid process of morphological change, increasing the intersexual differences already manifested by the original population. Therefore, it seems clear that the "malpaises" do not actually constitute a barrier, but simply a new ecosystem to be colonized.

The problem lies in the fact that the temporary persistence of the "malpaises" as differentiated ecosystems does not, in general, appear to be enough to complete the speciation process whose first stage of development takes place there. A generalized and well-distributed situation at the time of the volcanic events of this nature, which is not an unusual hypothesis in the context of volcanic islands like the Canaries, could, however, complete that process.

THE PROCESSES INVOLVED IN SPECIES SUBSTITUTION

Over the last 35,000 years at least on the eastern islands (Lanzarote and Fuerteventura) there has only existed one species of small lizard without any important variations in indivdual size or any other character having been noticed (MICHAUX *et al*, 1991). On the other hand, over the last 2,000 years on Gran Canaria no extinctions have been recorded but the size of the biggest individuals has decreased considerably (MATEO & LÓPEZ JURADO, 1992), thus adapting to a process that appears to have been common in various island areas during the Holocene (PREGILL, 1986).

Some of the 4,000 year old remains found in deposits in Gran Canaria correspond to individuals whose estimated head-tail length was over 35 cm. However, in deposits subsequent to the arrival of people (approximately 2,000 years ago), or between present day wild specimens it is very rare to find any over 25 cm SVL (two cases of captive lizards measuring 28 cm SVL are known - Figure 4).

However, on the rest of the western islands the situation was very different: all the large lizards became extinct, or are in a critical situation, as on El Hierro with *Gallotia simonyi* where there are less than 500 in the Wild (MACHADO, 1985). It would be meet to ask why the large specimens have disappeared or almost disappeared on the western islands while on Gran Canaria *Gallotia stehlini* can be found in almost every part of the island in densities that sometimes exceed 400 adults per hectare (unpublished data).

The answer appears to lie in the fact that in the western islands there are at least two species of lizard living at the same time, while in Gran

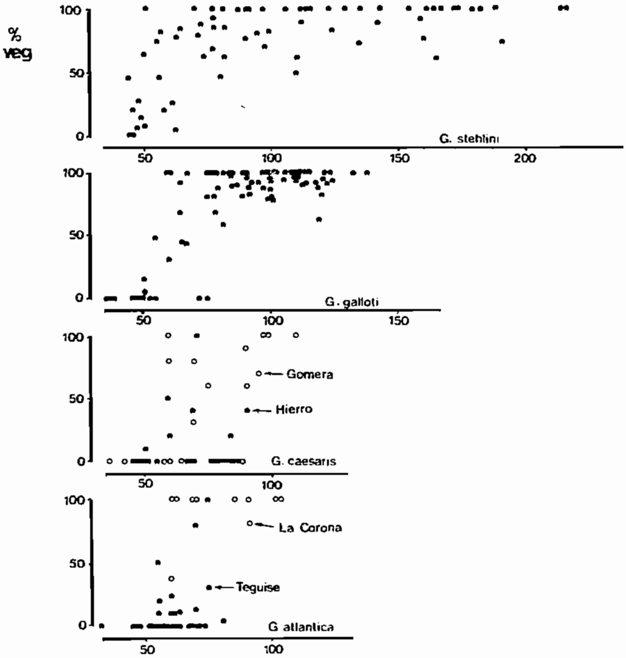

Figure 8: Percentage of vegetable matter in the total dry weight of the stomach contents of *Gallotia stehlini* specimens (several locations on Gran Canaria). *G. galloti* (San Andrés, Tenerife). *G. caesaris* (black circles: Parador of EL Hierro: white circles: Playa Santiago on Gomera). and *G. atlantica* (black circles: Teguise: white circles: La Corona "malpais").

Canaria there was only one with very special characteristics.

Figure 7 shows the differences in adult size between a *G. stehlini* population from the South of Gran Canaria and a *Gallotia Gallotia* one from the northeast of Tenerife. This confirms that most of the *Gallotia stehlini* lizards scarcely reach 13 cm SVL as in *G. galloti*, but some are very different from the average values and sometimes reaching 22 cm STL. The latter have a more vegetarian diet (Figure9) and make different use of space to the smaller lizards, to all effects, behaving like a different species (Mateo & López Jurado, 1992).

The arrival of people on Gran Canaria apparently gave rise to a reduction in size in the larger lizards due either to selective predation on the latter or through competition with the animals they brought (Mateo & López Jurado, 1992). Nevertheless, this has not affected the demographic viability of the populations since it left a very important part of the breeding population intact: lizards measuring 10 to 15 cm SVL.

The other large species about which we can still obtain data is *Gallotia simonyi*. The few individuals that still remain of this species behave very differently to *G. stehlini* as they only begin to breed after they have reached five when they measure over 15.5 cm SVL and the number of juveniles smaller than this is low (Castenet & Baez, 1989; Machado 1985); although from our own data we deduce that sexual maturity is attained from three years old, at least for animals kept in optimum conditions in captivity.

All this allows us to suppose that the stability of this and other species of giant lizards from the western islands must have been based on the longevity and size of the individuals. The arrival of people on these islands, without doubt, had a very different effect from that on the Gran Canaria lizards since the differences in some breeding parameters such as age and minimum size at maturity and the presence of another smaller lizard of the same genus on these islands, which behaved like a competitor, drove them directly to extinction.

In lizards an increase in body size obviously brings with it some related advantages that have repercussions on the demographic strategy of the population (Stearns, 1984; Sinervo, 1990), or on the trophic physiology of the animal (Pough, 1974), which, in this way, increases the efficiency with which it digests vegetables. However, it also involves some effects that may have a negative consequences: one of them is greater exposure of larger individuals to predation. Until the arrival of people, this effect must not have had serious consequences for the giant Canary Island lizards as there were no land predators on the islands and the birds of prey (*Milvus milvus, Buteo buteo and Falco tinnunculus*) do not appear to have been great specialists in prey that can weigh more than 4 kilograms. With the arrival of people and their domestic animals, their large size must have become a serious problem.

The three species of surviving large Canary Island lizard present diets with an important vegetarian component (See Machado, 1985, and Figure 8). On the other hand, both *Gallotia* *atlantica* and *Gallotia caesaris* (Gomera and Hierro) where the adults are considerably smaller than those on Tenerife and La Palma, even though they are also omnivorous, prefer insects. However, within this framework there is a very interesting fact: the lizard population that lives in the La Corona "malpais" on Lanzarote, which, as we have seen, underwent a rapid growth process, reproduces the feeding characteristics described for large and medium-sized species since the individuals become vegetarian to a great degree in an environment like the "malpaises" where the conditions particularly favour it.

The giant extinct lizards like *Gallotia goliath* or the big *Gallotia stehlini* of the La Aldea deposit (See LÓPEZ JURADO, 1985; MATEO & LÓPEZ JURADO, 1992), were associated, at least at the beginning, with especially favourable environments (= differentially wet areas) such as the "malpaises" or the bottom of ravines, but they were philogenetically very close to other populations of smaller lizards (perhaps what is today known as *Gallotia simonyi*), as occurs nowadays with *Gallotia atlantica*. According to this model, before people arrived on the western islands, there existed on all of them a varied mosaic of populations with lizards of different sizes and with equally different densities and geographical distributions.

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Molecular data on the phylogeny of African vipers -preliminary results-

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Abstract: Immunological blood serum albumin comparisons were carried out to investigate the phylogenetic relationships of the main groups of African viperine snakes. A total of 17 species from eight different genera were tested. Antisera were produced against the albumins of 16 taxa. To construct dendrograms, the Fitch-Margoliash algorithm and hierarchical cluster analysis were used. With the exception of *Echis*, both dendrogram patterns were congruent. The resulting phylogeny is: (((((*Bitis atropos* (*Bitis caudalis*, *Bitis. cornuta*)) *Bitis arietans*) (*Bitis gabonica*, *Bitis nasicornis*)) (((*Atheris nitschei*, *Atheris squamiger*) "*Atheris*" *superciliaris*) ((*Cerastes cerastes*, *Cerastes vipera*) (*Macrovipera*, *Daboia*))) *Causus*) *Bouleugerina*). In the Fitch-Margoliash dendrogram *Echis* is the sister group of the *Cerastes-Macrovipera-Daboia* group, following the cluster analysis dendrogram *Echis* is the sistergroup of *Cerastes* only.

Key words: African vipers, blood serum albumins, precipitin tests, phylogeny.

INTRODUCTION

African vipers, composed of the genera *Adenorhinos*, *Atheris*, *Bitis*, *Causus*, *Cerastes*, *Echis*, *Macrovipera* and *Vipera* are a very diverse group, including giant species such as *Bitis gabonica*, which may reach two meters in total length and dwarf species such as *Bitis schneideri*, the smallest viper known with an adult length of less than 30 cm. Some species have adapted to very different habitats, for example the sand dunes of deserts like *Bitis peringueyi* and *Cerastes vipera*, the trees and bushes of tropical rain forests like the arboreal *Atheris* species. Some are living on the bottom of the forests in leaf litter, perfectly camouflaged by their colour pattern as *Bitis gabonica* and *nasicornis*, others are rock dwellers under semidesert conditions as *Bitis xeropaga* and often *Cerastes cerastes* and *Echis* species, and others are inhabitants of swamps as some *Causus* species and "*Atheris*" *superciliaris*.

Morphologically, it includes very conservative species like the members of the genus *Causus*, showing an almost colubroid head scalation and round pupils and species of the genera *Cerastes* and *Echis* with strong modifications of the lateral scales together with a special defensive behaviour considered as highly derived.

A number of described species are extremely rare and known only from a very limited number of specimens, namely the monotypic genus *Adenorhinos barbouri*, *Bitis parviocula*, *B. heraldica*, *Atheris desaixi* and *A. katangensis*. Only recently, a still undescribed large species of *Bitis* turned up (BÖHME, 1990).

Despite the high interest African vipers have attracted throughout time, their phylogenetic relationships are still not satisfactorily resolved.

MARX & RABB (1965) investigated viperine snakes using internal and external morphological characters. They excluded the species *barbouri* from *Atheris*, transferring it to a new monotypic genus *Adenorhinos*, and transfered *superciliaris* and *hindii* from *Bitis* and *Vipera*, respectively, to *Atheris*. *Adenorhinos*, *Atheris* and *Bitis* were found to be a monophyletic entity whereas *Cerastes* and *Echis* were grouped together with Eurasian vipers.

GROOMBRIDGE (1980) in his extensive cladistic analysis of morphological characters of viperine snakes concluded that *Bitis*, *Atheris*, *Cerastes* and *Echis* are monophyletic. However, he stated that this arrangement is not permitted by a robust cladistic hypothesis but on balance seems to be most likely. Supported by his results he mentioned, that placing *barbouri* in a separate genus and treating *superciliaris* and *hindii* as members of *Atheris* is inconsistent, since he found *barbouri* to be the sister taxon of the true arboreal *Atheris*, whereas *superciliaris* and *hindii* are monophyletic with the former group but earlier side branches. He suggested excluding the problematic species *superciliaris* and *hindii* from *Atheris*. After the completion of his study he noted that the phylogenetic relation between *Echis* and *Cerastes* and between them and the other Viperinae remained as major question for future investigations.

ASHE & MARX (1988) tried to resolve the phylogeny of viperine snakes using a large set of characters analysed by a computer program based on the Wagner tree algorithm. Their work resulted in a phylogeny very different in many aspects to that found by GROOMBRIDGE or believed by most other scientists.

The intention of our study is to test the different hypotheses of African viper phylogeny using a different methodological approach, namely immunological blood serum albumin comparisons, and to add our results to the results of the former studies with the aim to bring light on some aspects of viperine taxa relationships.

MATERIALS AND METHODS

To obtain immunological distances (IDs) between blood serum albumins of the involved taxa we used quantitative precipitin tests (JOGER, 1984). Antisera were produced against albumins of all species listed below except *Echis leucogaster*. If more than one locality is mentioned specimens used for antisera originated from the locality marked with an asterisk. We used blood samples from the following species: *Bitis arietans* (Togo*, Rwanda, Namibia, South Africa), *B. nasicornis* (Togo*, Zaire), *B. gabonica* (Togo*, Zaire, South Africa), *B. atropos* (South Africa), *B. cornuta* (South Africa*, Namibia), *B. caudalis* (South Africa*, Namibia), *Atheris nitschei* (Rwanda), *A. squamigera* (Togo), *"A." superciliaris* (Malawi), *Echis cf. ocellatus* (West Africa, exact origin unknown), *E. leucogaster* (Algeria), *Cerastes cerastes* (Morocco*, Egypt, Jordan*), *C. vipera* (Morocco*, Egypt*), *Macrovipera deserti* (Tunisia), *Daboia russelli* (Pakistan), *Causus rhombeatus* (South Africa), *Causus resimus* (Burundi) and *Boulengerina annulata* (unknown locality). For albumin isolation we used preparative vertical discontinuous PAG electrophoresis. In *B. arietans* two electrophoretically distinct morphs of albumin were found and a monospecific antiserum against each was produced. Later precipitin tests showed no significant differences in the immunological reaction against heterologous albumins. Afterwards the ID values of the two antisera were averaged. With the exception of *A. squamigera* and *C. resimus* (only antiserum from one rabbit each) two or three rabbits were used to produce antiserum against each albumin. Pooling in reciprocal quantities relative to the measured immunological reactivity was performed for all other antisera against albumin from one snake taxon (species or local population in the *Cerastes* species) when more than one rabbit was used for antiserum production.

Each ID value in Table 1. represents a single or the mean of two measurements per antiserum and albumin. Because we were limited in quantity of blood serum samples of some species we used specimens from different localities in heterologous tests if necessary. *Echis* IDs in heterologous tests are either *E. cf. ocellatus*, *E. leucogaster* or average values of both. *Causus* antisera IDs are values obtained from both *C. rhombeatus* and *C. resimus* antisera. *Cerastes cerastes* and *C. vipera* antisera IDs are values measured with antisera produced against albumins of specimens from different localities (see above species account). The data were corrected for non random elements using the method described by SARICH & CRONIN (1976). As value for reciprocity we calculated the "% nonreciprocity" (SARICH & CRONIN 1976).

For dendrogram construction we used the Fitch-Margoliash method (FITCH computer program in the PHYLIP package by J. FELSENSTEIN, 1988) and hierarchical cluster analysis. For the later IDs were corrected for relative rates using *Causus* as an outgroup. F-values were calculated as goodness of fit parameters between input IDs and distances represented by the dendrograms (PRAGER & WILSON, 1976).

RESULTS AND DISCUSSION

In Table 1. reciprocal ID values are presented. The calculated "% nonreciprocity" for the original data is 14.8. After correction for non random elements the value dropped to 12.2. Minimum/maximum correction factors of 0.82/1.27 are within the limits found in other studies (HERRMANN 1990). In Figure 1 the dendrogram constructed with the Fitch-Margoliash method is shown. The F-value is 7.84. For hierarchical cluster analysis it is necessary to correct the ID values for different evolutionary rates since a similar rate of change in the albumins (i.e. amino acid substitutions) is implied by that method. The calculated relative rates using *Causus* as outgroup are given in Table 1. The albumin of *M. deserti* (1.31) and to a somewhat lower degree of *C. cerastes* (1.15) were found to be relatively conservative. Conversely the arboreal *Atheris* species *nitschei* (0.82) and *squamigera* (0.86) have relatively fast evolving albumins. The hierarchical cluster analysis dendrogram with an F-value of 11.66 is shown in Figure 2.

The phylogenetic pattern given by the two calculated dendrograms is congruent with the exception of the branch leading to *Echis*. Using the Fitch-Margoliash method *Cerastes* and the

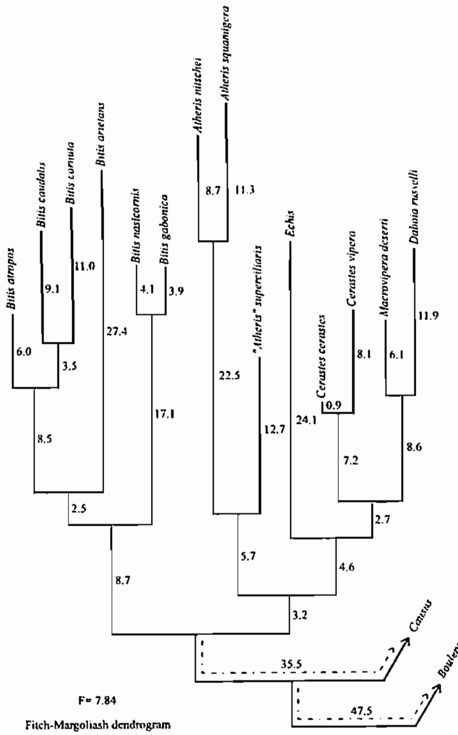

Figure 1: Dendrogram constructed using the fitch-margoliash method. The ID values indicate distances between branching points.

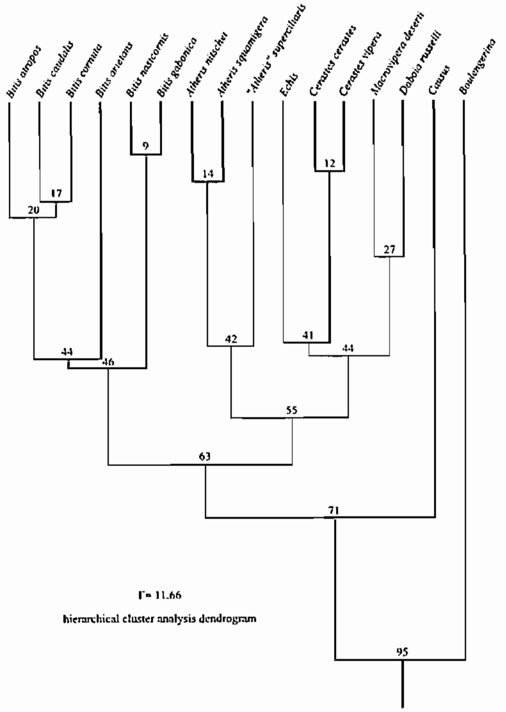

Figure 2: Dendrogram resulting from using hierarchical cluster analysis for relative rate corrected reciprocal IDs.

Eurasian vipers, represented here by *Macrovipera* and *Daboia,* are monophyletic with *Echis* as the sister group of them.

The cluster analysis tree results in a monophyletic group of *Echis* and *Cerastes,* with the Eurasian vipers being their sister group. From a morphological and behavioural viewpoint one could argue that the character complex of lateral scales with serrated keels combined with the very special defensive behaviour shown by *Echis* and *Cerastes* is highly derived and a synapomorphy characterising the monophyly of the two genera. GROOMBRIDGE pointed out that both are quite different regarding other morphological characters. The above mentioned character complex is observed to exist in some species of arboreal *Atheris* as well, although less perfectly developed .

The later view would support the opinion, that the mentioned characters are symplesiomorhic or convergent which makes the position of *Echis* shown Figure 1 (Fitch-Margoliash method) probable. In conclusion both arrangements for *Echis* are equally possible and neither one is rejectable because of available morphological or molecular data. Input/output data differences given in Table 2. show the highest differences where *Echis, Cerastes* and *Daboia* albumins are involved. Additional precipitin tests are needed to reduce methodological noise to clarify the relations of *Cerastes* and *Echis*.

Causus is situated at the basis of the viperine snakes tested herein and very early the remaining genera split into two main clades. This is in contrast to MARX & RABB as well as to GROOMBRIDGE who all considered *Bitis* and *Atheris* (sensu lato) to compose a monophyletic group. However, GROOMBRIDGE suggested a close relationship of the *Atheris* group and the *Echis-Cerastes* group was possible. In his interpretation this resulted in including *Echis* and *Cerastes* into the African clade together with *Bitis* and *Atheris* (s.l.). *Bitis* itself is composed of three main entities. These are (1) the tropical

antisera:	*Bari*	*Bnas*	*Bgab*	*Batr*	*Bcor*	*Bcau*	*Asup*	*Anit*	*Asqu*	*Ccer*	*Cvip*	*Eoce*	*Mdes*	*Drus*	*Caus*	*Boul*
albumins:																
B. arietans	0	51	42	36	48	41	5	77	77	68	62	78	74	72	77	93
B. nasicornis	49	0	7	31	36	38	50	66	78	54	53	57	51	63	70	93
B. gabonica	47	10	0	24	44	50	51	64	78	58	52	58	45	65	75	93
B. atropos	41	49	35	0	2	26	33	68	73	59	57	60	47	66	71	95
B. cornuta	54	53	65	28	0	32	47	71	78	43	44	74	38	68	80	89
B. caudalis	38	62	48	13	8	0	33	79	80	78	75	62	43	61	75	92
"A."superciliaris	59	70	40	51	57	51	0	31	62	48	31	58	14	53	73	89
A. nitschei	73	80	65	58	65	80	41	0	31	61	44	72	51	66	88	92
A. squamigera	73	69	62	67	70	77	52	10	0	66	51	71	46	67	83	92
C. cerastes	54	46	51	22	43	78	36	48	66	0	6	42	7	18	74	81
C. vipera	53	71	56	33	44	75	47	50	63	15	0	51	20	29	63	94
Echis	71	80	65	52	49	62	58	51	46	43	39	0	43	18	79	88
M. deserti	44	71	55	34	52	71	44	59	82	38	39	47	0	24	54	87
D. russelli	55	74	59	38	58	69	52	55	65	52	43	64	8	0	62	90
C. rhombeatus	64	64	67	49	63	72	58	74	84	58	64	79	54	42	0	93
Boulengerina spec.	95	90	91	94	89	92	92	92	94	84	88	92	95	89	88	0

"% nonreciprocity": 14.8"

correction for nonrandom elements

corr. factors:	**1.12**	**0.87**	**1.03**	**1.26**	**1.20**	**0.93**	**1.06**	**1.10**	**0.93**	**0.82**	**1.04**	**0.90**	**1.27**	**1.06**	**0.91**	**1.04**
B. arietans	0	44	43	45	57	38	59	85	72	56	64	70	93	76	70	97
B. nasicornis	55	0	7	39	43	35	53	73	72	44	55	51	64	67	64	97
B. gabonica	53	9	0	30	53	46	54	70	72	48	54	52	57	69	68	97
B. atropos	46	43	36	0	2	24	35	75	68	48	59	54	59	70	65	99
B. cornuta	60	46	67	35	0	30	50	78	72	35	46	67	48	72	73	93
B. caudalis	43	54	49	16	10	0	35	87	74	64	78	56	54	65	68	96
"A." superc.	66	61	41	64	68	47	0	34	58	39	32	52	18	56	66	93
A. nitschei	82	70	67	72	78	74	43	0	29	50	46	65	64	70	80	96
A. squamigera	82	60	64	84	84	72	55	11	0	54	53	64	58	71	76	96
C. cerastes	60	40	53	27	52	72	38	53	61	0	6	38	9	19	67	84
C. vipera	59	62	58	41	53	70	50	55	59	12	0	46	25	31	57	98
Echis	80	70	67	65	59	58	62	56	43	35	41	0	54	19	72	92
M. deserti	49	62	57	42	62	66	47	65	76	31	41	42	0	25	49	90
D. russelli	62	64	61	47	69	64	55	60	60	43	45	58	10	0	56	94
C. rhombeatus	72	56	69	61	75	67	62	81	78	48	67	71	68	45	0	97
Boul. spec.	106	78	94	117	107	85	98	101	87	69	92	83	120	94	80	0

"% nonreciprocity": 12.2

correction for relative rates

corr. factors:	0.93	1.10	0.96	1.05	0.89	0.98	1.03	0.82	0.86	1.15	1.06	0.92	1.13	1.31		
B. arietans	0	45	39	44	48	35	57	65	58	60	64	60	98	93	70	91
B. nasicornis	56	0	8	45	42	38	60	65	68	56	64	52	80	96	75	105
B. gabonica	47	9	0	30	45	44	53	55	59	53	55	46	62	87	70	96
B. atropos	45	49	36	0	2	25	38	64	61	59	66	52	70	96	73	87
B. cornuta	50	45	57	33	0	26	46	57	55	36	43	55	48	84	69	82
B. caudalis	39	58	47	17	8	0	35	70	62	72	81	50	60	83	72	100
"A." superc.	63	69	41	69	63	48	0	29	51	47	35	50	21	76	73	92
A. nitschei	62	63	53	62	57	60	37	0	20	47	40	49	60	75	70	92
A. squam.	65	57	53	75	64	60	49	8	0	54	48	51	56	80	70	100
C. cerastes	65	51	58	33	53	82	45	50	60	0	8	40	11	29	83	104
C. vipera	59	72	59	46	50	72	54	48	54	15	0	45	30	43	65	98
Echis	68	70	59	63	48	52	58	42	34	37	40	0	56	23	71	84
M. deserti	52	77	61	50	63	73	54	60	74	41	49	44	0	38	59	81
D. russelli	75	93	76	65	81	82	74	65	68	64	62	69	15	0	79	95
C. rhomb.	71	66	71	69	72	70	68	71	72	59	76	70	82	62	0	103
Boul. spec.	100	85	93	103	101	90	97	97	90	86	92	90	108	95	86	0

% nonreciprocity": 12.1

Table 1: Reciprocal ID values. Raw data, data corrected for nonrandom elements and data corrected for different rates of amino acid changes are listed. Relative rate corrections were made for columns and rows. Bold values indicate missing IDs compensated by using the reciprocal values.

forest species *nasicornis* and *gabonica*, (2) *arietans* and (3) the dwarf *Bitis* of Southern Africa here represented by *atropos*, *caudalis* and *cornuta*. Taking into account that these three groups are separated by IDs of more then 40 and compared with taxa separated by similar distances as *Echis*, *Cerastes* and the Eurasian vipers, taxonomical consequences might be justified. This is also true for the distance measured between the arboreal *Atheris* species *nitschei* / *squamigera* and *"A." superciliaris* (45 versus 42). Generic status for the species

	Bari	*Bnas*	*Bgab*	*Batr*	*Bcor*	*Bcau*	*Asup*	*Anit*	*Asqu*	*Ccer*	*Cvip*	*Eoce*	*Mdes*	*Drus*	*Caus*
B. arietans		5	3	1	5	7	3	1	1	0	1	1	12	21	0
B. nasicornis	1		0	1	2	2	2	1	0	9	5	2	16	32	0
B. gabonica	2	0		13	5	0	16	9	7	7	6	10	1	19	0
B. atropos	4	3	5		2	1	9	0	5	17	7	5	3	18	0
B. cornuta	9	2	13	2		0	8	6	3	18	16	11	7	20	0
B. caudalis	8	0	3	1	0		21	2	2	14	14	12	4	20	0
"A."superciliaris	3	5	3	3	3	13		9	8	9	10	1	17	20	0
A. nitschei	5	2	1	8	4	9	5		0	6	11	9	5	15	0
A. squamigera	4	7	4	8	1	2	10	0		2	4	12	10	19	0
C. cerastes	1	6	3	6	1	21	5	0	3		0	2	18	3	0
C. vipera	2	4	0	2	7	12	0	9	6	0		2	4	9	0
Echis	4	0	2	2	3	8	10	1	4	2	2		6	2	0
M. deserti	7	8	2	3	4	2	7	6	5	3	3	6		0	0
D. russelli	1	5	8	5	6	2	10	0	1	2	2	8	0		0
C. rhombeatus	3	5	8	4	4	0	7	5	1	4	1	4	7	16	

Table 2: Differences between input and output data for the Fitch-Margoliash dendrogram (lower left) and the hierarchical cluster analysis dendrogram (upper right).

superciliaris is also indicated by morphological evidence (GROOMBRIDGE, 1980).

Since ID values found here between *Macrovipera* and *Daboia* as well as between these and *Echis* and *Cerastes* correspond very well with values found in earlier studies (HERRMANN, *et al.* 1987, 1992a, 1992b) an approximate average evolution rate for blood serum albumins of viperine snakes of two IDs per one million years is proposed. Separation of the main three *Bitis* groups, of the line leading to arboreal *Atheris* and *"A." superciliaris*, and the separation of *Echis*, *Cerastes* and the Eurasian vipers must have taken place in early Miocene. The split up of *Bitis* and the other African vipers and of this whole group and *Causus* seem to be Oligocene events.

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3-D Reconstitution of the brain of *Typhlonectes compressicaudus* (Dumeril and Bibron,1841) (Amphibia, Gymnophiona)

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Abstract: Serial sections 10 µm thick have been realized from a brain of *Typhlonectes compressicaudus* perfused and fixed. After histological coloration about thirty sections are isolated for their morphological importance. Each section is then micrographed and the main elements and the limits of the brain are identified. From each micrograph, a numerization of the different elements is realized with digitalizer. For one section, the numeric points of a same element are joined by the creation of a curve. Each curve is joined to the curve of the previous section and to the following one to realize a transversal meshing which allows the generation of surfaces. Every numerized sections are then combined. The dressing of the surfaces end the phase of reconstitution. The 3D reconstruction can be visualized on different oriented views, new section levels can be defined, morphological data like distances or contact surfaces can be calculated, morphological elements can be isolated and they show their own morphology.

Key words: Brain, Amphibian, Gymnophiona, 3-D Reconstitution.

INTRODUCTION

The brain of *Typhlonectes compressicaudus* presents the typical organization of the brain of Gymnophiona.

The knowledge of the localization of the different parts of the brain and of the inner organization of the cellular groups will be interesting in order to understand the pathway of fiber tracts in three dimensions.

The use of 3-D reconstitution could be interesting to vizualise these organizations like it was made in parasitology (PALLUAULT, 1991 a and b).

HISTOLOGICAL PROCEDURE

Serial sections 10 µm thick have been realized from a brain of *Typhlonectes compressicaudus* transcardiacally perfused and post-fixed with P.A.F.

After histological staining about thirty sections were selected for their morphological importance.

Each section was micrographied and drawn with camera-lucida. Then the main elements and the limits of the brain were identified.

3 - D RECONSTITUTION WITH THE CATIA DASSAULT SYSTEM SOFTWARE

The images were displayed by using an IBM RISC/SYSTEM 6000 with a resolution of 928 x 928 pixels, developing 128 colors.

The program used was "CATIA DASSAULT SYSTEM" (Conception Assistée Tridimentionnelle InterActive), a program developed by DASSAULT for airplane C.A.D.

The cerebral walls, ventricle walls and the grey substance limits were defined on a screen with digitized points (Fig. 1a).

All points were connected to each other by a spline creation (Fig. 1b and c).

All splines (Fig. 1d) were used to create a network (Fig. 1e) used itself to realize a surface (Fig. 1f).

Then, polyedral complex was performed (Fig. 1g).

The reconstitution was ended by the shading of the surfaces (Fig. 1h).

CONCLUSION

The 3D reconstitution of the brain «in toto» can be visualized on different oriented views (Fig. 2a and b), new section levels can be defined (Fig. 2f), morphological data like distances or contact surfaces can be calculated (Fig. 2g and h), morphological elements can be isolated and they show their own morphology (Fig. 2c, d and e).

This technology will make possible a better understanding of the hightly complex brain structure.

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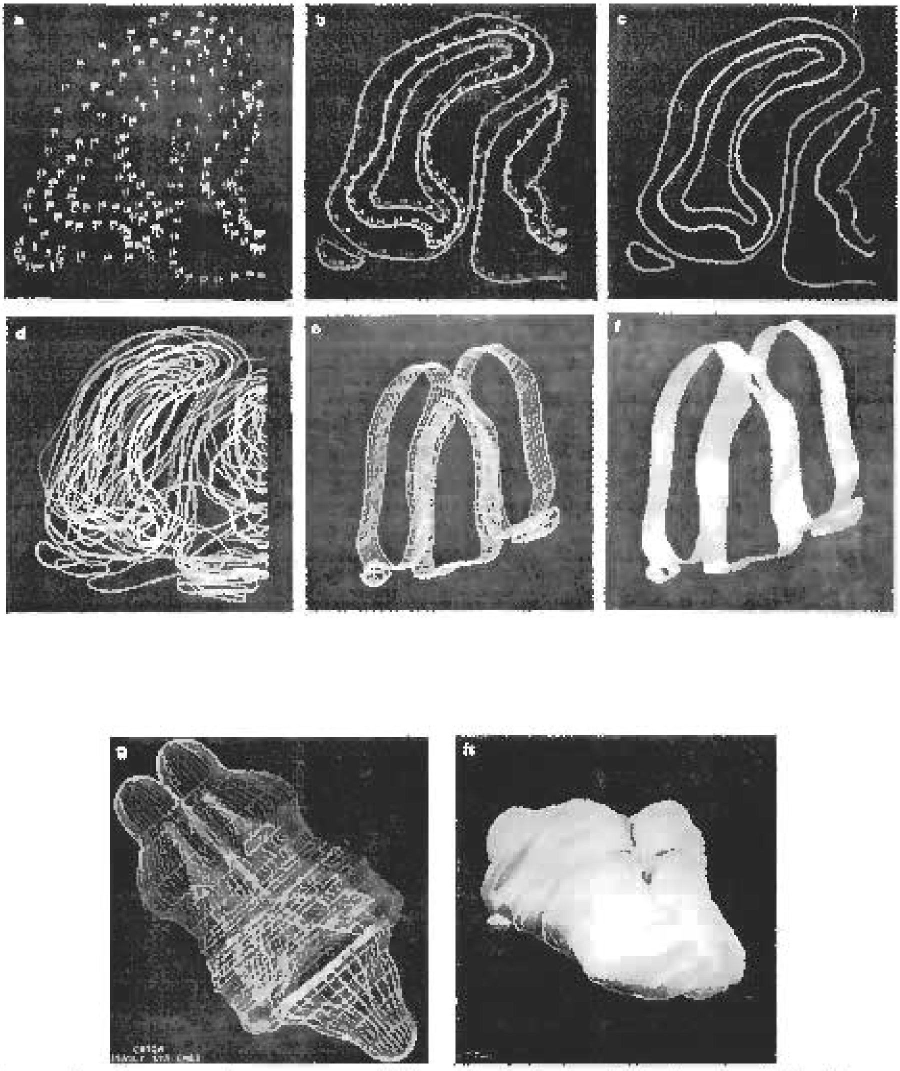

Figure 1: Steps in the computer-aided reconstitution of the brain of *Typhlonectes compressicauda* with the CATIA DASSAULT SYSTEM Software. a) Profiles were aligned by the best fit and entered into the computer using a digitizing tablet. b) Outlines were created. c) The points were erased. d) All the digitized serial sections were combined. e) The surface was created from the network. f) The surface was shaded. g) Each outline was bound to the ones above and below to make a network. h) Many surfaces were elaborated for the reconstitution of the brain.

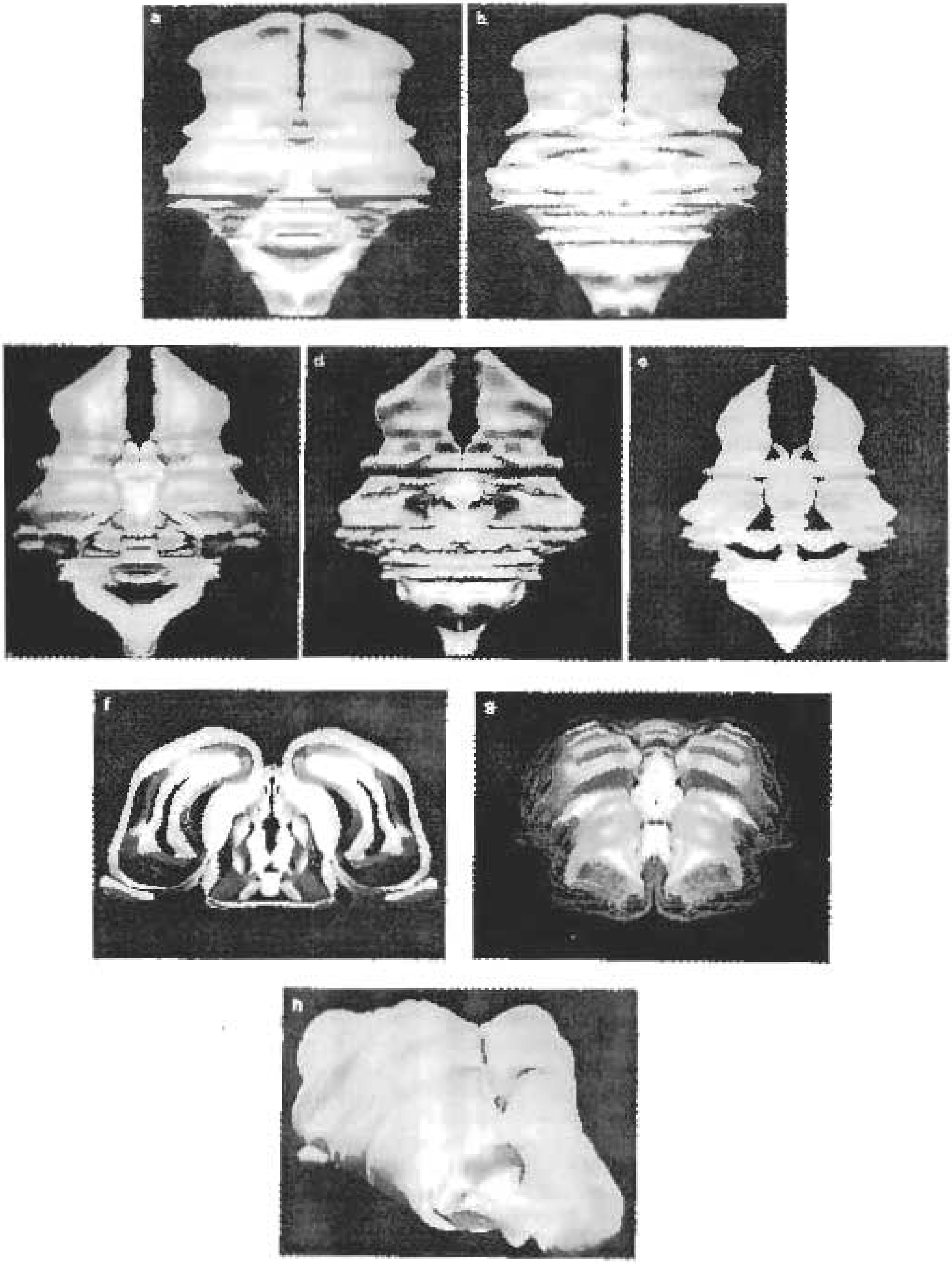

Figure 2: Different structures or surfaces can be artificially visualized. The brain can be visualized "in toto": **a)** Dorsal vue. **b)** Ventral vue. The inner surfaces can be shown: **c)** Internal limits of the grey substance: dorsal vue. **d)** Internal limits of the grey substance: ventral vue. **e)** Internal ventricles: dorsal vue. Artificial representations or orientations can be created: **f)** Artificial thick frontal vue. **g)** Inclined front vue. **h)** 3/4 front vue.

Anatomy and morphology of the brain of *Typhlonectes compressicaudus* (Dumeril & Bibron, 1841) Amphibia, Gymnophiona.

Preliminary stereotaxic Atlas

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Abstract: Sagittal, frontal and horizontal sections were realised on whole skulls and isolated brains of *Typhlonectes compressicaudus*. Different histological stainings were used to evidence the internal structures. Morphologically, the brain of *Typhlonectes compressicaudus* presents the typical elongated aspect of the gymniophona brain due to the large cerebral hemispheres. These telencephalic hemispheres are, anteriorly, prolongated by the globular olfactory lobes. At the limit between the olfactory lobe and the cerebral hemisphere, a globular protuberance corresponding to the accessory olfactory lobe is observed. Posteriorly to the telencephale, the diencephale is observed in ventral view, between the two hemispheres. The diencephale is longitudinally crossed by the third ventricule which is bordered with layers of pericarya constituting the diencephalic structures: - basal hypothalamus. - lateral thalamus. - dorsal thalamus. Dorsally to the third ventricule, the asymetric habenula is observed. It surrounds the impar pineal organ. This habenula, which covers the sagittal habenulary commissure is covered by the paraphysis. Ventrally, the diencephale is, anteriorely, limited by the reduced optic chiasma, and, posteriorly, prolongated by the well developped sagittal hypophysis. The brainstem is continuted dorsally by an large optic tectum and ventrally by the curved medulla oblongata. The brainstem is prolongated by the spinal cord. The internal morphology of the cerebral ventricules is also studied.

Key words: Brain, Amphibian, Gymnophiona, Atlas, Anatomy, *Typhlonectes compressicaudus* .

INTRODUCTION

The architecture of the brain of Gymnophiona is largely unknown. Only few studies have tried to describe the brain of Gymnophiona "in toto" or partly, included in general anatomical study (WALDSCHMIDT, 1887; BURCKHARDT, 1891).

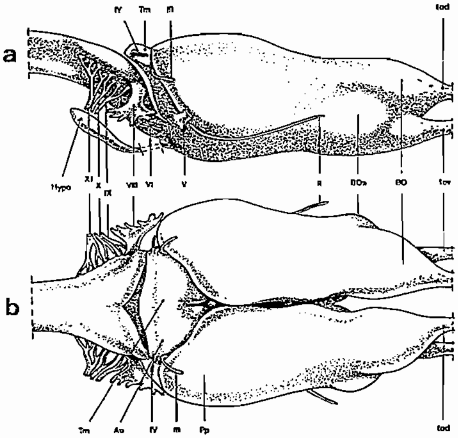

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Figure 1: Draw of the brain of *Typhlonectes compressicaudus* before the histological procedures. **a)** Lateral view. **b)** Dorsal view.

KUHLENBECK (1922) provided a detailed description of the brain of *Hypogeophis rostratus* and NOBLE (1931) realised a comparition between the brain of the three orders of Amphibians. More recently, some studies have tried to describe the cytoarchitecture of the different parts of the brain of Gymnophiona: the Forebrain (KUHLENBECK *et al.*, 1967; KUHLENBECK, 1969), the Diencephalon (WELSCH et al., 1976 ; FRITZSCH et al., 1985; WICHT and HIMSTEDT, 1986), the Mesencephalon (FRITZSCH et al., 1985; WICHT and HIMSTEDT, 1990) or the Hypophysis (KUHLENBECK, 1970 ; ZUBER-VOGELI and DOERR-SCHOTT, 1981).

The perspective of immunocytochemical studies of the brain of Gymnophiona has required the primary realisation of an atlas.

MATERIALS AND METHODS

Typhlonectes compressicaudus is an aquatic Gymnophion from french Guyana. The animals used for this study have been captured during a mission in march 1990 and aclimatized in a tropical room in our laboratory.

Height adults of both sex were used for the realisation of the atlas. The fixation of the brain was realised by transcardiac perfusion of paraformaldehyde-picric acid solution (STEFANINI *et al.*, 1967). The brains were quickly removed from the skull and post-fixed in the same fixative over a night, at 4°C.

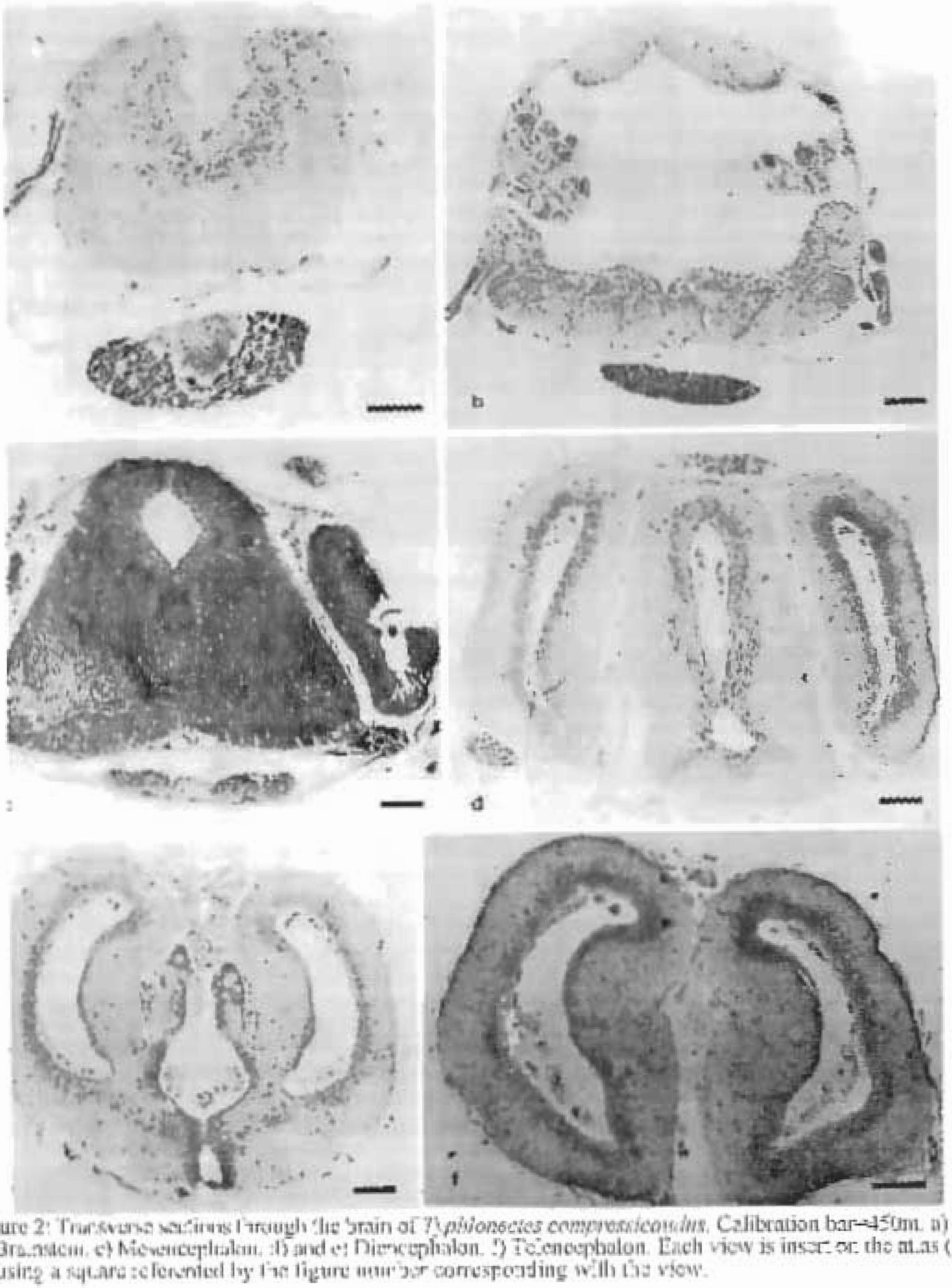
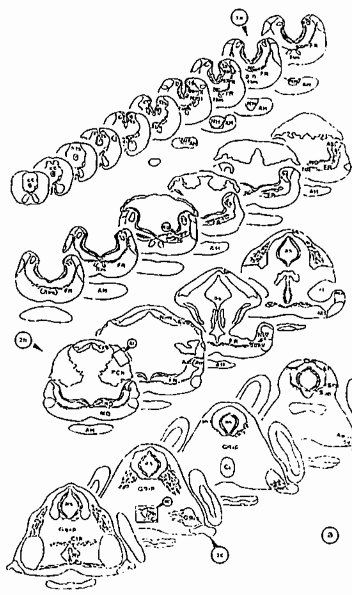


Figure 2: Transverse sections through the brain of *Typhlonectes compressicaudus*. Calibration bar=450m. a) and b) Brainstem. c) Mesencephalon. d) and e) Diencephalon. f) Telencephalon. Each view is insert on the atlas (Fig. 3) using a square referenced by the figure number corresponding with the view.



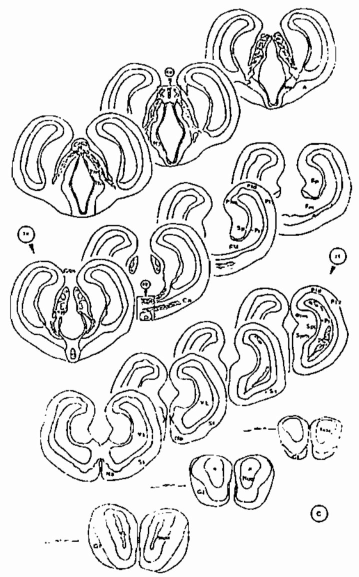


Figure 3 (a, b, c): Camera lucida draws of serial frontal sections through the brain of *Typhlonectes compressicaudus* depecting the topographical organisation of the different parts of the brain. Each section is separated from the next one by 100 m. Figures are arranged from caudal (upper left) to rostral (lower right).

Before the histological procedures, draws of the brain "in toto" were realised (Fig. 1a and 1b).

Then two histological procedures were used:

1) Four brains were embedded in paraffin and frontal, sagittal and horizontal serial sections of 10 m were realised.

2) Four brains were immersed in a solution of 25% of sucrose, pH 7,3 for 24 hours at 4°C.

The brains were then quickly frozen using isopentane cooled by liquid nitrogen. Frontal serial sections of 10 m were realised using a cryostat.

Three histological stainings were used:

- Trichrom of Masson
- Cresyl violet
- Luxol blue

Each frontal section was drawn with camera-lucida.

RESULTS

An atlas was realised using sections which had been taken every 100 m (Fig. 3). Frontal and horizontal sections were used to confirm the organization of the different brain structures.

For the rostral part of the brain, the distance between the sections was differente and was indicated on the draws.

BRAINSTEM (Fig. 2a and 2b)

The posterior part of the brain is made up of the Cerebellum and the Medulla oblongata and is rostrally prolongated by the spinal cord. The Medulla oblongata presents centraly a well demarcated Nucleus raphes (Fig. 4c) and the Formatio reticularis (Fig.4d). The small Cerebellum is made up of a dorsal paramedian prominence and a lateral pair of auriculary lobes which are anterior prolongations of the acousticolateral system of the Medulla.

MESENCEPHALON (Fig. 2c)

The Mesencephalon made up of the dorsal tectum (optic lobes) and the basal tegmentum.

DIENCEPHALON (Fig. 2d and 2e)

The Diencephalon made up of three major parts: Epithalamus, Thalamus and Hypothalamus.

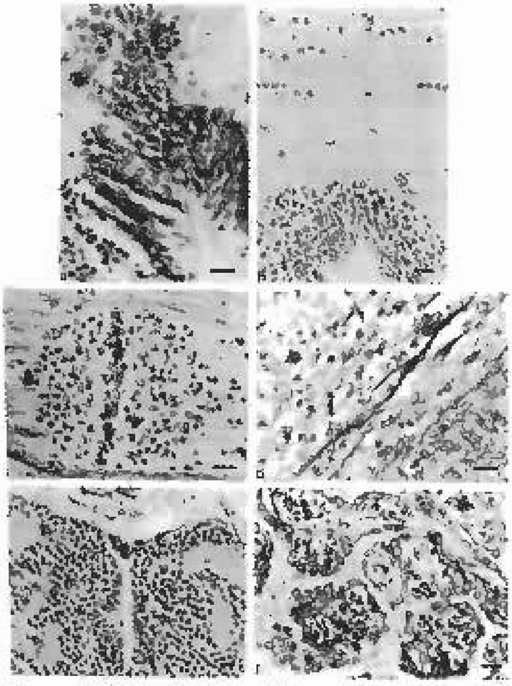


Figure 4: Calibration bar = 30m. a) Pineal organ. b) Commissura pallii and commissura anterior. c) Nucleus raphes. d) Formatio reticularis. e) Habenula. f) Plexus choroïdes.

The Epithalamus contains the Habenula (Fig. 4e), the Plexus choroides (Fig. 4f) which is a vascular invagination in the third ventricle, and a small Epiphysis (pineal organ) (Fig. 4a).

The Thalamus, located in the dorsal part of the Diencephalon, is subdivided into the dorsal Thalamus and ventral Thalamus. Rostrally, the Thalamus is separated from the mesencephalic tectum by the commissura posterior.

The Hypothalamus, localised in the ventral part of the Diencephalon, is divided into two major areas: the praeoptic area and the tuberoinfundibular area. The connections can be observed between the preoptic nucleus and the ventral lobe of the Hypophysis. Anteriorly, the Praeoptic nucleus is separated from the third ventricle by two fibers tracts: the ventral commissura anterior and the dorsal commissura pallii (Fig. 4b).

Dorsally, a large subcommissural organ stretching from the back of pineal organ to the commissura posterior can be observed.

TELENCEPHALON (Fig. 2f)

The Telencephalon made up of two elongated hemispheres, anteriorly prolongated by two olfactory bulbs. Anteriolaterally to each hemisphere, the characteristic accessory olfactory bulb forms a prominence.

CONCLUSION

This study has provided preliminary results. It is clear that additional work will be required (immunocytochemical and fonctional studies) to provide a more complete understanding of the brain of *Typhlonectes compressicaudus*. Moreover, these first observations are totaly in accordance with anterior studies realized on other species of Gymnophiona.

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ABBREVIATIONS

A	Amygdala
Aal	Area acustico-lateralis
AH	Adenohypophysis
Aol	Area octavo-lateralis
as	Aquaductus Sylvii
AsH	Area subhabenularis
Au	Auriculum
BO	Bulbus olfactorius
BOa	Bulbus olfactorius accessorius
Ca	Commissura anterior
cc	canalis centralis
Ccb	Corpus cerebelli
CH	Commissura Habenula
Ci	Commissura intertrigemina
Cp	Commissura posterior
Cpl	Commissura palliii
Cs	Commissura supra optica
Ctm	Commissura tecti mesencephali
Ctp	Commissura tuberculi posterior
E	Epiphysis
Ep	Ependym
flm	Fasciculus longitudinalis medialis
FM	Foramen Monroi
FR	Formatio reticularis
Fr	Fasciculus retroflexus
GgH	Ganglion Habenulae
Ggip	Ganglion interpedonculare
Ggis	Ganglion isthmi
Gl	Glomerula
H	Habenula
Hd	Hypothalamus dorsalis
Hv	Hypothalamus ventralis
Hy	Hypothalamus
Hypo	Hypophysis
I	Infundibulum
lml	Lemmiscus lateralis
MO	Medulla oblongata
MT	Mesencephales tegmentum
Na	Nucleus accumbens
Nc	Nucleus centralis
ND	Nucleus Deiterii
NH	Neurohypophysis
N XII	Nucleus XII
Nm V	Nucleus mot. V
Nm X	Nucleus mot. X
Nmt	Nucleus mesencephalicus trigemi
Noa	Nucleus olfactorius anterior
NPO	Nucleus praeopticus
Nr	Nucleus Raphes
NsV	Nucleus sens. V
Os	Organon subcommissurale
P	Pedunculus
PCh	Plexus choroides
Pld	Pallium dorsalis
Pll	Pallium lateralis
Plm	Pallium medialis
Pp	Polus posterior
Prl	Prominentia lateralis

pt	Praetectum
ri	recessus infundibuli
rl	recessus lateralis
rp	recessus praeopticus
Sap	Stratum album periventriculare
sd	sulcus diencephalus dorsalis
Sgp	Stratum griseum periventriculare
sm	sulcus diencephalus medius
Sm	Stria medullaris
Sp	Septum
Spl	Septum lateralis
Spm	Septum medialis
St	Striatum
sv	sulcus diencephalus ventralis
Thv	Thalamus ventralis
Thd	Thalamus dorsalis
Tis	Tectum isthmi
Tm	Tectum mesencephali
tod	tractus olfactorius dorsalis
Top	Tectum opticum
tov	tractus olfatorius ventralis
ts	tractus solitarius
VL	Ventriculus lateralis

Embryonic development of pineal organ in *Typhlonectes compressicaudus*, a viviparous Gymnophionan Amphibia.

Histological study

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Abstract: The embryonic development of the pineal organ of one Gymnophionan (*Typhlonectes compressicaudus*) has been studied. Morphological and cellular aspects of the pineal organ have been investigated in twenty embryos and four adults. The pinal organ appeared at the stage 26 as an evagination of the diencephalon roof. A lumen in connection with the third ventricle appeared (stage 27) and developped until the communication betwen lumen and the third ventricle disappeared (stage 31). Between stage 26 and stage 31, pineal cells became differenciated in photoreceptor-like cells and ganglion-like cells or intercalary-like cells. This cytological structure seemed to be a photoreceptor structure. After stage 31, the photoreceptor-like cells regressed and the structure changed complctly into a glandular structure. This correspond to the adult structure. This change could be in relation with the metamorphosis. These results suggest that the development of the pineal organ in *Typhlonectes compressicaudus* occurs like Caudata. Now it would be interesting to perform ultrastructural studies to confirm these observations.

Key words : Pineal organ, Embryonic development, Amphibia, Gymnophiona, *Typhlonectes compressicaudus*.

INTRODUCTION

Morphological and physiological aspects of the pineal complex of Amphibians have been studied mainly in Anura (EAKIN, 1961; OKSCHE and VON HARNACK, 1963, 1965; WAKE et al, 1974; KORF et al, 1981).

Fewer investigations have dealt with the pineal complex of Urodela (KELLY, 1962, 1965; KELLY and SMITH, 1964; HENDRICKSON and KELLY, 1969; KORF, 1976; HARTWIG and KORF, 1978).

Generally, the main difference between Anura and Urodela was shown as the lack of the frontal organ in Urodela (KAPPERS, 1965; KORF, 1976; HARTWIG and KORF, 1978; KIKUCHI and AOKI, 1984). However in one arcahaïc Urodela family (Hynobiidae), the embryonic development of the pineal complex has shown a frontal organ homologue (BJERRING, 1975; TAKAHAMA, 1992).

On pineal complex of Gymnophiona, no recent study has been published. No mention existed on the structure or the function of this complex. At the beginnig of the 20th century, several autors considered that the pineal organ of Gymnophiona looked like the one of Urodela. No observation has been confirmed. That is why we have observed the embryonic development of the pineal complex of one aquatic Gymnophiona, *Typhlonectes compressicaudus*.

MATERIALS AND METHODS

One male and one female were fixed by transcardiac perfusion with paraformaldehyde-picric acid solution (STEFANINI et al, 1967). Two other adults were fixed by immersion in Bouin solution. The brain of each animal was removed from the skull and post-fixed in the same fixative solution.

Twenty seven embryos of *Typhlonectes compressicaudus* were studied. These embryos have been taken in uterus of females captured in french Guyana. They have been fixed by immersion in Bouin solution. The embryonic stages have been studied according to the Sammouri's table (SAMMOURI et al, 1990).

All specimens were deshydrated and embedded in paraffin. For histological studies, frontal, sagital and horizontal serial sections were staining by different technics : Masson trichrome stain, azan, hemalun-eosin, cresyl violet and luxol blue.

RESULTS

Adult structure -fig 1-

The pineal organ of *Typhlonectes compressicaudus* looked like an invert pear. It was a dorsal impar evagination of the roof of diencephalon; localized in the medial plane. It standed on the ganglia habenulae. Both were covered by paraphysis.

A small lumen was present. The cells were globulous including a voluminous and round nucleus.

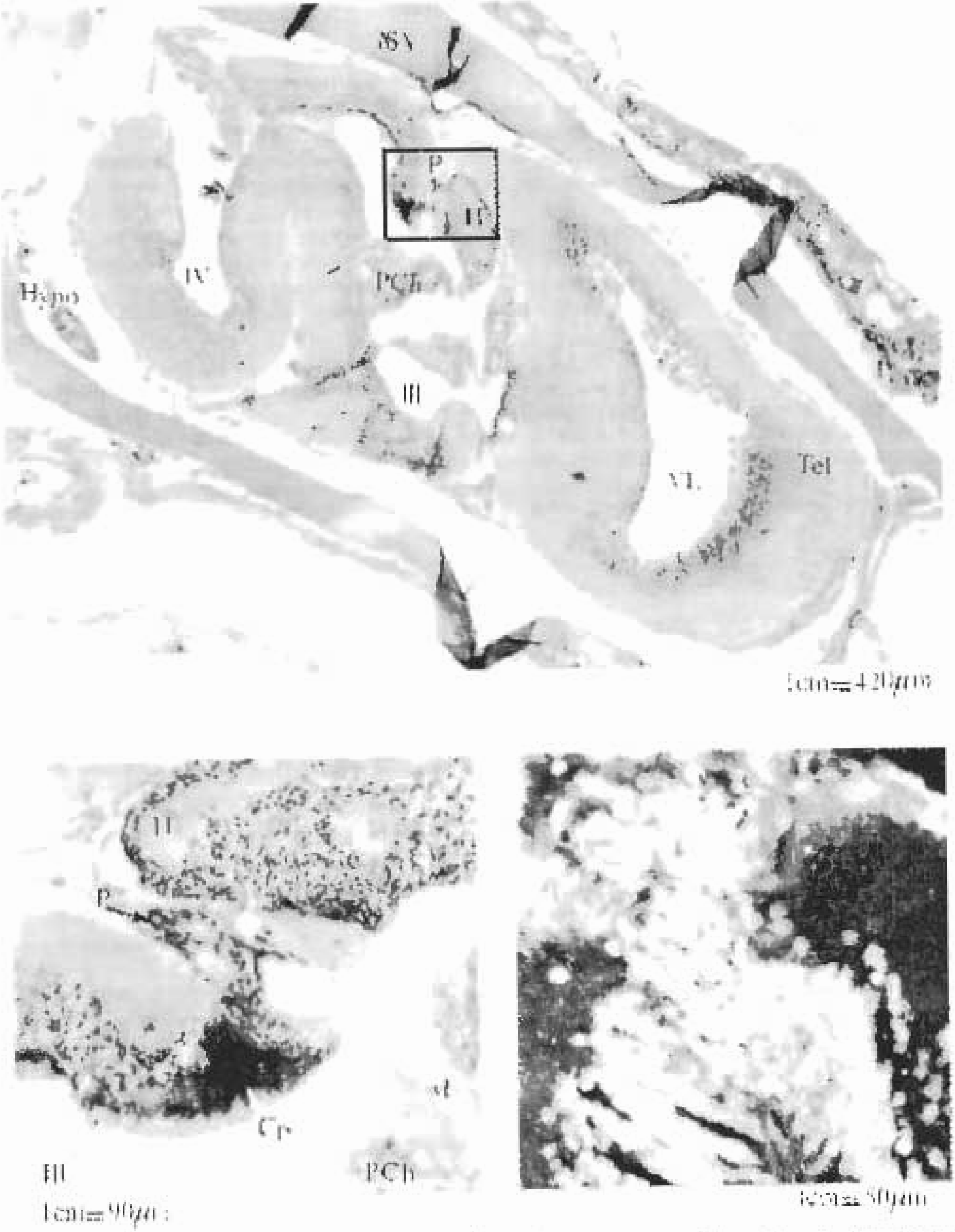

Figure 1: The brain of *Typhlonectes compressicauda*. a. In toto -parasagital section- b. Area if pineal organ -parasagital section- c. Pineal organ -frontal view- **Cp**: Commisura posterior; **H**: Habenula; **Hypo**: Hypophysis; **P**: Pineal organ;**PCh**: Plexus choroides; **S**: Skull; **Tel**: Telencephalon; **VL**: Ventriculus lateralis;**III**: III ventricle; **IV**: IV ventricle

Description of pineal development

Stage 26

The pineal organ appeared at this stage. It was an evagination of the roof of the diencephalon. It was initially undifferenciated without lumen.

Stage 27 -fig 2a-

The evagination swelled. A lumen in connection with the third ventricle appeared. The cells were still undifferenciated.

Stage 28 -fig 2b-

The pineal organ got bigger and looked like a drop. The lumen tended to develop. Few cellular types were observed : ovoid cells at the periphery and elongated cells near the lumen.

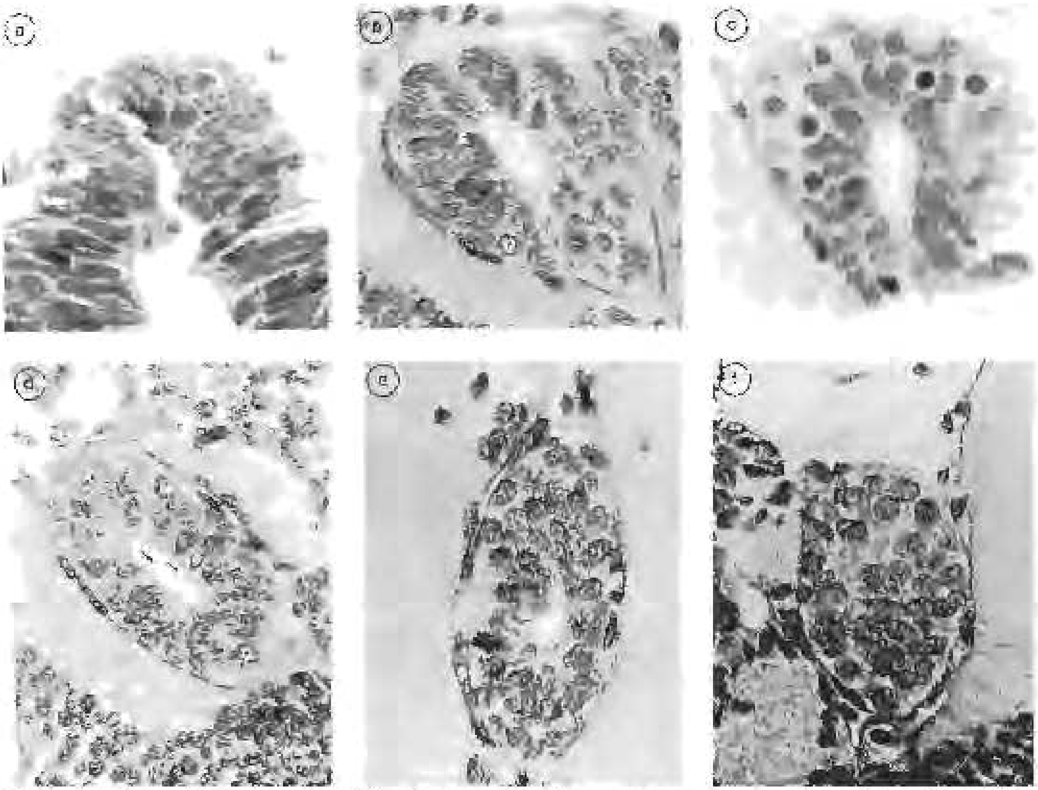

Figure 2: Embryonic development of pineal organ of *Typhlonectes compressicaudus*. a. Stage 27, d. Stage 31, b. Stage 28, e. Stage 32, c. Stage 30, f. Stage 33. O: Ovoid cells; E: Elongated cells; G: "Ganglion cells"; P: "Photoreceptor cells"; : Apical extension.

Stage 29

The pineal organ was swelling. The lumen was larger and the cellular differenciation continued.

Stage 30 -fig 2c-

The communication between lumen and third ventricle was closing. There was not any changement.

Stage 31 -fig 2d-

The communication lumen-third ventricle disappeared. Two cellular types could be found : - Few cells bording the lumen and having apical extension. They were similar to photoreceptor cells.

- The other cells looked like ganglion cells and intercalary cells.

Stage 32 -fig 2e-

The lumen was reduced. The "photoreceptor like" cells have regressed. No apical extension was observed in the lumen.

Stage 33 -fig 2f-

Only one cellular type was present. These cells were roughly round including a voluminous and round nucleus. The structure was typically glandular. This was the adult structure of *Typhlonectes compressicaudus*.

DISCUSSION - CONCLUSION

The pineal organ development of *Typhlonectes compressicaudus* looked like the one of Urodela.

At the stage 31, the observed cytological structure seemed to be anatomically similar to the pineal organ of Lacertidae described by COLLIN (1969). This structure was this of a photoreceptor organ. However in this case, we do not know if it was functional. Then, between stage 32 and 33, this structure changed completely into a glandular structure.

It seemed that this changing of structure would have been in relation with the metamorphosis.

Netherless several points remain obscure :

- The existence of a pineal tractus or a pineal nerve.
- The origin of glandular cells. Is it a transformation of "photoreceptor" cells or the differenciation of embryonic cells ?
- The exact function of the different cells.
- The disappearance of the continuity between the pineal lumen and the third ventricle could be linked with a structural and functional modification of this organ.

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The ontogeny of locomotion in marine turtles

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Abstract : The sea environment was probably an opportunity to occupy a spacious space for the ancestors of the living marine turtles. The extant forms have developed original strategies based on the perfection of the swimming. The great development of the forelimbs modified to form flippers, the hypertrophy of the pectoral muscles for powering them, their use for an anterior appendicular propulsion system by synchronized beating, separate the marine turtles from all other chelonians. They confer them aptitudes to swim faster and continually for longer periods of time. The terrestrial locomotion of the most heavy sea forms is derived from the swimming mechanisms. The primary pattern of Tetrapods, using alternate movements and a diagonal sequence of the limbs is replaced by a specific repertory of periodic and synchronous movements of the fore and hindlimbs. The synchronous movements of the forelimbs produces, with the associated action of the hindlimbs, the lifting and the forward progression of the body. This mechanism appears an efficient solution for such heavy animals when they leave the sea environment to walk on land. A discussion is led concerning the determinism of this system of limbs synchronization. Styles of life, physical constraints, morphological changes (as size and form of the flippers, body weight), gaits of other aquatic species, gaits of terrestrial forms, are discussed to explain this synchronized pattern of limbs coordination.

Key words: marine turtles, locomotion, marine adaptation

INTRODUCTION

Marine turtles are great and fast swimming living reptiles. Eckert and Martins, in 1989, reported the transatlantic travel of a juvenile loggerhead turtle, *Caretta caretta* of 84 cm. The crossing of sea was around 6900 km and the minimum rate of animal travel was about 45 km per day. In august 1992, scientists of U.S. National Marine Fisheries Service of Honolulu have detected, by means of transmitters attached to the shell of three adults of green turtles, *Chelonia mydas*, migration speeds of almost 50 km per day. These performances are attributed to remarkable simultaneous beating of their hypertrophied limbs. This synchronized flipper movement is probably the result of a long and complex history. However, the data concerning the early evolution of chelonians are imprecise. It is possible that the first representative forms of the group were amphibious. In this case, the terrestrial turtles were a first illustration of a secondary adaptation to a new environment. The marine turtles were another illustration of a new specialization. Chelonians have probably many times invaded the marine world, even if these attemps did not lead to modern sea groups. It is generally agreed that the existing marine turtles derived from heavy armoured amphibious cryptodiran ancestors, which occupy an habit similar to that of modern terrapins.

The analyzis of the locomotor abilities of marine turtles, and especially the knowledge of the determinism of such a locomotor pattern, constitute two complicate questions, because swimming is useful for different ecological strategies in sea environment, and these animals are able to use a temporary land locomotion, at birth and for nesting.

The first studies on chelonian locomotion concerned terrestrial gaits, with the works of WALKER (1963, 1971), ZUG (1971, 1972), JAYES & ALEXANDER (1980). The progression of marine turtles on the beach has been discussed by HENDRIKSON (1958), MC ALLISTER, BASS & VAN SCHOOR (1965), HUGHES, BASS & MENTIS (1967) and more recently, RENOUS, LESCURE, GASC & BELS (1989), RENOUS & BELS (1991). ZUG, in 1971, analyzes the swimming of some fresh water cryptodirans.

DAVENPORT, MUNKS & OXFORD (1984) compare the swimming actions and performances of young green tutles, *Chelonia mydas*, and fresh water chelonians of similar size, to evaluate the advantages conferred by the hypertrophied flapping forelimbs of marine species. DAVENPORT & CLOUGH (1986) complete the informations with young loggerhead turtles, *Caretta caretta*, and DAVENPORT (1987) with hatchlings of leatherback sea turtles, *Dermochelys coriacea*. Finally, RENOUS & BELS (1993) establish a comparison between aquatic and terrestrial locomotion in *Dermochelys coriacea*.

BASIC VERTEBRATE LOCOMOTION AND MORPHOFUNCTIONAL CONSTRAINTS IN TURTLES

In Vertebrates, the locomotion results from the functioning of two anatomical systems : the **axial system** constituted by the articulated vertebrae and the associated muscles and nerves, the **appendicular system** formed by the articulated limbs bones and the associated muscles and nerves. A propulsive force can be create by the lateral bending of the body or by the movements of fins or limbs against a solid (land) or a fluid (water) substrate. Generally, the action of one of these systems is predominant through an axial or an appendicular locomotion. However, both systems often associate their mechanic effects. For example, the curve of the back leads to amplify the limbs movements by increasing of the stride length. We can logically consider that axial locomotion was the primitive mechanism of progression adopted by the first Vertebrates, in aquatic medium. It is necessary to note that it remains the basic pattern for numerous primary swimmers which have improved such an adaptation, and also secondary swimmers as Tetrapods, which have returned to an aquatic mode of life.

The **appendicular locomotion** is basically made by the alternate movements of the four limbs. It corresponds to a sequential displacement in space and time. Each limb shows a propulsive phase (stance phase, retraction or downstroke), in which this limb exerts a thrust on the medium and a ballistic phase (swing phase, recovery phase, protraction or upstroke), in which the limb is flexed then extended to recover the biginning of the previous phase. These two distinct phases constitute a cycle (or stride) for each limb. Each limb follows one another in a sequential order, which is basically diagonal : two diagonal limbs execute the same action with a more or less time-lag. We know, since more than a century, that the time-lags between the diagonal limbs, the lateral limbs, and the relative duration of the two phases of the cycle, permit to detect the mode of coordination of the limbs movements and to define the gaits. These gaits are symmetrical when the time-lags between the same action of the two forelimbs and the two hindlimbs are identical.

Chelonians are exceptional Tetrapods on account of the rigidity of their trunk. Axial system cannot be used in locomotion and the appendicular system remains the only basic mechanism for all kinds of progression in terrestrial and aquatic environments. The edification of an external rigid box, requiring a drastic modification of the entire anatomical construction of the organism to be lodged into a bony shell box, cannot be explain by locomotor necessities. We must rather imagine a protective specialization to constitute a defensive system against predators. But, this armored device has probably induced a special evolutionary line, with serious limitations for future adaptational answers to new constraints. For locomotion, the presence of a bony shell has imposed modifications of girdles and limbs bones (orientation, reduction or development) associated to muscular changes (reduction of the dorsal units and development of the ventral). On the other hand, the shell is probably capable of precluding any collision between lateral or diagonal limbs. In spite of the mechanical and physical constraints create by the shell, the locomotor pattern of the great majority of the turtles is not very different of that found in many other Tetrapods. ROMER (1966) had even suggested that the extant chelonians may show a mode of progression evoking the pattern of primitive reptiles. He estimates that the shell had fixed an original pattern of walking. However, chelonians do not show a single locomotor pattern, but diverse ones, some of them being apparently more or less modified with regard to an hypothetical primitive reptilian pattern (Fig.1).

LOCOMOTION IN MARINE TURTLES

The living marine turtles form the Chelonioidea, among the Cryptodires. Seven species are placed in five genera, representing two families. Four genera, *Chelonia*, *Caretta*, *Lepidochelys* and *Eretmochelys*, composed the Cheloniidae. The genus, *Dermochelys*, is placed in another separate family, the Dermochelyidae. This systematical position is still discussed today, because many characters, as the number of chromosomes (2n=56) and some serological tests, are identical for all the marine forms. However, structural and morphological particularities, notably concerning the skin and the skeleton, justify such a division. To confirm the systematical choice, no paleontological document was found during the low jurassic concerning the marine turtles and we cannot know with a great assurance the phylogenetic relationships of the families of chelonians which lived at the end of this period.

In spite of minor or more important differences, these seven species of marine turtles have a great deal in common. The major common features are the use of an anterior appendicular system of propulsion and the modification of the forelimbs to form flippers. As described by DAVENPORT *et al.* (1984), in water, the propulsive force is generated by hydrodynamic lift produced as the flippers move through the fluid with different angles of attack. The forelimbs act like hydrofoils rather than oars or paddles. These properties confer noticeable aptituds to swim faster than other aquatic turtles and also to swim continually for longer periof of time.

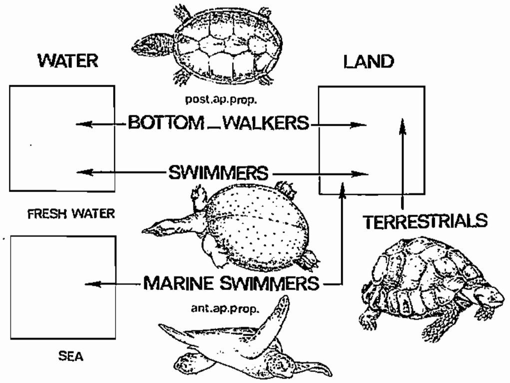


Figure 1: Basic modes of locomotion in turtles; ant.ap.prop., anterior appendicular propulsion; post.ap.prop., posterior appendicular propulsion.

A - The swimming

a) Data concerning the leatherback sea turtle

The swimming of hatchlings, large juvenile and adults of the largest living marine turtles, *Dermochelys coriacea*, mainly results from the synchronous flapping of the forelimbs. Asynchronous movements are only observed in association with changes of direction, but never during linear progression. Generally, in this last condition, the hindlimbs have synchronous movements with the foreflippers.

The foreflippers show a cyclic movement with two main phases, an upstroke in the course of it they move upward and anteriorly, and a downstroke in the course of it they move downward and posteriorly. These phases are separate by two periods of forelimbs repositioning, at the end of each phase.

As seen in hatchlings by DAVENPORT (1987), different types of swimming can be adopted : a **vigorous swimming**, with a velocity of 30 cm per second generated by forelimbs cycle of 0,5 or 0,6 s, a **routine swimming** when they came to the water surface, with a velocity of 8 cm per second for limbs cycle of 0,8 or

1 s. A juvenile of 60 kg, set in a vast aquarium, still reveals a slower routine progression, around 5 cm per second (RENOUS & BELS, 1993).

In **vigorous swimming**, each phase of the cycle has a propulsive action as suggested by an undulatory track, where upward motion appear during the upstroke and downstroke, and the increasing of the turtle velocity during both phases. The flippers moves more quickly on the downstroke than on the upstroke. The body axis makes an angle of 22° with the horizontal plane. During the downstroke, each forelimb follows a path that is about 75° to the horizontal plane and, in the middle of the trajectory, the blade formed by the flipper is 47° inclined with the same horizontal plane. The leading edge is ventral. During the upstroke, each forelimb follows a path that is about 82° to the horizontal plane. In the middle of the path, the blade is 32° inclined. The leading edge is dorsal. The foreflippers draw an arc of circle about 128° between the upper and downer positions.

In **routine swimming**, the body axis is near the horizontal plane. During the downstroke, the path of the forelimbs is about 54° with the horizontal plane and the blade is 54° inclined. During the upstroke, the path is now about 68° and the blade is inclined by 43°. The foreflippers draw an arc of circle more reduced, around 75°.

Markers evenly located and diametrally opposed on the two edges of each foreflipper of a juvenile of 60 kg highlight the limbs movement and reveal, during the upstroke, a combination of an elevation of the flippers tip and a rotation of the shoulders. The paddles are simultaneously twisted and the proximal part of their anterior edge, laterally oriented at first, becomes frontal. During the downstroke, the anterior edge of the flipper remains frontal during the main duration of the phase, suggesting a movement without axial shoulder rotation at the beginning of the phase. But a final axial shoulder rotation sets the medial surface of the limb forward.

A hydrodynamic model was proposed by DAVENPORT (1987) to explain the efficiency of the foreflippers. With the direction of the path, the direction of the body progression, the velocity of the flippers and the body, he found a resultant which materializes the direction of the water flow and the drag which is exerted on each paddle in the middle of their path. The angle of attack can be calculated and the direction of the lift known. In vigorous swimming, the resultant for the drag and the lift generates two hydrodynamic forces, one for the downstroke and one for the upstroke, which have propulsive components. The propulsive force created during the downstroke is greater. In routine swimming, only the upstroke seems to give such a propulsive component. However, in the very slow routine swimming of a juvenile of 60 kg (RENOUS & BELS, 1993), the body moving up during the main part of the downstroke, and down during the upstroke, suggests a propulsive force only during the downstroke. The swimming gaits of hatchlings and juveniles reveal a good synchronization of the movements of the fore and hindlimbs.

b) Data concerning the green turtle

In young green turtles, *Chelonia mydas*, which have also long, smooth and flattered hypertrophied forelimbs, the complete pectoral limbs cycle of 0,87 s produces a speed between 10 and 50 cm per second, in vigorous swimming (DAVENPORT *et al.*, 1984). A cycle of 1,13 s produces a speed between 9 and 14 cm per second, in routine swimming. As *Dermochelys coriacea*, the forelimbs are simultaneously flapped up and down, while the hindlimbs function as rudders and do not contribute to the propulsion. The foreflippers always move more quickly (80 cm per second in vigorous swimming and 42 cm per second in routine swimming) on the downstroke than on the upstroke (66 cm per second in vigorous swimming and 34 cm per second in routine swimming). As for *Dermochelys*, the hydrodynamic model ables to calculate the angles of attack and to estimate the propulsive components. In routine swimming, the cambered flippers generate a considerable horizontal propulsive force during their downstroke, but because a negative angle of attack, a smaller propulsive component during their upstroke. In vigorous swimming, a great horizontal propulsive component is only given by the downstroke.

It is important to note that, for very slow speeds, *Chelonia mydas* uses sometimes a diagonal alternate paddling action.

c) Data concerning the loggerhead turtle

DAVENPORT & CLOUGH (1986) observe that the hachlings of *Caretta caretta* employ simultaneously their foreflippers in only three situations : feeding, diving and fright. They adopt a vigorous swimming with a speed of 20 cm per second. In all other conditions of the sea life, they use a routine swimming by synchronous hindlimbs kicks. The speed is about 5 cm per second. The foreflippers, which are long and covered with a large number of small, tough but slightly flexibles scutes, are folded against the carapace. The simultaneous action of the hindlimbs probably avoid lateral yawing movements of the body. The position of the forelimbs reduces probably the drag. This type of swimming disappears as the young animals overstep 1500 g. They recover a synchronous beating of the foreflippers, but they also occasionnally use a diagonal alternate paddling action, even for the adults.

No studies on the locomotion of *Eretmochelys* and *Lepidochelys*, but we know that the species use synchronous limbs movements, when adults.

In spite of incomplete data, we can notice that in the swimming of marine turtles, the diagonal alternance of the limbs movements disappears for the benefit of a simultaneity of the forelimbs and/or the hindlimbs. This alternance, which is occasionally used by *Caretta caretta*, and rarely by *Chelonia mydas*, does not exist in the swimming of *Dermochelys coriacea.*

The use of the foreflippers as synchronous hydrofoils allows the adjustment to the diverse circumstances of the marine life, the speed and the type of the swimming to change the hydromechanical conditions to generate the propulsive component.

B - The terrestrial locomotion

When the females of *Dermochelys coriacea* come to nest on the beaches, they use a peculiar mode of locomotion. As described by RENOUS & BELS (1991, 1993), this mode does not involve a raising of the entire body. A specific repertory of cyclic and synchronous movements of the fore- and hindlimbs is adopted. The simultaneous movements of the forelimbs produce the lifting and the forward progression of the body as if the turtle is "walking on crutches".

The forelimbs have a swing and a stance phases. The stance phase involves two stages : a retraction and a repositioning. During retraction, the cutting radial edge of the distal part of each forelimb exerts a pressure against the sand. When the distal extremity reaches the flank, the limb adopts a position favouring the subsequent lifting. The swing phase also shows two stages : an effective protraction and a repositioning. During the protraction, the paddles are lifted and propelled forward. When they reach their maximal forward position, a humeral axial rotation sets their radial edge on contact with the sand

The stance phase of the hindlimbs, which coincids with the start of the forelimbs retraction, shows two main distinct periods : an active retraction and a passive dragging along. The active retraction corresponds to a pushing down against the sand. The swing phase coincids with the swing phase of the forelimbs.

The body progression is discontinuous. A vertical rising appears during the simultaneous retraction of the fore- and hindlimbs. These last limbs seems to give an effective complementary mechanical impulse, because their vertical and horizontal speeds increase prior the elbows contact. The rising is combined with a forward progression, at the end of the forelimbs retraction. The body falls down during the simultaneous phase of the two pairs of limbs.

A great deceleration of the horizontal progression and rising of the body, during the simultaneous stance phase of the two pairs of limbs, is explained by the backward motion of the limbs pushing against the substratum. A sudden acceleration coincids with the elbow contact which produces the body buttressing. A constant speed characterizes the end of the forelimbs stance phase before the body fall. This speed reaches 35 cm per second, for a specimen of 8 kg and an average duration of 2 s for the forelimbs cycle (0,72 s for protraction and 1,28 s for retraction).

The comparison between swimming and terrestrial locomotion is not easy, because the propulsive force is mainly generated by the forelimbs retraction on land, but by the downstroke, the upstroke, or the two phases in swimming, according to the type of progression and speed. We can only compare with the very slow routine swimming of large juveniles (60 kg), because only the downstroke generates a propulsive force, and the forelimbs cycle duration ranges between 2,88 s and 5,25 s, the average duration for the adults in terrestrail walk being 3,3 s. In this case, the terrestrial locomotion appears as a result of the use of swimming mechanisms and movements on land, and also a modulation of the swimming gait, by the gravitational constraints on the ground. To transport a heavy body, the modulation consists to use a propulsive system which now associates the hindlimbs, and to change the modulators. For example, the duration of the forelimbs cycles and the body speed, are regulated by the duration of the retraction phase on land instead of the upstroke in swimming. The vertical amplitude of the forelimbs beating becomes constant on land instead of the lateral amplitude in swimming. By using "crutches", the body is elevated as a reaction of the forelimbs pushing forces against the substratum. The vertical and longitudinal components of these forces are only effective, because the simultaneous movement makes inefficient the lateral opposite components. During the stance phase, the turtle gains the kinetic energy generated by the limbs pushing action. Because the turtle elevates, this energy is gradually transformed into potential energy, which is then transformed again in kinetic energy, during the body fall. The simultaneous limbs displacement appears as a kind of energy-saving system to move the heavy body mass.

In *Dermochelys coriacea*, in opposition to the opinion of HUGHES *et al.* (1967), this peculiar mode of locomotion still appears in the hatchlings. However, the alternate limbs movements can be demonstrated in experimental conditions, when the hachlings are set on a smooth support (paper or plexiglas) as seen by RENOUS & BELS (1991).

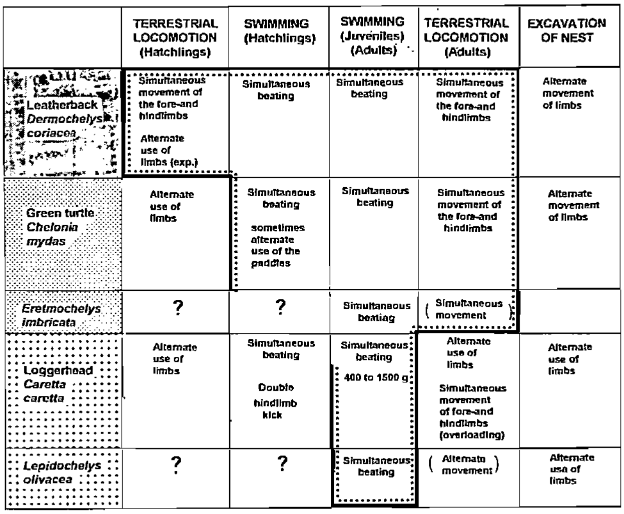
The terrestrial locomotion of *Chelonia mydas* adults corresponds to simultaneous movements of the fore-and hindlimbs, but the hatchlings use alternate limbs movements. The hatchlings or the adults of *Caretta caretta* show a crawling walk by alternate action of the four limbs, as described by MC ALLISTER *et al.* (1965). While the belly rests on the sand to support the body weight, a forelimb is laterally rotated and moves forward until the humerus axis forms an angle of 45° with the body axis. The hindlimb on the opposite side acts simultaneously. During their propulsive phase, the foreflippers dig into the sand. During the same phase, the hindlimbs seem only to be set on the substrate and they do not probably exert an efficient thrust.

DETERMINISM OF THE MARINE TURTLES SIMULTANEOUS LIMBS MOVEMENTS IN WATER AND ON SAND

A - Informations on the ecological strategies of the sea turtles

HENDRICKSON (1980) explains that the diversity of marine turtles ecological niches is suggested by comportemental differences. He proposes three major strategies. A first group, primitively herbivorous in mainland shallow waters, has energy-expensive migrations to reach beaches for nesting. *Chelonia mydas*, *Chelonia depressa*, although it is carnivorous and *Eretmochelys imbricata*, although it is omnivorous, constitute this group. The second group, with *Caretta caretta*, *Lepidochelys olivacea* and *Lepidochelys kempi*, has a carnivorous life on the continental shelves without travel to reach nesting beaches. The third group, with *Dermochelys coriacea*, jelly-feeder adapted to pelagic life, effects long travels by sea. The swimming is differently used to eat. For example, for the benthic form *Chelonia mydas*, the active swimming is used to tear away pieces of food, whereas the pelagic form *Dermochelys* uses small forelimbs displacements to maintain the mouth near the prey (BELS & RENOUS, 1992).

The alternate limb paddling action seems to be employed by forms as *Caretta* which does not perform great migrations. But, these more coastal species, need a great manoeuvrability. The simultaneous beating of the forelimbs matches with the necessity of great migrations with speed and endurance, as in *Chelonia mydas* and *Dermochelys coriacea* (Fig.2).



	TERRESTRIAL LOCOMOTION (Hatchlings)	SWIMMING (Hatchlings)	SWIMMING (Juveniles) (Adults)	TERRESTRIAL LOCOMOTION (Adults)	EXCAVATION OF NEST
Leatherback *Dermochelys coriacea*	Simultaneous movement of the fore-and hindlimbs Alternate use of limbs (exp.)	Simultaneous beating	Simultaneous beating	Simultaneous movement of the fore-and hindlimbs	Alternate movement of limbs
Green turtle *Chelonia mydas*	Alternate use of limbs	Simultaneous beating sometimes alternate use of the paddles	Simultaneous beating	Simultaneous movement of the fore-and hindlimbs	Alternate movement of limbs
Eretmochelys imbricata	?	?	Simultaneous beating	(Simultaneous movement)	
Loggerhead *Caretta caretta*	Alternate use of limbs	Simultaneous beating Double hindlimb kick	Simultaneous beating 400 to 1500 g	Alternate use of limbs Simultaneous movement of fore-and hindlimbs (overloading)	Alternate use of limbs
Lepidochelys olivacea	?	?	Simultaneous beating	(Alternate movement)	Alternate use of limbs

Figure 2:- Alternate and simultaneous movements of the limbs in terrestrial and aquatic locomotions of marine turtles. Grey, pelagic form; dotted, species which perform great migrations; crosses, coastal forms which do not perform great migrations.

B - Influence of the body size and weight

We can observe a gradient of size (length of the carapace) and weight in marine turtles : *Lepidochelys* (less than 100 kg), *Eretmochelys* (130 kg), *Caretta* (450 kg), *Chelonia* (250 to 500 kg) and *Dermochelys* (more than 500 kg). For Hendrickson (1958) the ability to move rapidly by alternate movements of limbs in reptiles is a function of weight. For Hughes *et al.* (1967), the great weight of *Dermochelys* can prevent if from moving in this manner, whereas the small body weight of *Caretta* ables to do so.

To demonstrate the influence of the weight, MC ALLISTER *et al.* (1965) had placed large weights (more than 68 kg) on the backs of *caretta* diagonally walking on land, and the animals adopted simultaneous limbs movements. Size and weight are not limiting factors in sea environment. The greater size of the body unables to lodge a large pectoral musculature in relation to a greater swimming power. In Leatherback, which manifests simultaneous limbs movements since birth, the exceptional alternate locomotion which appears when the hatchlings and juvenils are set on smooth surfaces, is lost with growth (45 to 2500 g).

An increase of the weight facilitates the use of a synchronous coordination of the limbs in land propression and a lost of eventual capacities of alternate coordination.

C - Influence of the forelimbs morphology and function

In marine turtles the forelimbs are true natatory paddles by modification of skeleton and associated muscles to change their surface and shape. The anterior flattened edge becomes very efficient to plough through the water. DAVENPORT and collaborators (1984) have compared the direct measurements of the thrust developped by the active simultaneous swimming of young *Chelonia mydas* and by the alternate swimming of some Terrapins of similar size (*Mauremys caspica, Chrysemys scripta, Kinosternon subrubrum*). The thrust is more important for the green turtle. The speed is also significantly greater for the marine species, for similar size and limbs cycle duration.

For the length of the foreflippers, the aquatic turtles constitute a morphological range where the leatherback occupies the top, with a high value of the ratio : limb length/body length.

In adults the hindlimb length is shorter, but the difference between the length of the fore and the hindlimbs increases for *Dermochelys* and significantly decreases for *Caretta*. But how explain the change of limbs coordination in this animal. Mc Allister *et al* (1965) supposed modifications in musculature and innervation during the passage to the adult life. However, the adults also use alternate limbs movements when they dig their nest. The only change concerns probably the allometric growth of the foreflippers and a variation of the rigidity with this growth. No precise data exists, only some informations from hughes and MENTIS (1967) suggesting a relative greater length in juveniles than in hatchlings. DAVENPORT & CLOUGH (1986) note that young *Caretta* seems to possess relatively smaller, less streamlined and more flexible flippers than those of *Dermochelys* or *Chelonia*, whereas the hindlimbs are large and flexible as those of fresh water turtles.

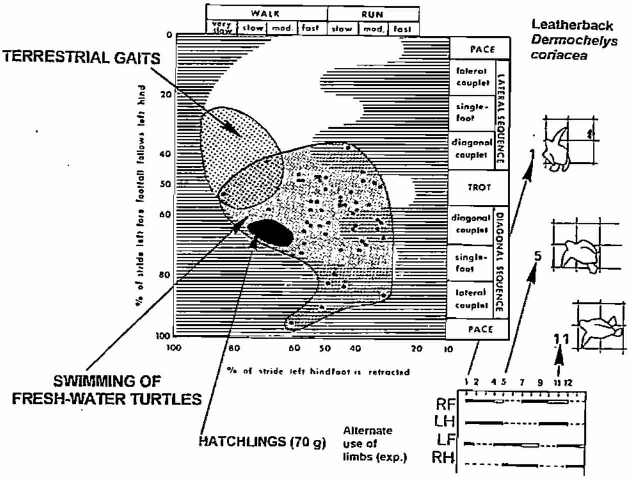


Figure 3: Symmetrical gaits of cryptodiran turtles during terrestrial and aquatic progressions (cf. HILDEBRAND, 1966) and situation of the experimental gaits of hatchlings of leatherback set on smooth substrate (as paper or plexiglas).

D - Informations given by the locomotion of the other turtles

The gaits of the terrestrial turtles and the fresh water forms when they leave their aquatic conditions correspond to very slow walks or slow walking trots in which the lateral sequence is dominant. The stance phase represents 70 to 84 per cent of the cycle. The time-lags between the lateral and also the diagonal limbs are less than 50 per cent of the reference cycle. These gaits leads to maximize the period of tripodal support commonly used in slow tetrapods.

All the fresh water turtles, bottom-walkers or swimmers use a walk, a fast walk or a moderate run in which the diagonal sequence is dominant, and reduce the yawing. The limbs of the former are drag forward in a protraction and push against the bottom during a retraction as on land. The latter show a paddle action drag based propulsion system. During the propulsive stroke the web of the toes form a geat angle of attack (90°) and a very low (10-20°) during the protraction. A thrust is generated and perhaps a small lift nullified by a drag which reduces the speed.

The terrestrial gaits of hatchlings, youngs and adults of leatherback have only been studied. The alternate gaits of hatchlings and adults of *Caretta* are not identified. We do not know the duration of the stance phase and the time lag between the lateral limbs. However, the exceptional alternate progression of the hatchlings of *Dermochelys* reveals a walking trot characterized by a diagonal sequence as for the bottom-walkers (Fig.3).

The passage to the simultaneity of the limbs supposes an intermediate asymetrical gait where the hindlimbs are asynchronous, as seen in juveniles. But the simultaneity of the limbs in each diagonal is it the solution to leads to a total synchronization? The simultaneity of the limbs movements is really a functional advantage in sea to effect great migrations. The associated increasing of the foreflippers length and the global body size and mass favorise the efficiency of this pattern of swimming. This mode of limbs coordination also remains a functional advantage in terrestrial conditions, when the body size and mass have raised. The data reinforce the idea of two distinct great adaptations in chelonians, one towards the terrestrrial life and one towards the marine life. It is generally agreed that the bottom-walkers use the more primitive locomotion, which was perhaps that of the amphibious ancestors of the existing marine turtles. The synchronous mode of locomotion adopted only in swimming by the neritic forms, in swimming and on land, with the growth, by the migratory forms, and in all conditions by the pelagic species, supposes a modification of the interneuronal control of the motor centers which govern the limbs movements. More informations are necessary to understand correctly the evolutionary process.

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Geographical patterns of italian *Emys orbicularis*: a biometrical analysis.

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Abstract: Two geographical populations of the European pond terrapin have been compared, from both sides of Northern Appennines of Italy. On average, Emilia Romagna (NE Italy) populations are longer, larger and heavier than Tuscany ones (W central Italy) both under ANOVA of linear and ratios, and it is assessed that at least 2 geographical populations occur along Thyrrenian and Adriatic coasts of Italy.

Key words: Emys orbicularis; biometry; Italy; NW populations; NE populations

INTRODUCTION

Reptile populations living on islands or peninsulas, or separated from the inland population by natural barriers or that simply are common in different habitats (i.e. prairies, wooded lands, hilly areas), can display different behaviours, biological traits and/or be different in one or more morphological features (THORPE, 1976, 1987; FRITZ, 1989, 1992, 1993), depending also on the time ellapsed from colonization of the considered area, on habitat resources and competitors, altitude and on climate (THORPE, 1987). It has been widely described how modern forms of Emys orbicularis had colonised in a relatively short period (8000-2300 b.C.) almost all the Central, North and Western Europe (MLYNARSKI, 1956; QUAGLIERI, 1991), coming probably from a Southwestern area of Asia, during tertiary period, via Middle East.

During ecological studies on some European pond terrapin populations from Emilia Romagna (NE Italy, towards the Adriatic Sea) and Tuscany (NW Central Italy, towards the Thyrrenian Sea) (DI TRANI & ZUFFI, unpublished data; ZUFFI & GARIBOLDI, unpublished data), separated by the Appennines, we planned to verify also if these Emys orbicularis populations were different in morphological traits.

MATERIALS AND METHODS

Ninetyfour adult males and 123 adult females (54 males, 72 females from ER, 40 males, 51 females from T) were measured and weighed as reported in ZUFFI & GARIBOLDI (1993). Linear parameters (N=8) and ratios (N=9) (already described in ZUFFI & GARIBOLDI, 1993) were compared between sexes for the same region and between regions for the same sex, using ANOVA in comparing linears and ratios, Multiple Regression Analysis (Stepwise Backward Selection, with F remove equal 4) (SOKAL & ROHLF, 1981) to select characteristic and diagnostic parameters of sex and population. Localities from each region (Emilia Romagna=ER; Tuscany=T) were plotted together for general pattern analysis and then studied separately for interregional comparison (Figure 1).

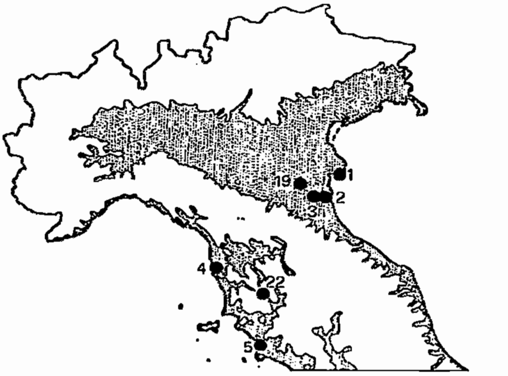

Figure 1. Study area. 1="Bosco della Mesola"; 2="Bassa del Bardello"; 3=Campotto; 4=Arnino; 5="Parco dell' Uccellina"; 19=Alfonsine; 22=Siena environs; scale 1:5000000; pointed area: below 200 m asl.

	N	EmiliaRomagna	P	N	Tuscany	P	t	P
CL	54	12.25 ± 0.18	<0.0001	40	11.69 ± 0.20	<0.0001	2.096	0.02
	72	13.76 ± 0.19		51	12.65 ± 0.14		4.36	<0.001
CW	54	8.71 ± 0.12	<0.0001	40	8.77 ± 0.13	<0.01	-0.287	0.39 NS
	72	9.94 ± 0.14		51	9.42 ± 0.14		2.5	<0.01
PL	54	10.55 ± 0.16	<0.0001	40	10.09 ± 0.18	<0.0001	1.908	<0.05
	71	12.82 ± 0.18		51	11.46 ± 0.15		5.56	<0.001
PW	19	6.65 ± 0.19	<0.0001	40	6.58 ± 0.14	<0.001	0.29	0.38 NS
	27	8.1 ± 0.24		50	7.31 ± 0.12		3.386	<0.001
SH	54	4.30 ± 0.06	<0.0001	15	4.40 ± 0.12	<0.001	-0.725	0.24 NS
	72	5.78 ± 0.09		10	5.44 ± 0.29		1.25	0.11 NS
TL	26	7.88 ± 0.18	<0.0001	36	5.72 ± 0.21	0.59 NS	7.47	<0.001
	33	6.61 ± 0.18		41	5.86 ± 0.15		3.26	<0.001
CT	25	5.32 ± 0.13	0.72 NS	10	4.74 ± 0.32	0.86 NS	1.99	<0.05
	29	5.40 ± 0.17		6	4.63 ± 0.52		1.75	<0.05
BW	54	275.02 ± 10.32	<0.0001	27	231.45±11.01	<0.0001	2.63	<0.01
	72	467.18 ± 19.06		48	340.71±10.97		5.05	<0.0001

Table 1: Sex comparison of linear parameters for the same region (sample size of male/female, mean, SE, probability level of ANOVA) and difference between regions for the same sex ("t" Student) (CL-CT in cm, BW in g).

	N	ER	P	N	T	P	t	P
CL/BW	54	0.047 ± 0.002	<0.0001	27	0.052 ± 0.003	<0.0001	-1.57	0.059 NS
	72	0.032 ± 0.001		48	0.038 ± 0.001		-4.36	<0.0001
CW/CL	54	0.71 ± 0.008	0.314 NS	40	0.75 ± 0.005	0.319 NS	-3.6	<0.0005
	72	0.72 ± 0.005		51	0.74 ± 0.006		-2.73	<0.005
PW/PL	19	0.62 ± 0.008	0.61 NS	40	0.65 ± 0.007	0.399 NS	-2.77	<0.005
	26	0.63 ± 0.007		50	0.64 ± 0.011		-0.85	0.198 NS
SH/CL	54	0.35 ± 0.004	<0.0001	15	0.36 ± 0.005	<0.0001	-1.26	0.104 NS
	72	0.42 ± 0.004		10	0.41 ± 0.001		0.49	0.31 NS
SH/CW	54	0.49 ± 0.005	<0.0001	15	0.49 ± 0.006	<0.0001	0.77	0.22 NS
	72	0.58 ± 0.005		10	0.57 ± 0.01		1.0	0.158 NS
SH/ PL	54	0.41 ± 0.003	<0.0001	15	0.41 ± 0.006	<0.0005	-0.85	0.19 NS
	71	0.45 ± 0.003		10	0.46 ± 0.01		-1.42	0.079 NS
TL/PL	26	0.74 ± 0.013	<0.0001	36	0.56 ± 0.015	<0.02	8.36	<0.0001
	30	0.55 ± 0.05		41	0.51 ± 0.014		-1.34	0.09 NS
CT/TL	25	0.64 ± 0.011	<0.0001	9	0.70 ± 0.015	<0.02	-3.03	<0.005
	29	0.75 ± 0.01		6	0.79 ± 0.032		0.65	0.258 NS
PL-TL	26	2.77 ± 0.18	<0.0001	36	4.39 ± 0.18	<0.0001	-6.32	<0.0001
	33	5.83 ± 0.29		41	5.61 ± 0.2		1.0	0.158 NS

Table 2: Sex comparison of ratios for the same region (sample size of male/female, mean, SE, probability level of ANOVA) and difference between regions for the same sex ("t" Student) (CL-CT in cm, BW in g).

	ER	Coeff.	R^2 ± SE	N		T	Coeff.	R^2 ± SE	N
males	CW PL SH PW	55.58 33.12 76.31 -58.45	0.9762 ± 14.02	13	males	CL	54.73	0.8652 ± 21.85	25
females	CL SH	32.98 95.47	0.92 ± 34.04	18	females	PL CW TL	56.29 23.49 36.68	0.7935 ± 37.30	38

Table 3: Multiple Regression Analysis of linears (BW as dependent variable).

	ER	Coeff.	R^2 ± SE	N		T	Coeff.	R^2 ± SE	N
m + f	PL-TL PW/PL	0.64 -0.009	0.505 ± 0.028	34	m + f	CW/CL TL/PL PL-TL	1.01 -0.28 -0.03	0.7169 ± 0.047	69
males	CW/CL PW/PL TL/PL PL-TL	-0.24 0.18 -0.21 -0.01	0.9213 ± 0.0005	14	males	TL/PL PL-TL	-0.27 -0.02	0.39 ± 0.01	25
females	TL/PL PL-TL	-0.19 -0.01	0.709 ± 0.006	18	females	TP/PL PL-TL	-0.18 -0.01	0.578 ± 0.004	38

Table 4: Multiple Regression Analysis of ratios (CL/BW as dependent variable).

RESULTS

Considered parameters analyzed by ANOVA are shown in Tables 1, 2. The clearcut difference in male-female was underlined almost for all the linears and ratios and the evident difference between regions embraced 5 linears for both sexes, 5 ratios for males and 2 for females. Multiple Regression Analysis of linears (Table 3) puts in evidence that a positive correlation does exist for both sexes, particularly of ER and T males. Multiple Regression Analysis of ratios shows a higher positive correlation in males than in females of ER and a lower positive correlation of males than females of T. Differently from what observed for linears, two characters are common to both ER and T males and females, with the exception of ER males which are also characterised by CW/CL and PW/PL (Table 4). Both populations were relatively variable-often not significantly -among localities (ANOVA).

DISCUSSION

It is worth referring that diagnostic parameters of sexes and, as a consequence, of populations, were particularly related to the morphology of reproductive biology (e.g. SH/CL) and, almost surely, to growth rates. On average, ER populations are longer, larger and heavier than T ones (from both linear and ratios) and it could be assessed that at least 2 geographical populations occur along Thyrrenian and Adriatic coasts of Italy.

Available data from Liguria (NW Italy), Friuli-Venezia Giulia (NE Italy), Corse, Sardinia and Sicily (ZUFFI & GARIBOLDI, unpublished data; U.Fritz, pers. comm., June 1993) appear going along the same hypotethical trend, to say a distinct biometric, also morphometric (U. Fritz, pers. comm., June 1993), separation of Emys orbicularis of Italian range. Main migratory flow coming from the Eastern and Central Europe divided probably in two: the first reached the Eastern coasts of Italy and the Po plain area; the second, passing North to the Alps, came Westward to France, probably down to Southern France and Northwestern coasts of Italy (Liguria and Tuscany).

If we suppose that we are facing not only to a variation pattern per se, but also to an evolutive one, probably it shall be necessary to test most variability along latitude and longitude to find how and where variation does appear and, finally, examine populations under biometric, biochemical and ecological approaches.

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Sexual dimorphism of the European Pond Terrapin, *Emys orbicularis* (L., 1758) from Italy.

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Abstract: Nevertheless a large amount of information on *Emys orbicularis* is available from several topics, almost anything has been stressed about sexual dimorphism. To understand much more especially from field experiences, some linear parameters and ratios were evaluated, to describe 110 adult males, 151 adult females, 31 subadults and juveniles, previously sexed according to papers of general interest. Adult males of the European pond terrapin were described, on average, shorter, narrower and lighter than adult females, with longer tail, in absolute terms; males were also relatively lighter and slimmer than females.

Key-words: *Emys orbicularis*; Italy; sexual dimorphism.

INTRODUCTION

A few description exists on sexual dimorphism of the European pond terrapin, Emys orbicularis, all over its Italian range (LANZA, 1983) and most of the available data do not carefully show how dimorphism plays its role and which features of the species are determinant (ROLLINAT, 1934; FRITZ, 1989). The present paper has been aimed at testing how discriminate both sexes especially from field experiences and describing how the considered parameters vary. Available data on juvenile and immature stages are shown.

MATERIALS AND METHODS

110 adult males, 151 adult females, 31 subadults and juveniles were measured; among them 79.09% of males, 78.15% of females and 19.35% immatures have been captured in the field; remaining specimens came from museum and university herpetological collections. Field stations were at Alfonsine (MAZZOTTI, 1990) and "Bassa del Bardello" (Province Ravenna), Campotto and "Bosco della Mesola" (Province Ferrara), "Arnino" (Province Pisa), "Parco Naturale della Maremma" (Province Grosseto). Preserved specimens were from Abruzzi (N=4), Campania (N=2), Emilia Romagna (N=3), Friuli-Venezia Giulia (N=13), Latium (N=2), Liguria (N=9), Lombardy (N=2), Apulia (N=2), Sardinia (N=15), Sicily (N=8), Tuscany (N=8), Veneto (N=4), and are present in the "Museo civico di Storia naturale " (Milan), "Museo Friulano di Storia naturale" (Udine), "Museo civico di Storia naturale" (Trieste), "Museo civico di Storia naturale" (Genua), "Società Romana di Scienze Naturali" (Rome), "Museo zoologico 'La Specola'" University of Florence, "Museo di Storia Naturale e del Territorio" University of Pisa, "Museo di Zoologia" and "Museo di Anatomia Comparata" University of Bologna, "Naturhistorisches Museum" University of Bern.

A Pesola dynamometer (±5 g for adults; ±1 g for juveniles) and a precision calliper (±0.02 mm accuracy) were used. All living terrapins were carefully handled and no head and/or neck measurements were taken, because each captured specimen usually retained its head inside the carapace, during and after handling. Mass was calculated to the nearest 1 g for juveniles and nearest 5 g for adults; distances (e.g. length, width) were taken to the nearest 1 mm. Were considered, as adults, only specimens with evident sexual characters: as male each specimen with concavity of the plastron and tail longer than half of carapace length; as female each specimen with tail shorter than half of the carapace or as long as half of that (LANZA, 1983: 151). Terrapins with intermediate morphological features were not considered as adults. Age (i.e. the number of countable rings on the carapace or plastron surface) was difficult to establish with more than 10-11 years old terrapins, because of total overlapping of rings, and was not considered in this preliminary paper.

The following characters were considered:

1.Mass, in grams (BW). Also preserved specimens were weighed (see Results)

2.Carapace length (CL), as the minimum

distance between the nuchal scale and the supracaudal scale (Figure 1, a-a)

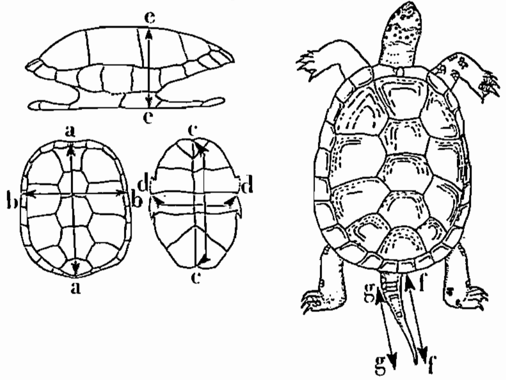

Figure 1: View of standard measurement points.

3.Carapace width (CW), as the distance between the carapace borders, passing from the sixth marginal through the second lateral and the caudal margins of the second central to the other side of the carapace (Figure 1, b-b)

4.Plastron length (PL), as the minimum distance between the anterior and the posterior convexities (i.e.: gular and caudal ventral shields) (Figure 1, c-c)

5.Plastron width (PW) as the distance between the bridge sutures, calculated from the line separating the pectoral and the abdominal shields (Figure 1, d-d)

6.Scute heigh (SH), as the distance between the caudal margin of the second central, and the median point of a virtual straight line joining both plastron margins (Figure 1, e-e)

7.Total tail length (TL), as the distance from the posterior margin of the plastron, to the tail tip (Figure 1, f-f)

8.Cloaca-tail tip length (CT), as the distance from the cephalic margin of the cloacal opening to the tail tip (Figure 1, g-g) .

The following ratios and differences were evaluated, to describe relative relationships:

1.CL/BW as dimensional ratio

2.CW/CL as shape of carapace

3.PW/PL as shape of plastron

4.SH/CL as thickness of the body related to its dorsal length.

5.SH/PL as thickness of the body related to its ventral length.

6.SH/CW as thickness of the body related to its dorsal width.

7.TL/PL as possible sexual relationship.

8.CT/TL as cloaca relative position along the tail.

9.PL-TL as relative tail length vs. plastron length.

Data from adults were compared statistically, whilst data from joung and subadult specimens were only shown. One tailed Student "t" test for each paired feature at =0.005 (i.e. male vs. female CL) was used; Multiple Regression Analysis (Stepwise Backward Selection) was used to establish which group of parameters best fit sexual characteristics and if differences exist among sexes in relation to body mass (as dependent variable). SOKAL & ROHLF (1981) and Statgraphics package (vers. 4.0) were used in statistical analyses.

RESULTS.

Two randomly chosen groups of preserved adult specimens (N=9 males and N=9 females) were compared to 6 randomly selected groups (3 of males and 3 of females, N=9 each group) of living animals to test if difference in body weight exists, that is, if data from collections were comparable. Relatively or absolutely dehidrated specimens were not considered at all. Any difference did not exist in such a comparison (Table 1).

Males		
Sample (N=9,9)	t	P value
preserved-fresh 1	0.7121	0.2433
preserved-fresh 2	-0.3236	0.3752
preserved-fresh 3	-0.5493	0.2951
Females		
Sample (N=9,9)	t	P value
preserved-fresh 1	-0.5688	0.2886
preserved-fresh 2	1.39	0.091
preserved-fresh 3	1.4749	0.079

Table 1: Comparison between fresh and preserved terrapin weight.

Adult males of the European pond terrapin were shorter, narrower and lighter than adult females, with longer tail, in absolute terms (Table 2a); males were also relatively lighter and slimmer than females, with a TL/PL ratio greater than that of females (Table 3a). All observed differences were highly significative (Tables 2b, 3b).

On average sexual dimorphism appeared evident in linear parameters and in evaluated ratios and differences. Only in two ratios the comparative difference male-female, usually with lesser values of males, was null or lesser in females (e.g. CW/CL, TL/PL). Very often, upper values of juvenile linear records overlapped

	CL	CW	PL	PW	SH	TL	CT	BW
Males								
	110	110	108	73	83	75	47	89
	11.9±0.13	8.71±0.09	10.37±0.12	6.64±0.10	4.33±0.05	6.59±0.17	5.09±0.16	4.89±8.09
	8.5÷16.5	6.8÷11.6	7.6÷15.1	4.8÷9.4	3.3÷6.5	3.8÷10.1	2.8÷6.9	90÷460
Females								
	151	151	149	104	106	96	57	141
	13.19±0.13	9.60±0.09	12.15±0.12	7.54±0.10	5.64±0.08	6.20±0.10	5.23±0.13	406.50±12.43
	9.4÷19.0	6.7÷15.5	8.0÷16.1	5.1÷11.0	3.6÷8.7	2.7÷8.5	2.3÷7.3	135÷1100
Juveniles								
	31	31	31	31	27	28	23	17
	5.26±0.41	4.42±0.29	4.77±0.37	3.51±0.24	2.19±0.17	3.21±0.17	2.9±0.16	45.41±11.24
	2.4÷9.7	2.2÷7.2	2.1÷8.8	1.8÷5.9	1.2÷3.8	2.0÷5.1	1.8÷4.5	5÷140

Table 2a: Variation per selected character per sex (sample, average ± SE, range).

Measure	CL	CW	PL	PW	SH	TL	CT	BW
P value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.05	N.S.	<0.0001

Table 2b: Statistical results of male-female difference (one tailed "t" test)

CL/BW	CW/CL	PW/PL	H/CL	SH/PL	SH/CW	TL/PL	CT/TL	PL-TL
Males								
9	110	73	83	83	83	75	46	75
0.048±0.13	0.73±0.05	0.64±0.005	0.36±0.003	0.41±0.003	0.49±0.004	0.63±0.013	0.70±0.09	3.80±0.15
0.03÷0.11	0.5÷0.88	0.54÷0.79	0.22÷0.42	0.35÷0.47	0.43÷0.6	0.38÷0.87	0.58÷0.87	1.3÷8.4
Females								
141	151	103	106	105	106	96	57	96
0.035±0.001	0.73±0.003	0.63±0.006	0.42±0.003	0.45±0.003	0.58±0.005	0.53±0.009	0.81±0.008	5.66±0.15
0.02÷0.07	0.63÷0.89	0.56÷0.95	0.36÷0.54	0.4÷0.62	0.49÷0.79	0.23÷0.77	0.64÷0.95	2.3÷10
Juveniles								
17	31	31	27	27	27	28	23	28
0.28±0.05	0.86±0.01	0.76±0.02	0.43±0.01	0.47±0.01	0.50±0.01	0.76±0.04	0.89±0.006	1.48±0.27
0.06÷0.74	0.72÷1	0.57÷1	0.32÷0.54	0.38÷0.62	0.36÷0.59	0.46÷1.18	0.84÷0.94	-0.4÷4.1

Table 3a:Variation per selected character per sex (sample, average ± SE, range).

Measure	CL/BW	CW/CL	PW/PL	SH/CL	SH/PL	SH/CW	TL/PL	CT/TL	PL-TL
P value	<0.0001	N.S.	N.S.	<0.0001	<0.0001	<0.05	<0.0001	<0.0001	<0.0001

Table 3b: Statistical results of male-female difference (one tailed "t" test)

adult data (Table2a) and lower values of juvenile ratio records overlapped those of adults (Table 3a). Moreover, immatures showed a similar pattern than that of females (e.g. SH/CL) or males (e.g. SH/CW). In males CL, CW, PL (MRegr. Analysis, R^2=0.8533, N=27) and in females CL, CW, SH (MRegr. Analysis, R^2=0.9, N=41) were selected showing that only two parameters were similarly important for both sexes when at adult stage (i.e. CL and CW), that PL was important in describing male, that SH appeared exclusive characteristic in describing female (Table 4). On average some ratios were observed to be near to one (e.g. CW/CL: 0.72÷1.00; Table 3a), typically of newborn and 1-2 years old juveniles. Among ratios and differences, in males CW/CL, SH/CL, SH/CW, TL/PL, PL-TL (R^2=0.735, N=27) and in females SH/PL, TL/PL, PL-TL (R^2=0.73, N=41) were selected showing that several parameters were involved in explaining sexual differentiation (Table 5a, b).

DISCUSSION

We did not use the method of CARR (1952: 49) and FRITZ (1989: 145) because it foresees the maximum distance between the two most distant points of a considered area of the body and, in practice, it does not follow a real standardized procedure, such as the same measure at the same point of the animal's body (Figure 1), necessary in all population studies.

Model	Coeff.	F-remove	not in the model	Pcorr.	F-enter
CL	59.43	71.91	PW	0.08	0.15
CW	72.14	10.53	SH	0.36	3.29
PL	-53.19	7.83	TL	0.11	0.25
CT	0.15	0.53			

$R^2=0.85$ SE=33.23

Table 4a: Multiple Regression Analysis (Stepwise Backward Selection) of linear parameters of males.

Model	Coeff.	F-remove	not in the model	Pcorr.	F-enter
CL	29.68	5.00	PL	0.09	0.34
CW	43.70	7.92	PW	0.16	0.90
SH	40.87	6.41	TL	0.17	1.02
CT	0.24	2.21			

$R^2=0.90$ SE=43.40

Table 4b: Multiple Regression Analysis (Stepwise Backward Selection) of linear parameters of females.

If a parameter (e.g. carapace width) is only taken where the body is just slightly greater or where a particular specimen is relatively larger, we could much more overestimate (or underestimate if narrower) the considered record, change the fixed point or line from which take measurements and mislead the overall and averaged measure. Whilst male carapace and plastron are different with respect to that of female (Table 2a), male carapace and plastron shapes are not different from that of female (Table 3a). Moreover it is so simply possible standardize most bias of data recording, and this method provides a fixed point from which to take most parameters. On the contrary, it is evident that we can underestimate maximum values, not necessary in this study and approach. As a trial, we measured some specimens under CARR (1952) and FRITZ (1989) suggestions obtaining no statistical difference around mean and standard error values, whilst obvious discrepancy does exist for extreme values (i.e. maximum and minimum). In other words it should be possible compare available mean values of a given population, if necessary.

Among selected parameters PL was considered typical in identifying male and SH was typical in identifying female. Moreover males were described by carapace shape and tail length, TL beeing important for only its first portion, characterized by penis presence (e.g. from the posterior border of the plastron to the anterior margin of the cloacal opening): as expected, the remaining part (i.e. CT) was not significantly sex determinant (Table 2a). Females were described by CL, CW and SH, where SH was much greater and significantly different from that of male (the difference between sexes was 23.22%, the highest among selected parameters) (cf. Figure 2). Diagnostic sexual ratios for males and females allowed also the comparison among adult specimens of different size (and age), avoiding most of allometric relationship of little forms (Figure 3, 4) (ZUFFI, unpublished data).

As a consequence, the use of ratios should be more favourable than the only use of linears, in analyzing and comparing geographical populations (ZUFFI & GARIBOLDI, 1993).

Among larger immature specimens, we observed a wide and evident overlap with respect to some adults as if these former had not developed yet external sexual features, having just reached a proper size (Table 2a, 3a).

Model	Coeff.	F-remove	not in the model	Pcorr.	F-enter
CW/CL	0.0895	14.39	CW/PL	0.08	0.14
SH/PL	-0.4496	24.92	SH/CL	0.36	2.98
SH/CW	0.3482	20.28	CT/TL	0.15	0.48
TL/PL	-0.3847	61.1			
PL-TL	-0.0347	64.08			

$R^2=0.74$ SE=31.76

Table 5a: Multiple Regression Analysis (Stepwise Backward Selection) of ratios parameters of males.

Model	Coeff.	F-remove	not in the model	Pcorr.	F-enter
SH/PL	420.51	4.07	CW/CL	0.08	0.25
TL/PL	2027.25	110.66	PW/PL	0.03	0.02
PL-TL	168.410	233.63	SH/CL	0.19	1.36
SH/CW	0.20	1.40			
CT/TL	-321.83	4.72			

$R^2=0.73$ SE=0.006

Table 5b: Multiple Regression Analysis (Stepwise Backward Selection) of ratios parameters of females.

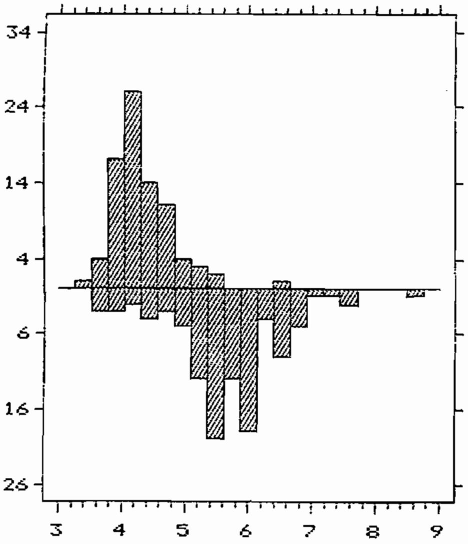

Figure 2: SH comparison between sexes in *Emys orbicularis* (males, N=83; females, N=106). y axis: number of animals.

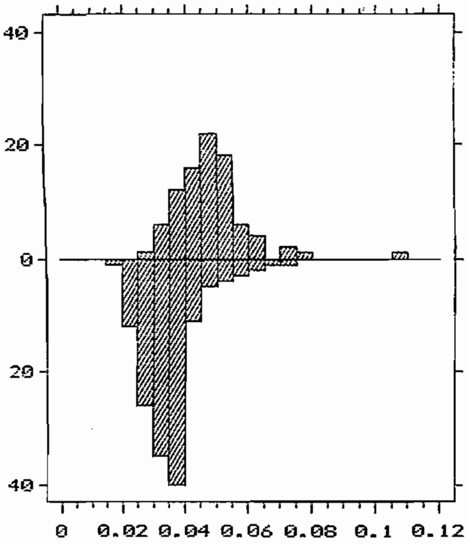

Figure 3: CL/BW comparison between sexes in *Emys orbicularis* (males, N=89; females, N=141). y axis: number of animals.

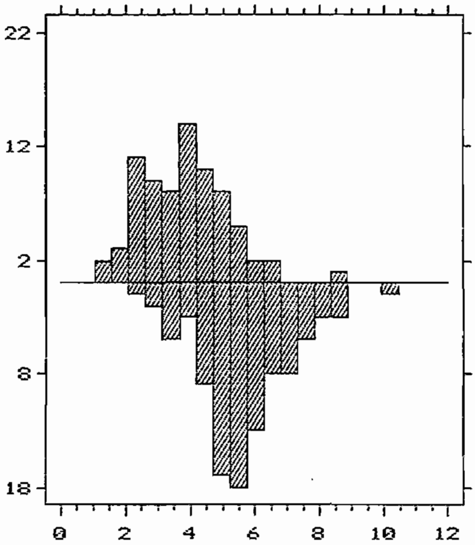

Figure 4: PL-TL comparison between sexes in *Emys orbicularis* (males, N=75; females, N=96). y axis: number of animals.

The physiological change between the juvenile-immature condition and the immature-adult one should be carefully investigated, under both biochemical and ethological experiments.

It must be stressed that sexual genotypic determinism is temperature dependent (BULL & VOGT, 1979; ZABORSKI et al., 1988), that intersexes can occur and their morphological phenotypes are not still described. We could in fact be expected in observing, for instance, specimens morphologically feminine and genetically masculine (as already suggested by B.Lanza, March 1992, pers. comm.). Morphological differences should be investigated also throughout a gonad development control (ZABORSKI et al., 1988), specific chromosomal analysis and ecological comparisons of home habitat.

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The structure of the Adrenal gland in eight species of the family Scincidae. Electron microscopic studies.

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Abstract: The adrenal morphology and fine structure, the ratios between adrenalin and noradrenalin cells and their relationship with philogenetic position were studied in eight species of the family Scincidae: *Eumeces schneideri, Eumeces inexpectatus, Eumeces obsoletus, Scincus scincus, Chalcides chalcides vittatus, Sphenops sepsoides, Mabuya quinqueteniata* and *Tiliqua gigas*.
The two kinds of chromaffin cells are easily distinguished in all the species. The noradrenalin cells outnumber the adrenalin cells in *C. chalcides vittatus* in *E. inexpectatus* and in *T. gigas* . In *E. obsoletus* and *S. sepsoides* the NA and the A cells are almost even,while in the other species the A cells prevail on the NA cells.
In *S. sepsoides*, together with A and NA cells, are present also SGC (small granule containing) cells.
Also the steroidogenic tissue shows at the E.M. some differences in the different species examined. The greatest differences regard the morphology of mitochondria, which are usually roundish in most species, but appear elongated in *Mabuya quinqueteniata* and do not always show the tubular cristae typical of the mitochondria of steroid producing cells.

Key words: adrenal gland, chromaffin cell, reptiles, Scincidae.

INTRODUCTION

The adrenal gland of squamates is usually made up by a steroidogenic parenchyma and by a ribbon of chromaffin cells located on the dorsal margin of the gland. The chromaffin ribbon is generally completed by small chromaffin islets scattered between the steroidogenic cords. While the general organization of the gland is rather uniform in most species, noticeable variations can be noticed as far as the number and the distribution of the adrenalin and noradrenalin cells is concerned. (VARANO and LAFORGIA, 1976)

A study of the NA/A ratio in numerous species of squamates has pointed out that there is a relationship between this ratio and the philogenetic position of the species. Species with a ratio close to 1/1 are usually recently evolved species,while species with high numbers of NA cells are those of more ancient origin.(LAFORGIA and VARANO, 1982, LAFORGIA et al., 1982, 1983, 1990a, 1990b, 1991, VARANO and LAFORGIA 1991)

In a preceeding study on six species belonging to the family Scincidae, subfamily Scincinae (LAFORGIA et al., 1990b) the AA found fairly high NA/A ratios (around 2/1) , in accordance with GREER (1970) who considers the subfamily Scincinae the most primitive of the group.

In the present paper were studied specimens belonging to six species of the subfamily Scincinae and to two species of the subfamily Lygosominae.

MATERIALS AND METHODS

The adrenal glands of the following species were investigated: *Scincus scincus, Eumeces schneideri, Eumeces obsoletus, Eumeces inexpectatus, Sphenops sepsoides, Chalcides chalcides vittatus.*

For each species,at least two specimens of each sex were examined.The animals were killed by decapitation. For light microscopy the adrenals were fixed in a mixture of potassiumdichromate and sodium sulphate buffered at pH 4.1 with 5M acetate buffer,to which 10% formaldehyde was added before use (WOOD, 1963).The glands were embedded in paraffin,sectioned at 6-7 um and stained with one of the following solutions which allow to differentiate NA cells from A cells:

1) a mixture of eosine-aniline blue,buffered at pH 4 with 5M acetate buffer ,staining NA cells gold and A cells orange-red.

2) Giemsa solution modified according to PEARSE (1960),staining NA cells dark green and A cells light green.

3) Mallory trichromic stain.NA cells appear gold-yellow and Acells appear red.

For electron microscopy the adrenals were fixed in 2.5% glutaraldehyde in phosphate buffer, post fixed in 1% OsO_4 and embedded in Epon 812 (COUPLAND et al., 1966). The sections were stained with uranyl acetate or lead citrate and observed under a Philips EM 301 electron

Figure 1. x22000. Adrenal gland of *Chalcides chalcides vittatus*. Notice in the steroidogenic cells the elongated mitochondria and some very large lipid droplets

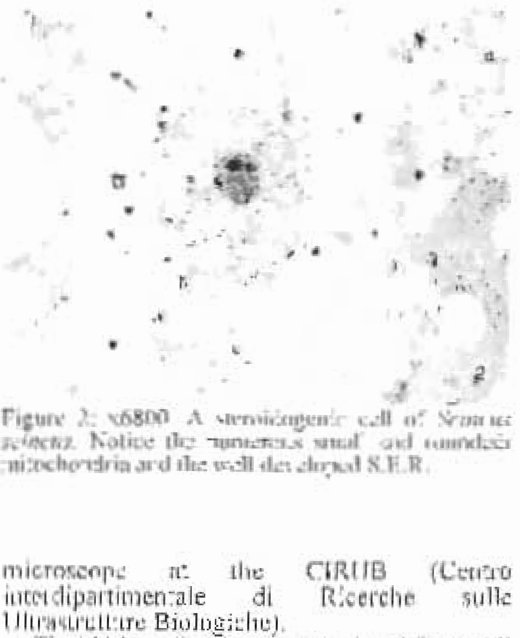

Figure 2: x6800. A steroidogenic cell of *Scincus scincus*. Notice the numerous small and rounded mitochondria and the well developed S.E.R.

microscope at the CIRUB (Centro interdipartimentale di Ricerche sulle Ultrastrutture Biologiche).

The NA/A cell ratio was calculated from cell counts,usingevery tenth transverse section from the whole gland of each specimen.

The data reported here apply to both sexes since differences between sexes and between specimens were very small and hence not quantified.

RESULTS

Light microscopy

The glands of the species examined are not very elongated and are always surrounded by a thin envelope of connective tissue.In all the species the steroidogenic tissue is made up by prismatic cells rich in lipid droplets with basal nuclei. The steroidogenic cells are arranged to form anastomosing cords, generally consisting of two cell rows, separated by small blood vessels

Histochemical methods allow to identificate two different chromaffin cells : the noradrenalin cells (NA) and the adrenalin cells (A). They are generally arranged to form a dorsal ribbon with digitations deepening between the steroidogenic cords and small islets scattered between the interrenal cords. Small clusters of chromaffin cells, made of A and NA cells, are present on the ventral surface of the gland.

Eumeces schneideri and *Tiliqua gigas* make exception to this pattern: in *Eumeces schneideri* the chromaffin envelope is continuous and covers all the gland; in *Tiliqua gigas*, instead, the chromaffin cells are present mainly at the two poles.

The NA cells are mostly located in the outer layer of the ribbon or of the envelope while the A cells are present in the inner layers and in its digitations.

The chromaffin islets are exclusively made of A cells ; they are numerous and of medium size in all the species examined with the exception of *Tiliqua gigas* where they are almost absent.

The different distribution of the chromaffin cells in these species influences the adrenalin/noradrenalin cell ratios (NA/A), which were calculated as follows:

SUBFAMILY Scincinae	
Scincus scincus	0.37/1
Eumeces schneideri	0.44/1
Eumeces obsoletus	1.3/1
Eumeces inexpectatus	2.4 /1
Sphenops sepsoides	1.2 /1
Chalcides chalcides vittatus	5.8 /1
SUBFAMILY Lygosominae	
Mabuya quinquetaeniata	0.65/1
Tiliqua gigas	2.0 /1

Electron microscopy

The comparison of the fine structure points out that there are clear morphological differences in the steroidogenic cells of most of

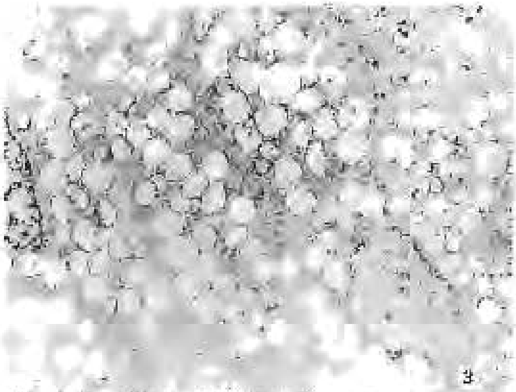

Figure 3: x6800. The mitochondria are small, roundish and numerous also in *Eumeces shneideri*. In the steroidogenic tissue are present an extremely large number of lipid droplets.

Figure 5: x3025.Notice the well developed tubular cristae in the mitochondria of *Eumeces obsoletus*.

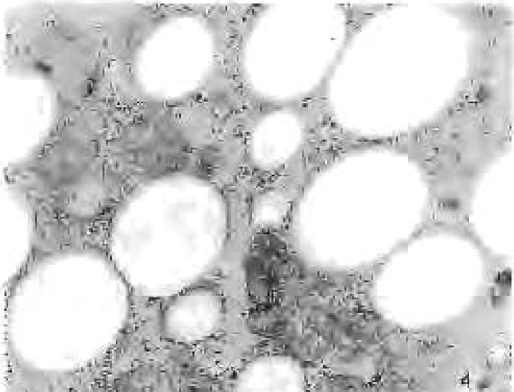

Figure 4: x2200.The steroidogenic cells of *Sphenops sepsoides* show large lipid droplets and roundish mitochondria with tubular cristae.

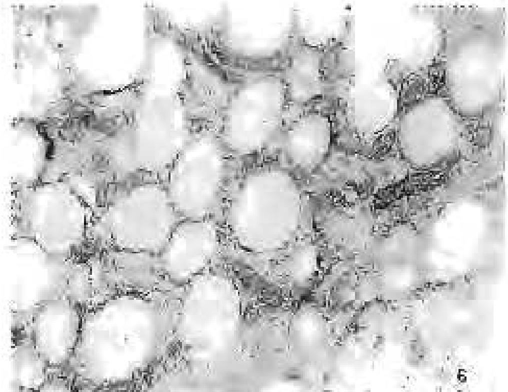

Figure 6: x22000.Despite of the other species the mitochondria of *Mabuya quinquetaeniata* are elongated with narrow cristae.

the species studied; all the species of the subfamily Scincinae, with the exception of *C. chalcides vittatus* (Figure 1) show steroidogenic cells with roundish mitochondria which are small in the species with low NA/A ratios (*S. scincus* and *E. schneideri*), (Figures 2, 3) . The mitochondrial morphology is very similar in *S. sepsoides* and *E. obsoletus* (large and numerous tubular cristae) which have similar NA/A ratios (Figure 4, 5).

In the subfamily Lygosominae, instead, *Mabuya quinquetaeniata* features in the "cortical" cells elongated mitochondria with narrow cristae (Figure 6), while *Tiliqua gigas* shows roundish mitochondria with large tubular cristae (Figure7).

The lipid droplets are particularly large in *S. sepsoides* (Figure 4) and in *C. chalcides vittatus* (Figure 1).

The two kinds of chromaffin cells have in all the species studied a very similar fine structure. The main difference between A and NA cells is constituted by the chromaffin granules. In A cells the granules are roundish, of medium electron density and show a core made up by very fine granules, separated from the limiting membrane by a small electron lucent space. In NA cells the granules are of variable shape and very electron dense. (Figure 9) The core doesnot show any structure and adheres strictly to the

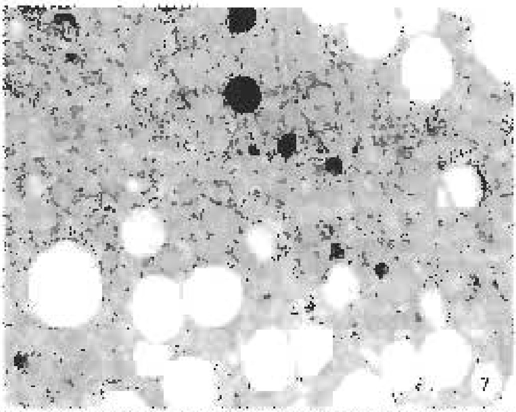

Figure 7: x18200. Steroidogenic cells of *Tiliqua gigas* show roundish mitochondria with large tubular cristae.

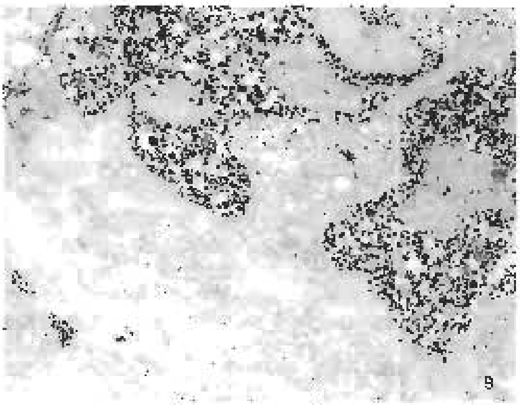

Figure 9: x3800. The chromaffin tissue of *Tiliqua gigas*. Notice the two kinds of chromaffin cells. The NA cells, very numerous, show electron dense granules, while the A cells feature in their cytoplasm granules of lower electron density.

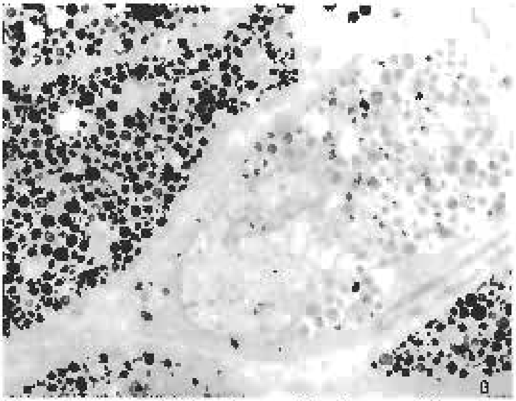

Figure 8: x9000. The chromaffin tissue of *Eumeces obsoletus*.In the cytoplasm of noradrenalin cells (NA) are present very electron dense granules, while in the adrenalin cells (A) the granules appear less electron dense

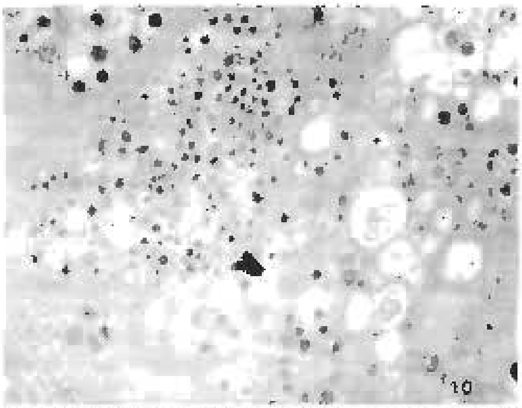

Figure 10: x19500. Notice a small granule containing cells (S.G.C.) in the chromaffin tissue of *Sphenops sepsoides*.

limiting membrane which is not distinguishable (Figure 8). Sometimes the NA granules appear enlarged with the electron dense core eccentrically placed .

S. sepsoides features also some S.G.C. (small granule containing) cells wich were not observed in all the other species studied (Figure 10).

DISCUSSION

The distribution of the adrenalin and noradrenalin cells and the NA/A ratios observed in the specimens of the two subfamilies in the present paper confirm only partially the results of a preceeding paper on Scincidae (LAFORGIA et al., 1990) and those of GREER (1970). Actually, while GREER (1970) considered the subfamily Scincinae and the genuses *Eumeces* and *Scincus* the most primitive of the family,our results and the NA/A ratios calculated are in agreement with Greer's findings as far as the primitivity of the subfamily Scincinae is concerned but do not agree with him on the primitivity of the genuses *Eumeces* and *Scincus*

Other species (*C. chalcides vittatus*) show much higher NA/A ratios, indicative of a more primitive ancestry. Some species of the genus *Eumeces* (*E. obsoletus* and *E. schneideri*) and the *Scincus* studied appear, on the basis of the NA/A ratios, recently evolved species despite the ancestry of the subfamily; our morphological data are confirmed by a caryological study (CAPUTO et al., 1993) which points out the heterogeneity of the genus *Eumeces* and the close similarity of the caryotypes of *E. schenideri* and *S. scincus*. Finally the high NA/A ratio of *C. chalcides vittatus*, which, according to PASTEUR and BONS (1960) and to Pasteur (1981), is a species belonging to the fourth group of *Chalcides* confirms our statement that the first group is more evoluted than the fourth (LAFORGIA et al., 1990).

Ultrastructural studies on steroidogenic and chromaffin cells point out some morphological differences and similarities between all the species studied; all the species of the subfamily Scincinae, with the exception of *C. chalcides vittatus*, show steroidogenic cells with roundish mitochondria. The fine morphology of the steroidogenic tissue is particularly similar in the two species with low NA/A ratios (*S. scincus* and *E. schneideri*). A similar fine structure is also featured by *S. sepsoides* and *E. obsoletes* which also have similar NA/A ratios. In *S. sepsoides* are present also some S.G.C. (small granule containing) cells which were occasionally described in other reptiles (UNSICKER, 1976). Some ultrastructural similarities were also observed in the steroidogenic cells of the two species of the subfamily Lygosominae despite the very different NA/A ratios.

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Use of the skeletochronological method for ecodemographical studies on *Salamandra salamandra gallaica* from Portugal

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Abstract: Several problems may be encountered when using the skeletochronological method for age determination. We attempt to show how they were solved for the study of a *Salamandra salamandra gallaica* population from Sintra, Portugal. A three-year capture program (from October 90 to April 93), which yielded a total of 642 captures of 397 individually marked animals, allowed us to infer about the period and periodicity of formation of the Lines of Arrested Growth (LAG). Comparisons between LAG patterns of successive years in different phalanges from the same animal showed that these are normally formed at the rate of one LAG per year, during summer (the period of inactivity). LAG decipherment problems included: presence of endosteal bone in almost 90% of the individuals; partial endosteal resorption of the innermost LAGs, and the enumeration of the peripheral LAGs in old animals. Since these problems were solved, we used the data to present some demographic parameters of this population. The longevity maximum was 18-19 years and 1/3 of the sample was 10 or more years old. There was a sexual dimorphism - males tended to be smaller (mean SVL 96.4 vs. 101.6 mm for females), to mature one year earlier (at 2-3 vs. 3-4 years), and to live longer (mean adult age 9.6 vs. 7.6 years). These differences might result from the adoption of different life history strategies by each sex.

Key words: Caudata, Salamandra, skeletochronology, demography, sexual dimorphism

INTRODUCTION

The skeletochronological method of age determination is based on the presence of periodic growth marks in the skeletal tissue (CASTANET *et al.*, 1977). It is assumed in amphibians, and was demonstrated for some species (FRANCILLON, 1979; HEMELAAR, 1981; CAETANO & CASTANET, 1987), that the cyclic alternation between periods of arrested and active growth that happens yearly in the temperate areas will correspond, in the bone tissue, to the alternation of a darkly staining band - the Line of Arrested Growth (LAG) - with a lighter and broader band - the zone (CASTANET, 1975) and represents one cycle of growth, normally one year.

This method has been used with considerable success in several urodele species (e.g. SMIRINA & ROCEK, 1976; HALLIDAY & VERRELL, 1988; FRAGEOLE & LECLAIR, 1992; CAETANO, 1990) and the use of phalanges has also been successfully tested (SMIRINA, 1983; GIBBONS and MCCARTHY, 1984; RYSER, 1988; BASTIEN & LECLAIR, 1992; CAETANO & CASTANET, 1993).

We tested the viability and validity of the method for *Salamandra salamandra gallaica* using phalanges from a population that was sampled during 3 consecutive years. The data were then used to estimate some demographic parameters, such as age at first reproduction, longevity and relation of size to age.

Although *Salamandra salamandra* is a well known and widespread urodele in Europe, skeletochronological analyses using long bone cross-sections are just beginning (WARBURG, 1992). These are the first data obtained for this iberian subspecies by skeletochronology.

MATERIAL AND METHODS

The animals were captured in a deciduous forest of Sintra, Portugal, about 20 Km NW of Lisbon (altitude 350m). The area is characterised by a mild and humid climate, only interrupted by the summer drought.

Monthly visits were made from October to April of three successive years (1990 to 1993). Simultaneously, a search for dead animals was conducted in wells and alongside the roads. A total of 397 different animals were caught.

Each animal was dorsally photographed, measured (snout-vent length, to the nearest mm), sexed, toe-clipped and released back in the place of capture. Recaptures were individually recognized due to its unique dorsal spot pattern. When the time gap between two captures of the same animal exceeded one year, it was toe-clipped again. We also took the femur, humerus and phalanges of 10 dead animals in order to make comparisons between different long bones.

A subsample of 156 fingers was taken from the 1991-92 sample; after being cleaned, the bones were decalcified in 3% nitric acid for 4 to 6 hours, rinsed overnight in running water and cross-sectioned at the mid shaft diaphisis in 15m width sections. These were stained with Ehrlich's haematoxylin for 20 minutes and then mounted in Aquamont.

Individual ages were estimated by counting the number of LAGs present in the periosteal bone, the bone periphery being not counted as a LAG. Ages were established independently by the two authors, after which they were compared with each other and confronted with the ecological and morphometrical data.

RESULTS AND DISCUSSION

Histological description

The long bone diaphyses of *S. s. gallaica* were similar to those of other urodeles of similar size (RICQLÈS, 1975; CASTANET, 1975; FRANCILLON & PASCAL, 1985; CAETANO *et al.*, 1985; MIAUD, 1991), consisting of avascular compact bone rarely crossed by radial blood vessels that reach the marrow cavity. Osteocyte densities were low and their distribution was random within the bone tissue. In almost all sections, endosteal bone was present. This is produced centripetally to the inside of the marrow cavity, while the centrifugal, appositional growth of the periosteal bone is the responsible for the increase in diameter of the diaphisis as the animal grows old (Fig . 1).

All the different long bones from the same animal exhibited the same number and relative arrangement of LAGs, except for the third, more distal phalanx that is subject to a more intense remodelation. The humerus seemed to be the best piece for skeletochronology, both because of the symmetry of its sections and because it displayed well defined and contrasting LAGs. The posterior choice of the phalanges to work on was done because their use didn't implied the sacrifice of the animals.

Encircling the often irregular marrow cavity, and establishing the separation between endosteal and periosteal bone, there was a very haematoxylinophilic and irregular line - the cementing line of resorption (CASTANET, 1975) - an aperiodic structure whose asymmetry and irregularity tended to be greater in older individuals. It's progression towards the periphery depends upon the endosteal resorption process (Fig. 1).

There was no evidence for a metamorphosis LAG - several larvae were raised in captivity under natural conditions of photo and thermoperiod and none of the newly metamorphosed animals showed any evidence of it. Nevertheless, in animals older than 2 years, the marrow cavity and the cementing line of resorption were usually surrounded by a darker band (Fig. 1). This should not be considered as a LAG, but as a local differentiation of the bone tissue laid in the earlier phases of the terrestrial life.

The first 1, 2 or 3 LAGs were comparatively broader and less defined than the others, which could be due to the high growth rate that is thought to be characteristic of the young immature animals, the blurring being the result of a reduction in the speed and not of an arrestation of bone growth.

The sudden decrease in bone growth that was suggested by the approaching of the peripheral lines in opposition to the first 3, 4 or 5 ones is usually related to the attainment of sexual maturity (CAETANO *et al.*, 1985; CASTANET, 1986; AUGERT, 1991).

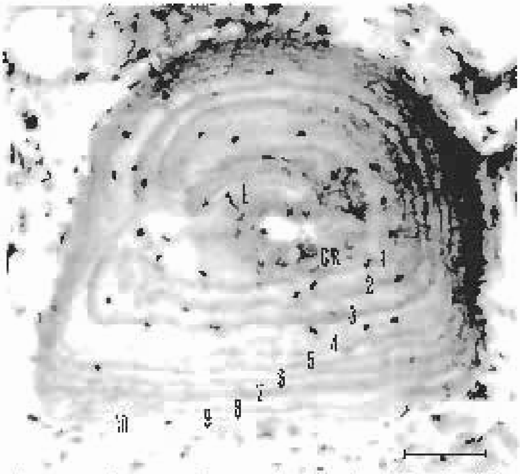

Figure 1: Cross-section at the diaphysis level of the first phalange of a 10 year old *Salamandra salamandra*. Numbers refer to Lines of Arrested Growth (LAG). E- endosteal bone, CR- cementing line of resorption. Scale bar = 10µm.

As the animal gets old, the structure of the newly formed bone changes gradually from a parallel fibers type to lamellar. This kind of bone tissue, deposited when the growth rate is very slow (RICQLÈS, 1975), was characteristic of animals older than 8-9 years, and it's appearance coincided with an extreme approaching of the LAGs.

Periodicity of LAG formation

Confirmation that only one zone and one LAG are formed annually was provided by the comparison of the LAG pattern of phalanges from the same animal, when this was captured in different years (the same was done with *Bufo bufo* by HEMELAAR, 1981), complemented with the histological observations of some animals that were raised in captivity, under semi-natural conditions, for two years. We found that whenever there was a one summer or a one summer/one winter gap between the two captures, there was also present one additional LAG; two additional lines were seen in the sections with a two summer difference; no additional line was seen in sections with one winter difference. These results confirm both the rhythm and period of LAG formation - one LAG laid per year during the summer - and are in accordance to what we know about the activity cycle for this population: the period of LAG formation (of arrested growth) should coincide with the inactivity period, the summer.

LAG interpretation problems

We have already seen that aperiodic lines such as the cementing line of resorption and the metamorphosis LAG constituted no problem to LAG enumeration.

Endosteal resorption was a very common phenomenon - 95% of the animals showed partial LAG resorption. In the majority of the cases resorption is asymmetrical and so there were cases of partial resorption of the first (71%), second (21%), and even the third LAG (3%); nevertheless, there was never a total resorption of any LAG. This means that in every case ages could be directly estimated.

Occasionally (in less than 10% of the sections), the regular LAG pattern was disturbed by the presence of two LAGs very close to one another, often showing fusion at some particular point. These were considered as only one LAG that was doubled, corresponding to just one growth arrestation period (CAETANO *et al.*, 1985; CAETANO & CASTANET, 1987). The recognition of double lines was easy among the first 7-8 LAGs, but then the lines were so close that the distinction between simple and double LAGs became very difficult.

The enumeration of the peripheral LAGs in old animals was rendered difficult by its extreme proximity, a result of the slow growth characteristic of the older ages. This problem was only solved by a cautious observation of the sections at different magnitudes, but it was still the responsible for virtually all the disagreements between the countings.

The majority of the problems found during the process of LAG counting could thus be easily solved, which explains the high degree of agreement between the independent readings - 89%; 82% of the discordances were due to a one-year difference miscounting of the peripheral lines.

This high degree of agreement, together with the confirmation of the period and rhythm of LAG formation constitute good indicators of the validity of the method for this population.

Demographical data

Age structure

The age structure is depicted in Fig. 2. There was a paucity of juveniles that is also commonly found in skeletochronological studies (SMIRINA & ROCEK, 1976; FRANCILLON & PASCAL, 1985; MIAUD, 1991) and is usually explained by differences in conspicuity, and therefore in catchability. In this population this difference could be due to a higher mobility expressed by the adults during the breeding period.

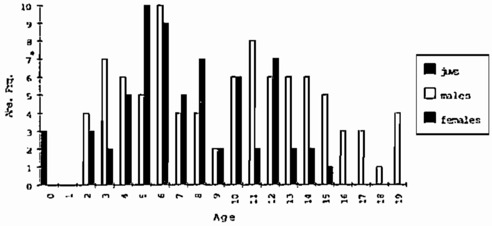

Figure 2: Age frequencies of the sample. Males, females and juveniles are shown separately. Note the bimodal distribution.

The age distribution was roughly bimodal, with a peak at 5 years to females and 6 to males, and another at 11 years to males and 12 years to females. This heterogeneity may be caused by inter-annual differences in juvenile recruitment and this is probably related with the abundance/shortage of bodies of water during the reproduction period. Similar patterns were found for other species under a fluctuating environment (SMIRINA & ROCEK, 1976; DODD, 1993).

It is also interesting to note the high longevity of this population: more than 1/3 of the animals were 10 or more years old; the longevity maximum was of 18-19 years to males and 14-15 years to females. For urodele species, longevity usually increases with medium adult size (FRANCILLON *et al.*, 1990; MIAUD, 1991), the values we've came to are in agreement with this hypothesis.

Males appear to live longer than females: they were both the oldest individuals and presented higher frequencies (relative as absolute) in the highest age classes; their mean adult age was 9,6 vs. 7,6 years for females (Mann-Whitney-U test, P<0.04). The difference in longevity might explain the unbalanced sex-ratio of the sample - approximately 1:1,4 in favor of males. Another cause might be a sampling error due to different conspicuity of the sexes, specially due to sexual differences in activity at the beginning of the breeding period.

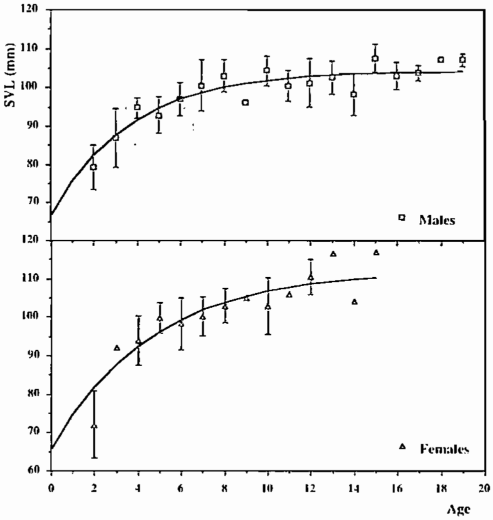

Figure3: Relation of age to snout-vent length. Vertical lines show the mean 1 s.e. Curves show Von Bertalanffy's model. For ages under 2 years, data were pooled. Values for the growth curves were obtained from the equations:
Females $Lt=112.2[1-e \exp[-0.215 (t+4.09)]]$
Males $Lt=104.2 [1-e \exp[-0.275 (t+3.69)]]$

Age and size, age at sexual maturity

There was a considerable overlap of body sizes within every post-maturation age-class, for males as for females (fig. 3), which confirms the inadequacy of utilizing body size frequencies for age estimation in this species. The amount of overlap suggests the importance of epigenetical, unpredictable factors in the control of the growth process.

Females tended to grow bigger than males (fig. 3), but this difference was found to be of no significance (t-test for pooled adult -more than 5 years old- age-classes). If we take Von Bertalanffy's theoretical growth curves, females have an infinite length of 112 vs. 104 mm for males. This difference is determined by an earlier inflexion of the male's growth curve that may be related with the attainment of sexual maturity.

Age at first reproduction can also be related with the sudden decrease in bone growth that is suggested by the approaching of the peripheral lines in opposition to the first ones. We counted the last line after which the sudden approach of the LAG could be easily seen; intermediate classes were used for cases of difficult interpretation: 54% of the animals started to reproduce during the third, and 38% during the fourth year of life. Males tended to mature 1 year earlier than females, at 2-3 vs. 3-4 years old (Mann-Whitney-U test, P<0.05).

Life histories

For this population it is now possible to point a different life history strategy for each sex. Males tend to be smaller, to mature one year earlier and to live longer, while females tend to grow bigger, to mature later, but to live shorter. The earlier maturation of males, commonly found in amphibians (e.g. MIAUD, 1991; BRUCE, 1993) results in an earlier shift in resource allocation from somatic growth to reproduction. The consequent reduction of growth rate is thought to be the cause of the smaller adult size of the males. Females keep rapid growth for one year more, which enables them to become larger. The delay of the first reproduction should be advantageous in a long lived species because, as in many other herp species, female size is probably positively correlated with fecundity (HALLIDAY & VERRELL, 1988).

The sexual difference found for the longevity is not a very common phenomenon - usually the later maturing sex grows bigger and lives longer (BRUCE, 1993) but here females live clearly shorter than males. The answer to this problem lies perhaps in the ecology and reproductive biology of the species. While the other urodeles are semi-aquatic and oviparous, *S. salamandra* is entirely terrestrial and ovoviviparous, and both of these reasons might explain a higher mortality among females. In opposition to other urodele species, only female salamanders need to move yearly to the water bodies. So, while males remain in their relatively safe home ranges, females are more prone to accidents, such as drowning in natural pitfalls or wells and being runned over by cars. Furthermore, perhaps there is an ovoviviparity-related cost that oviparous species do not have to support.

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Llorente *et al.* (Eds). *Scientia Herpetologica 1995:141-153*

Population density, space arrangement and sex ratio for sympatric populations of the three species of newts in two puddles in Žumberak, Croatia

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Abstract: This work refers data concerning with an investigation of the sympatric populations of the Southern Smooth newt (*Triturus vulgaris meridionalis*), the Alpine Crested newt (*Triturus carnifex*) and the Alpine newt (*Triturus alpestris*) in Žumberak, near Zagreb, Croatia. The investigations has been accomplished between 1988.and 1990. at two habitats (the puddle by Budinjak and the puddle in Gornji Oštrc). The number of males and females of all three species and population density has been measured in both puddles. The most frequent species in the puddle by Budinjak. Was established to bee Alpine newt, and in the puddle in Gornji Oštrc Alpine Crested newt. Probably, more specimens and larger population density in the puddle by Budinjak is the consequence of the larger surface, deeper bottom and less aquatic vegetation. By netting and observing newts during aquatic faze of life the space arrangement of all three species has been recorded. The high degree of disconnection among the Smooth and the Alpine Crested newts populations exist in the puddle near Budinjak, while the Alpine newts population is spread all over the puddle. All three species are equally spread over the puddle in Gornji Oštrc. The effects of these differences are probably the differences in competition among species on these two localities. The sex ratio for all of the three species of newts has been established to be near 1:1 (it differ significantly from 1:1 only in the Alpine newt population from the puddle by Budinjak). During the whole season the number of the Alpine newts males in the both puddle has been slightly over the number of females, and in the both puddles the whole number of Alpine Crested newts females has been slightly over the number of males. The sex ratio in the Smooth newts and the Alpine Crested newts populations has been significantly change several times during the season.

Key words: Triturus, population density, sex ratio, space arrangement, Croatia.

INTRODUCTION

The objectives of this work were investigations of: number, density, sex ration, and in puddles distribution of sympatric populations of Southern Smooth Newt, *Triturus vulgaris meridionalis* (Boulenger, 1882); Alpine Crested Newt, *T. carnifex* (Laurenti, 1768); and Alpine Newt, *T. alpestris alpestris* (Laurenti, 1768).

The research were conducted on two puddles, one by Budinjak village and one in Gornji Oštrc village, at the Žumberak Mt. area (Figure 1). This mountainous region is a border area between Slovenia and Croatia.

Budinjak village is situated 720 m above sea level at the center of Žumberak Mt. (UTM mark: WL37), about 30 km eastward of Zagreb. Syntopic populations of newts were investigated in puddle located 500 m from the village and 50 m of the road to Dane village. Nearby are several other puddles. One is just by the road to Gornja Vas, about 250 m from Budinjak village. Only the alpine newt was found in it. Second one is in Bratelji village, one kilometer W-NW from Budinjak village, with the alpine newt too. Third puddle is a complex of two smaller puddles and one spring, located near Dane

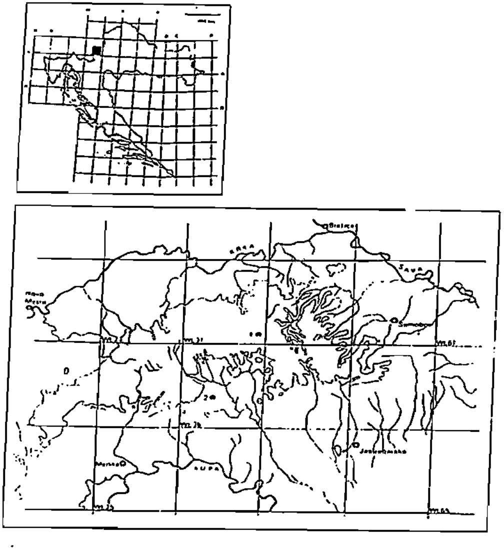

Figure 1: Settlement of the Žumberak region and the Žumberak mountain: site 1 - Budinjak site 2 - Gornji Oštrc.

village about 1.5 km W-SW from Budinjak village. All three species of newts were found in it.

The researched puddle is placed on karst foundation, more precise, on Cretaceous limestone ([iki} et al. 1972). Closely are several distinct karstic shapes. This foundation was in favor of the front of Dinaric Alps mountainous beech forest (*Dentario enneaphylle - Fagetum*) development (Marin~ek 1987). About 200 m S-SW from the puddle, this forest community changes to related, somewhat thermophilic *Haquetio - Fagetum* community.

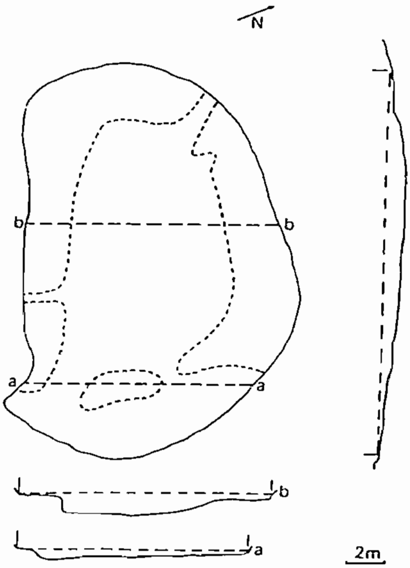

Figure 2: Puddle near the Budinjak: ---- vegetation border.

This puddle (Figure 2) has length of 19.80 m, with widest transect of 13.90 m, and total area of 220 m^2. It is east - west extended. The depth increases from the east to the west. The deepest spot is 100 cm. Almost 28 percent of the puddle (62 m^2) is deeper than 60 cm. The water level fluctuation was minimal during research period, even during the drought in summer of 1989, only for ± 5 cm. Most of the puddle bed is covered with a mud layer, 20 to 25 cm deep. Rest of the bottom is covered with sand and rock fragments. In shallow parts, water vegetation is growing: at eastern edge, on depth of 10 to 40 cm, *Zannichellia palustris*; along the most part of left bank, and by the western bank edge *Scirpus sylvaticus*. Along the right bank and on protruding part of the left bank, *Gliceria fluviatilis* is present. Other plants: *Juncus conglomeratus, Rumex cf. conglomeratus, Ranunculus acris, R. repens, and Carex hirta* are randomly distributed at different parts of the puddle edge or nearby area. In deeper parts of the puddle plants are not present. The puddle is a permanent watering place for cattle, so the water transparency is low (not more than 10 cm) and quite eutrophic (hypertrophic). In the puddle and its nearby surroundings we recorded: *Tubifex tubifex*, Cladocera, Odonata and Trichoptera larvae, Notonectidae imagoes, eggs, tadpoles and adults of *Bombina variegata, Bufo bufo, Hyla arborea, Rana temporaria*, adult specimen of *Natrix natrix, Sorex araneus*; some of them are prey of newts and some their predators. Recorded were *Microtus arvalis, Apodemus sylvaticus*, and several song bird species as well.

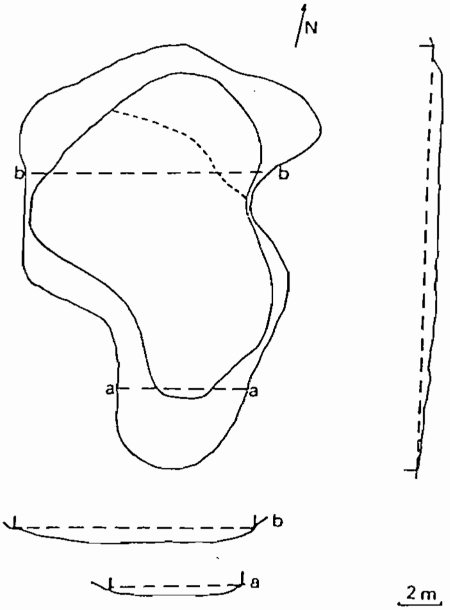

Figure 3: Puddle in the Gornji Oštrc: __ pond borders (high and low water levels) ----- vegetation border.

Gornji Oštrc is a village 5 km SE of Sošice village (about 40 km eastward from Zagreb). The puddle is located 7 km from a road, on altitude of 410 m above sea level (UTM mark: WL36). The puddle is extended in north - south direction, parallel with a road. Immediate by the puddle is *Hacquetio - Fagetum* forest. It is of irregular shape (Figure 3), 19.60 m long, and widest 13.60 m. At the beginning of research, the puddles area was 172 m^2 (what is 48 m^2 less than the puddle by Budinjak). The deepest spot, at the beginning of research, was between 60 and 70 cm. About 40 percent of the puddle area (about 69 m^2) is deep about 60 cm. In May of 1990, when catch is performed in order to count newts in this puddle, water level was much lower, so that the puddle area was only 104 m^2. Water depth increases toward the northern bank and from side toward the puddle center. The majority of puddle bed is covered with different thickness of mud layers (20 cm at most). Boulders are placed at the bottom on few places. Southwestern part of the puddle is partially fill in with gravel, for easier cattle approach. In this puddle, free water area is limited because the most part is overgrow with algae of the genus *Chara*. Except this algae, in the puddle are dominant: planktonic green alga *Oedogonium sp.*, bluegreen algae *Oscillatoria princeps*, and diatom alga *Fragilaria crotonensis*. At the northern puddle part, *Typha latifolia* is penetrating into the water. Except this species, in and around the puddle, following plants are randomly distributed: *Juncus articulatus, J. effusus, J. conglomeratus, Agrostis stolonifera, Alisma plantago aquatica, Trifolium fragiferum, Bidens tripartitus, Ranunculus sardocis, and Polygonum nite*. This puddle is also very eutrophic and rich with organic matter in process of decay. In the puddle and around it, significant number of different animal species were recorded too. The most numerous insect larvae were those belonging to dragonflies and caddis flies. The imagoes of *Notonecidae*, *Dytiscus marginalis*, which is quite numerous and other water beetles are present too. In the water were recorded: larvae of *Bombina variegata, Bufo bufo, Hyla arborea*, and *Salamandra salamandra*; adult specimen of *Natrix natrix*; and by the water *Lacerta viridis*.

MATERIAL AND METHODS

The field research were conducted in period from April 1988 to May 1990. In 1990 only one, three days long, field excursion was organized. The exact dynamic of field data collection is presented in Table 1 (see appendix). Precise ground plan and profile of both puddles are shown in Figures 2 and 3. During each field excursion, water temperature at different depths, temperature of a surface soil layer, and air temperature were measured. The temperature were measured by mercury thermometer exact on 0.2 centigrade.

Newts were caught exclusively with a deep net, 30 cm in diameter and 60 cm deep (Ø 0.5 x 0.5 cm). The animals were caught always in a same way for about two hours, throughout the whole puddle; gradually from one to another imaginary segment of approximate area of 2.0 x 2.0 m. First, edged segments were depleted, and than deep water. There were no indication than this way of collecting increases mortality of adult specimens (not for the period of catching in order to count newts as well). In Gornji Oštrc, puddle collecting was difficult because of a thick vegetation. Sex of specimens were determined, and displacement of species in area, were recorded. Hunting method is not selective per depth (exact depth of caught specimens was impossible to determine, but depth of every depleted segment was recorded). Since all newts were caught by the same method and throughout entire puddle area, we estimated that possibilities of catching any species, as well as catching male or female specimen are more or less equal. By this way, species numerical ratio and sex ratio during the season was established, for all three species. During research, the presence of all three species larvae were recorded, based on observation and catch.

At the beginning of research, caught specimens were only recorded in a field data sheet and then released into the puddle. During last few field excursions, by employed standard Petersen's "capture-recapture" method and its modification (BEGON 1979), number of specimens was established, and based on number, population density was estimated. Separately was calculated number of males and number of females, and accordingly the sex ratio. At the puddle by Budinjak capture lasted four days in 1989 (April 20 to 21 and April 26 to 27), when animals were marked by cutting off the longest finger on the front left leg. This method does not increases mortality of specimens, not even after a year period (GRIFFITHS 1984). The catch in the puddle in Gornji Oštrc lasted three days in 1990 (May 22 to 24), and animals were marked firs day by cutting off the longest finger on the front left leg, and second day by cutting off the same finger on the front right leg. All the animals were turned into the puddle after they have been marked and signed in a field data sheet.

Statistical analysis

A significant differences in number of caught males and females, of every species on each localities have been tested by x^2 - test.

To establish number of animals, "Petersen's method" (or "Lincoln's index") and "weighted mean" (BEGON 1979) have been used.
Data analysis, collected at the puddle in Gornji Oštrc, has been done by "triple catch" method (BEGON 1979) as well.

Population density of all three species in the puddles by Budinjak and in Gornji Oštrc was expressed by number of specimens/m². Total number of animals in each puddle, for all three species, was obtained by summing values calculated by expanded Petersen's method for each species. To a maximal value, a standard error value was added, and to a minimal value subtracted.

After calculated mean values and standard deviations for the temperature of water, soil, and air on both localities, correlated were mean values of water and air temperature then water and soil temperature, by using regression analysis.

RESULTS

Number and density of specimens in researched populations

Puddle by Budinjak

Results obtained by catching newts in order to count them, are shown in Table 2 (see appendix).

After the last, fourth day of catch, percent of recaptured animals were: 71.43% (*T. vulgaris* males), 75.68% (*T. vulgaris* females), 66.67% (*T. carnifex* males), 94.74% (*T. carnifex* females), 50.62% (*T. alpestris* males), and 54.45 (*T. alpestris* females). High percentage of recaptured animals, enabled determination of specimens number, with a high confidence. According to Begon (1979), in Petersen's method of estimated number, at least 55% of recapture is needed to reach significance factor $p<0.1$, what is satisfied in this case, except for the alpine newt. Nevertheless, even for this species, the p is very close to a necessary value (males $p=0.108$, females $p=0.101$).

Results of counting animals after first two days of catching, calculated by Petersen's method were:

T. vulgaris (males): N = 186.0 SE_N = 84.9
T. vulgaris (females): N = 44.1 SE_N = 9.6
T. carnifex (males): N = 74.7 SE_N = 25.4
T. carnifex (females): N = 162.0 SE_N = 63.0
T. alpestris (males): N = 1245.6 SE_N = 171.2
T. alpestris (females): N = 1305.6 SE_N = 200.3

After four days of catching, results of counting animals calculated by extended Petersen's method ("weighted mean") were:

T. vulgaris (males): N = 94.8 SE_N = 11.8
T. vulgaris (females): N = 60.3 SE_N = 7.9
T. carnifex (males): N = 84.4 SE_N = 17.6
T. carnifex (females): N = 113.2 SE_N = 17.6
T. alpestris (males): N = 1281.0 SE_N = 73.8
T. alpestris (females): N = 1249.5 SE_N = 69.6

From the above results, it is obvious that the largest population in this puddle has *T. alpestris* (87.8% of total number of calculated animals), then follow populations of *T. carnifex* (6.8%) and *T. vulgaris* (5.4%).

Puddle in Gornji Oštrc

Results obtained by catching newts in order to count them, are shown in Table 3 (see appendix).

After the last, third day of catch, percentage of recaptured animals were: for *T. vulgaris* 100% (males) and 86.67% (females), for *T. carnifex* 74.44% (males) and 62.50 (females), and for *T. alpestris* 88.89% (males) and 86.11 (females). In all cases, significance factor was $p<0.1$. In this case too, there was no mortality ($n = r$ for every day).

Results of counting animals after first two days of catching, calculated by Petersen's method were:

T. vulgaris (males): N = 31.6 SE_N = 7.6
T. vulgaris (females): N = 31.3 SE_N = 3.9
T. carnifex (males): N = 169.5 SE_N = 38.3
T. carnifex (females): N = 191.6 SE_N = 50.2
T. alpestris (males): N = 49.9 SE_N = 2.5
T. alpestris (females): N = 53.6 SE_N = 7.6

After three days of catching, results of counting animals calculated by extended Petersen's method ("weighted mean") were:

T. vulgaris (males): N = 29.1 SE_N = 9.02
T. vulgaris (females): N = 37.4 SE_N = 7.63
T. carnifex (males): N = 153.6 SE_N = 17.17
T. carnifex (females): N = 158.6 SE_N = 20.65
T. alpestris (males): N = 58.6 SE_N = 9.26
T. alpestris (females): N = 59.4 SE_N = 8.24

In the Gornji Oštrc puddle, the most numerous population is *T. carnifex* (63% of total calculated number of specimens in the puddle), followed by *T. alpestris* (23.7%) and *T. vulgaris* (13.3%).

The catch results were calculated by "triple catch" method too. Following results are obtained:
(symbols: b_1, b_2 - growth rate
$\varnothing_1$, $\varnothing_2$ - survival rate)

T. vulgaris (males): $N_2 = 20.3$ $SE_{N2} = 54.4$
$\emptyset_1 = 0.64 = \emptyset_2$
$b_2 = 0.27 = b_1$
$N_3 = 17.8$ $N_1 = 23.1$
T. vulgaris (females): $N_2 = 24.2$ $SE_{N2} = 5.8$
$\emptyset_1 = 0.77 = \emptyset_2$
$b_2 = 0.52 = b_1$
$N_3 = 38.8$ $N_1 = 15.1$
T. cristatus (males): $N_2 = 137.9$ $SE_{N2} = 26.3$
$\emptyset_1 = 0.81 = \emptyset_2$
$b_2 = -0.08 = b_1$
$N_3 = 103.4$ $N_1 = 183.9$
T. cristatus (females): $N_2 = 163.9$ $SE_{N2} = 43.4$
$\emptyset_1 = 0.86 = \emptyset_2$
$b_2 = 0.02 = b_1$
$N_3 = 143.8$ $N_1 = 186.8$
T. alpestris (males): $N_2 = 45.6$ $SE_{N2} = 6.6$
$\emptyset_1 = 0.91 = \emptyset_2$
$b_2 = 0.53 = b_1$
$N_3 = 88.2$ $N_1 = 23.5$
T. alpestris (females): $N_2 = 50.8$ $SE_{N2} = 10.0$
$\emptyset_1 = 0.95 = \emptyset_2$
$b_2 = 0.79 = b_1$
$N_3 = 229.8$ $N_1 = 11.2$

In the puddle by Budinjak, the mean animal number value was 13.1 specimens/m^2, maximal value 14.0 specimens/m^2, and minimal value 12.2 specimens/m^2.

In the puddle in Gornji Oštrc, the mean animal number value was 4.8 specimens/m^2, maximal value 5.5 specimens/m^2, and minimal value 4.1 specimens/m^2.

Areal displacement of newts in researched puddles

Puddle by Budinjak

Areal displacement of all three newt's species in the puddle by Budinjak are shown in Figures 4 (a., b., c.). The depth at figure indicate the puddle depth in place of caught, but not the exact depth on which newts were caught.

Specimens of *T. vulgaris*, were more often found in shallow water, up to 40 cm depth, but only on two occasions were caught in water depth less than 10 cm.

Specimens of *T. carnifex*, were most often caught in water depth between 60 and 100 cm, less often in depth of 20 to 60 cm, and never over 20 cm water depth.

The alpine newt (*T. alpestris*) has been found in every depth, during entire period of research, but less often in very shallow water.

By summing caught specimens of each species per depth, during all field excursions, became obvious that some species were caught more often on certain depths. Majority of the southern smooth newt were caught in depth between 10 to 20 cm, the alpine crested newt in depth between 80 and 100 cm, and the alpine newt in depth between 60 and 80 cm (Table 4) see appendix).

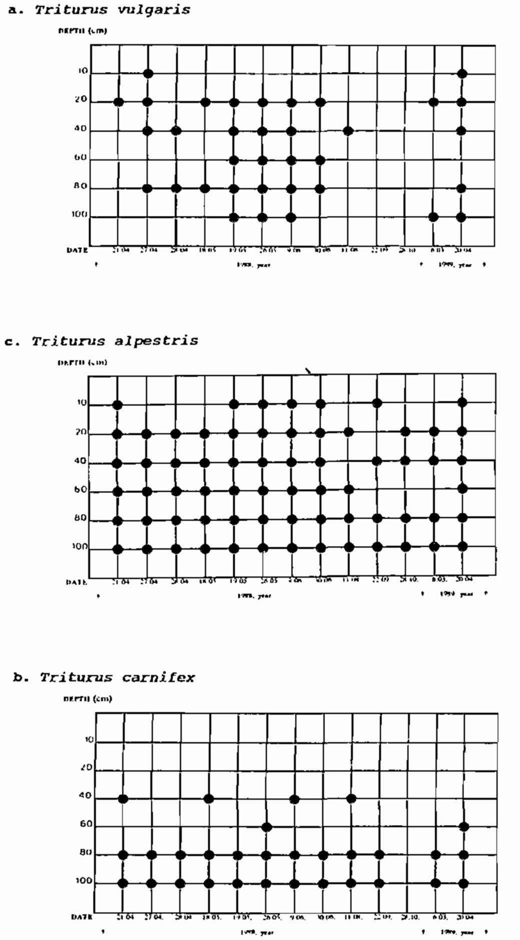

Figure 4: Space arrangement of the newts at the puddle by the Budinjak: a. *Triturus vulgaris*, b. *Triturus carnifex*, c. *Triturus alpestris*

Puddle in Gornji Oštrc

Based on observation and catch during population density determination, it can be concluded that newts of all three species are equally present in all parts of the puddle. All three species are somewhat more numerous in deeper puddle parts, and the alpine crested newt specimens have not been observed or caught at the most distant southern puddle part with less than 10 cm water depth.

The temperature of water, soil, and air

In tables 5 and 6, the water temperatures in both puddles, temperatures of surface soil layer, and air temperature are shown.

The regular decrease of water temperature with depth in the puddle by Budinjak is obvious, less regularity is present in the Gornji Oštrc puddle (during two measurements, water temperature was equal in all depths).

Calculated are mean values and standard deviations of those temperatures. Following values are obtained:

BUDINJAK	GORNJI OŠTRC
$X_{tw} = 13.4$ $S_{tv} = 4.4$	$X_{tw} = 19.4$ $S_{tv} = 6.6$
$X_{ta} = 17.1$ $S_{ta} = 5.6$	$X_{ta} = 21.3$ $S_{ta} = 5.7$
$X_{ts} = 16.0$ $S_{ts} = 7.1$	$X_{ts} = 20.0$ $S_{ts} = 6.0$

X_{tw}, X_{ta}, X_{ts} - mean temperature of water, air, and soil

S_{tw}, S_{ta}, S_{ts} - standard deviation

The results show that water, air, and soil temperatures, during the research period, were permanently lower in the puddle by Budinjak. The water temperature at both localities was highly correlated with air temperature, and soil temperature ($r = 0.887$-0.958, $p<0.0001$).

The sex ratio of investigated population of newts

Results are shown in tables 7 and 8. Number of caught animals of all three species varied from one catch to another, from which can be supposed that number of animals present in a puddle permanently varied as well.

In the puddle by Budinjak, the arithmetic mean of the southern smooth newt males and females ratio, calculated for all captures, was 51.5% : 48.5%, what practically can be expressed as 1 : 1 ratio ($X^2 = 0.150$, $P = 0.6988$) (Table 7, see appendix). During May and June, the ratio varied several times in favor of males, that is, in favor of females. The sex ratio obtained by counting animals by Petersen's method, in April of 1989, showed that males were dominant in the population at that time (80.9% : 19.1%; $X^2 = 7.903$; $P = 0.0001$). For the alpine crested newt, calculated sex ratio of all captures was 47.4% (males) : 52.6% (females) ($X^2 = 0.474$;
$P = 0.4912$). Females were dominant in the puddle, specially in April of 1988. Results of counting animals by Petersen's method, from where the ratio is calculated: 42.6% (males) : 57.4% (females), pointed out a small but statistically significant dominance of females at that particular moment in the puddle ($X^2 = 4.269$; $P = 0.0388$). In the alpine newt population, during a whole season, males are dominant in the puddle, and arithmetic mean of all captures was 56.8% : 43.2% and number of males is significantly higher ($X^2 = 25.190$; $P < 0.0001$). From results obtained by employed Petersen's method the ratio 50.6% : 49.4% was calculated, what is almost equal to 1 : 1 ratio ($X^2 = 4.405$;
$P = 0.5246$).

The alpine newt males and females have been caught in this puddle during all field excursions, while the alpine crested newt specimens have not been found at all in September of 1988. The southern smooth newt specimens have not been caught in September and October of 1988. Only males of this species were caught on April 21, 1988 and June 3, 1989, and only one female was caught on August 11, 1988.

Based on less number of data for the *puddle in Gornji Oštrc*, following results were obtained (Table 8, see appendix). After all captures, the ratio of the southern smooth newt males and females was
48.1% : 51.9% ($X^2 = 0.117$; $P = 0.7324$). Nevertheless, number of animals was not large enough to establish more certain sex ratio. After a number of animals has been calculated, by Petersen's method, after three days of catching in May of 1990; for the southern smooth newt, following sex ratio was established: 43.9% (males) : 56.1% (females) ($X^2 = 0.970$; $P = 0.3247$). For the alpine crested newt, it can be said that females prevail for a little, because the arithmetic mean for all captures was 42.7% : 57.3%
($X^2 = 2.184$; $P = 0.1394$). The ratio calculated after animals were counted by Petersen's method was 49.2% 50.8% ($X^2 = 0.080$;
$P = 0.7774$). Arithmetic mean of the alpine newt specimens caught number, shows that sex ratio is 56.2% : 43.8% in favor of males
($X^2 = 3.329$; $P = 0.0680$). Calculated ratio after counting animals was 50% : 50% ($X^2 = 0.000$; $P = 1.0000$).

Newts' larvae

Besides research of adult part of newt's populations in two investigated puddles, presence of their larvae were recorded as well.

During 1988 in the puddle by Budinjak, very small number of larvae were noticed. Since we were not able to observe them in the puddle, the data are based on capture only. First larvae have been caught in May 19, 1988; and in June 9, 1988 and August 30, 1988 their presence were not confirmed. On August 11, 1988, seven larvae have been caught, on September 22, 1988, 19 larvae, and on October 28, 1988, only one. In the spring of 1989, larvae were not found in the puddle, and on October 11, of the same year, a great number of all three newt's species larvae were noticed in submersed vegetation.

In the puddle in Gornji Oštrc, during all field excursions (except October 28, 1988) it has been noticed that larvae of all three species of newts were present in a large number and displaced through entire puddle area. In October of 1988, only one specimen of recently metamorphosed alpine newt has been caught, and larvae were not present any more.

DISCUSSION

GLANDT (1982), by using same methods in research of number and density of specimens in sympatric populations of the southern smooth newt, alpine crested newt, and alpine newt near Munster in West Germany, showed a high correlation between specimens density and average yearly puddle temperature. Not as strong correlation he has between specimens density and area, and between depth and puddle overgrowth. The most numerous specimens and their density he recorded in a puddle with the highest average yearly temperature, the largest area and depth, and with a small water area overgrow with vegetation. With decrease of water temperature in a puddle, there were less specimens per m^2.

The puddle by Budinjak has a large area and depth up to 100 cm, and only its edges, shallow parts are overgrow with vegetation. The puddle in Gornji Oštrc has less area and it is shallower, and almost entirely overgrow with vegetation. Temperature measurements in the puddles, surface soil layer, and air, during research period, indicated that the water temperature in both puddles is highly correlated with the soil and air temperature, and that the puddle temperature by Budinjak is constantly, for few centigrade, lower than temperature in the Gornji Oštrc puddle. The same is pointed out by thermophilic vegetation around Gornji Oštrc. Number of specimens, as well as density, that were much higher in sympatric population of newts in the puddle by Budinjak than one in Gornji Oštrc, showed that the rule about influence of higher temperatures to increase number and density of specimens, is not applicable for the two researched puddles. It can be assumed that number and specimens density in these puddles are more effected by their shape: area, depth, and vegetation overgrowth. There were no indication about differences in food supply for newts in both puddles, as well as no differences if human impact on them. Both puddles are used as a watering place for cattle, and nearby both puddles are low traffic roads. In nearby area of both puddles the montane beech forests are developed. Probably, those factors have no major influence on differences in number of newts in these two puddles.

In works dealing with sympatric populations of the southern smooth newt and alpine crested newt in Great Britain (BELL 1979), and in Norway (DOLMEN 1988); population ecology, number and density of sympatric populations of the southern smooth newt, alpine crested newt, and alpine newt (GLANDT 1978 and 1980) in Germany, are indicated that in such syntopic populations most often and most numerous species is the southern smooth newt, with less specimens of the alpine and alpine crested newts. GLANDT (1978) stated a shift of these relations during a season, suggesting several captures through a season, in order for more precise estimation of specimens number between species. Nevertheless, according to his results, during entire season prevail the southern smooth newt. HAGSTROM (1979), working on ecology of sympatric populations of the southern smooth newt and the alpine crested newt, on locality near Gotteborg in southwest Sweden, find out that specimen number of both species is almost equal (350 specimens of the alpine crested newt and 250-300 specimens of the southern smooth newt).

On researched localities by Budinjak and in Gornji Oštrc, the ratio of specimens number for three species does not coincides with mentioned works. In the puddle by Budinjak distinctly prevail the alpine newt. That this ratio did not significantly changed through a season, we can conclude based on captures during all field excursions, because in all samples this species was distinctly dominant. Results obtained by estimated number of newts in the puddle by Budinjak, we can consider quite close to a real condition, because during captures, decrease of specimens number were not noticed, and number of recaptured animals increased permanently. What is a reason that in this puddle number of specimens and population density of alpine newt prevail so obviously is not clear jet. Maybe this ratio is related with the altitude increase. With altitude increase, maybe is not only number of the alpine newt populations increased related to number of the southern smooth and alpine crested newt populations (GLANDT 1980), but this also leads to increase specimen number of the alpine newt in syntopic populations with the other two species. In the puddle in Gornji Oštrc, during counting, in population prevailed the alpine crested newt, followed by the alpine newt and southern smooth newt. Number estimated by "triple catch" method has to be taken with reserve. Results pointed out on a possible greater emigration of the southern smooth and alpine newt specimens, which obviously disturbed by catch started to abandon the puddle. From day to day number of caught specimens decreased and number of recaptured animals increased. But employed Petersen's and extended Petersen' methods for number estimation, resulted in a similar species ratio in the puddle, and we can assume that calculated ratio is close to real one.

In this work, daylight areal displacement of three newt species in the same puddles was studied as well. It has been established that adult animals have, in general, permanent areal displacement in the puddles. Research results in the puddle by Budinjak confirm, in general, observations of some other authors (DOLMEN 1983b, 1988; DOLMEN & KOKSVIK 1983) who, between other issues, deal with nutrition and areal displacement of *T. cristatus* and *T. vulgaris* in Norway, and paper by COOKE & FRAZER (1976) where characteristics of puddles with sympatric populations of *T. cristatus, T. vulgaris*, and *T. helveticus* in Great Britain are analyzed. In these works are indicated that adult specimens of the alpine crested newt prefer puddles with water depth over 50 cm. In independent populations, as well as in sympatric ones with *T. vulgaris*, for their stay choose deeper parts of a puddle, stick to a bottom, and feed mainly on benthic organisms. This kind of displacement was confirmed for the alpine crested newt population in the puddle by Budinjak. The southern smooth newt, in sympatric populations, for its stay chooses more often upper water layer and feed mostly on planktonic species (DOLMEN & KOKSVIK 1983). VERELL (1985) concluded that gradually, throughout the phases of aquatic life, the southern smoothed newt uses for food more and more benthic organisms and its presence near a puddle bottom is getting more often. Majority of free water, occupied in syntopic populations of the southern smooth newt and the alpine crested newt by the southern smooth newt (DOLMEN 1983a, 1988; DOLMEN & KOKSVIK 1983), is occupied by the alpine newt in the puddle by Budinjak. This dominant species of newts in the puddle was registered with considerable number in all parts of the puddle, from shallower parts with depths less than 10 cm to the most deeper parts. In the most shallower parts, parts without vegetation, only specimens belonging to this species were recorded. To areal displacement of newts, in this puddle, probably some influence has also a water temperature. Namely, during research period, it was established that water temperature near the puddle bottom is permanently lower than water temperature at its surface, and according to DOLMEN (1988), somewhat lower temperature is more suitable to the alpine crested newt then to the southern smooth newt.

The puddle in Gornji Oštrc does not represent a typically suitable habitat for the alpine crested newt, with characteristics mentioned by COOKE & FRAZER (1976), meaning there are no enough "open" water (free of vegetation), and depth is on a border (50 to 50 cm) of the depth considered ideal, by mentioned authors, for this species of newts. Nevertheless, in Gornji Oštrc in "untypical" habitat, the population of this species is more numerous than populations of other two species. This can be partially explained by results of BEEBEE (1985), who researched amphibians in southeastern England. This author pointed out that limiting factors, for presence of this newt species, were not find out in researched puddles. In this puddle, observations and catches did not confirm space displacement regularity between three species of newts. That is, all three species were present in all parts of the puddle. Only the alpine crested newt was not registered in the most shallower (5 to 10 cm) puddle parts. During catch, in order to count newts, it was noticed that majority of the alpine crested newt specimens choose deeper water as a place of residence, but this was a case with other two species as well. In the puddle in Gornji Oštrc, as a more homogenous habitat (more uniform vegetational overgrow) and habitat where all three species of newts are mixed in space, the competition among species is probably more expressed, what can be a cause for differences in mutual population density relationship of these species on both localities. It is possible that in conditions that prevail in the puddle in Gornji Oštrc, the alpine crested newt is an important predator of other two species, specially of the southern smooth newt, as it was confirmed for syntopic population of these two species in the puddle near Gothenburg in Sweden (HAGSTROM 1971 and 1979).

There is a high possibility that in puddles with syntopic populations of several newt species, on their areal displacement influence have interspecific relations (DOLMEN 1980). Different displacement in space makes "easier" joint life of these, biologically, very similar species; that is, probably decreases food competition and cannibalism among species (DOLMEN 1983a).

During research in 1988, small number of all three species larvae were observed and caught in the puddle by Budinjak, while at the same time, quite numerous and relatively stable sympatric adult population remained in the puddle. To maintain established great number of specimens, considerable influx of juvenile specimens is essential. HAGSTROM (1973) in Denmark and BELL (1977) in England find out that survival rate through the year is about 50 percent in stable populations for adult specimens of the southern smooth newt.

According to this and assumption that the same rule is valid for other two studied species in this work, we can assume that noticed number of larvae during 1988 in the puddle by Budinjak, is not sufficient to maintain stable populations. So, it can be assumed that certain number of newts find in this puddle were not hatched in it, but they inhabited it coming from surrounded puddles. According to BELL (1977) and GLANDT (1985), adult specimens of all three species show distinct connection for the puddle where they have spent previous years, that is they express "homeland behavior" (KALEZI 1985), and juveniles migrate between puddles to inhabit new habitats. Possible explanation of "stable population" of newts in the puddle by Budinjak is in metapopulation theory, according to which most of the specimens originate in one or several puddles used as a reproductive center for the rest of puddles in nearby area (GILL 1978).

In the puddle in Gornji Oštrc a great number of larvae belonging to all three species were noticed, indicating that larvae production in this puddle is probably sufficient to maintain "stable" population.

This research included only the aquatic phase of newts' life. The beginning of aquatic phase in newts' life, in Europe, mostly starts in the spring and it is connected with temperature and moisture increase (DUELLMAN & TRUEB 1986). Because of this, time in the spring, when newts enter in the water varies depending on latitude, altitude, and meteorological conditions (GISLEN & KAURI 1959, THORN 1968, BELL 1977), and might also be different between close placed populations and between particular species as well (GLANDT 1978). Some species, such as *T. vulgaris*, might have additional immigration phase during the summer (HAGSTROM 1974, BELL 1977). The same factors have influence during emigration from the water. Part of population of some species (*T. vulgaris*) does not enters in puddles to mate (GRIFFITHS 1984), and equally there are cases that some specimens, through the year, enters a puddle and coming out (HAGSTROM 1979, GRIFFITHS 1984). All of these factors have influence on sex ratio fluctuation during a season, differently with various species.

The total calculated sex ratio of the southern smooth newt, on both localities, was approximately 1 : 1, what is quite similar to population ratios from Great Britain (BELL 1977) and Germany (GLANDT 1978). The appearance of males first (March and April) and than females (but for some time males still prevail), in the puddle by Budinjak, correspond to results mentioned by GRIFFITHS (1984). Sex ratio has changed few times through the season, so we can assume that were several emigrations and immigrations through the season in studied population, as it is known for other populations of this species (HAGSTROM 1979, GRIFFITHS 1984).

Considering the alpine crested newt, there is no difference in sex ratio between two researched localities. In both puddles, somewhat females are dominant, although the ratio is not significantly different from 1 : 1 ratio, what corresponds to literature data about populations of this species in Germany and Sweden (GLANDT 1978, HAGSTROM 1979). On both localities the sex ratio changed significantly during research period (particularly in the puddle by Budinjak), from significant female dominance to significant male dominance.

Results obtained for alpine newt populations in both investigated localities correspond to literature data as well (GLANDT 1978). In both puddles there were slight dominance of males. Only during two occasions in the puddle by Budinjak and once in Gornji Oštrc, minimal dominance of females were recorded (not statistically significant).

Sex ratio changes, in all three species of newts, indicate existence of migration in and out of a puddle during the aquatic life phase. The newts mating intensity changes with the change of sex ratio, and this is maybe one more way (if these ratios harmonize among species in syntopic population) of competition decrease among species. ZUIDERWIJK & BOUTON (1987) proved that changes in the sex ratio and changes in specimens number, during the aquatic life phase, have an important role in competition decrease and in increase of stability inside syntopic populations of highly competitive species - *Triturus cristatus* and *Triturus* *marmoratus* in France. Different times of entering a puddle and coming out of it have the same influence on mating. It was confirmed that three species of newts enter the puddle by Budinjak, at different times, indicating possible differences in mating time, and maybe having influence to decrease competition among species.

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APPENDIX

YEAR	SEASON	DATE	LOCALITY		No. OF DAYS
			Budinjak puddle	Gornji Oštrc puddle	
		21.04.	*		1
		27.-28.04.	*		2
		18.-19.04.	*		2
	spring	25.05.		*	1
		26.05.	*		1
1988.		9.06.	*		1
		10.06.		*	1
		30.06.	*	*	1
		11.08.	*		1
	summer	8.09.		*	1
		21.09.		*	1
		22.09.	*		1
	fall	28.10.	*	*	1
	winter	6.03.	*	*	1
1989.	spring	20.-21.04.	*		2
		26.-27.04.	*		2
1990.	spring	22.-23.05.		*	2
		24.05.	*	*	1
				TOTAL =	23

Table 1: Fieldwork dynamics.

		male				female			
SPECIES	DAY	n_i	m_i	r_i	M_i	n_i	m_i	r_i	M_i
	1	31		31		21		21	
Triturus vulgaris	2	17	2	17	31	20	9	20	21
	3	45	24	45	46	41	22	41	32
	4	56	40		67	37	28		51
	1	14		14		15		15	
Triturus carnifex	2	31	5	31	14	53	4	53	15
	3	32	9	32	40	39	20	39	64
	4	15	10		53	19	18		83
	1	224		224		230		230	
Triturus alpestris	2	178	34	178	224	192	33	192	230
	3	382	105	382	368	419	131	419	389
	4	332	163		645	292	159		677

Table 2: Results obtained by catching newts in order to count them, in the puddle by Budinjak:

n_i - number of specimens captured i^{th} day
m_i - number of recaptured specimens i^{th} day
r_i - number of specimens captured and marked i^{th} day
M_i - number of specimens marked until i^{th} day.

		DAY						
		1		2			3	
SPECIES		$n_1 = r_1$	n_2	m_{21}	r_2	n_3	m_{31}	m_{32}
Triturus vulgaris	male	17	12	6	12	10	3	7
	female	25	14	11	14	15	5	8
Triturus carnifex	male	31	81	14	81	90	16	51
	female	34	61	10	61	80	12	38
Triturus alpestris	male	31	28	17	28	27	7	17
	female	28	43	22	43	36	3	28

Table 3: Results obtained by catching newts in order to count them, in the puddle in Gornji Oštrc:

n_1, n_2, n_3 - total number of specimens captured first, second and third day
r_1, r_2 - total number of specimens marked and released first and second day
m_{21} - number of specimens marked first day and recaptured second day
m_{31} - number of specimens marked first day and recaptured third day
m_{32} - number of specimens marked second or first and second day and recaptured third day.

SPECIES	*Triturus*	*vulgaris*	*Triturus*	*carnifex*	*Triturus*	*alpestris*
DEPTH (cm)	No.of sp.	%	No.of sp.	%	No.of sp.	%
0 - 10	3	1.8	0	0	50	3.7
10 - 20	83	49.7	0	0	166	12.4
20 - 40	27	16.2	8	4.6	221	16.4
40 - 60	29	17.4	8	4.6	111	8.3
60 - 80	19	11.4	73	42.2	489	36.4
80 - 100	6	3.6	84	48.6	307	22.8
TOTAL	167	100	173	100	1344	100

Table 4: Number of newts captured on different depths, in the puddle by Budinjak.

DEPTH (cm)	10	20	40	60	80	100		
DATE			T_w	(°C)			T_a (°C)	T_g (°C)
21.04.88.	14.5	13.5	13.0			11.0	14.0	13.5
27.04.88.	18.5	18.0			12.0	10.5	14.5	13.5
28.04.88.	11.5	10.5			9.5	9.0	10.0	8.5
18.05.88.	18.5	16.5		15.0	14.5	14.5	18.5	17.0
19.05.88.	17.5	15.5	16.5	15.5	15.5	15.0	17.0	14.5
26.05.88.	16.0	15.0	14.5	14.0	13.2	12.2	22.0	16.0
9.06.88.	21.0	21.0	16.0	15.5	15.0	14.5	22.0	26.0
30.06.88.	24.2	22.0	20.2	19.0	18.0	16.5	27.0	26.5
11.08.88.	27.0	25.0	19.5	18.5	17.0	16.0	25.0	31.0
22.09.88.	15.0	12.5	11.5	11.2	11.0	11.0	15.0	15.0
28.10.88.	6.0	5.5	5.0	5.0	5.0	5.2	9.8	6.2
6.03.89.	7.5	7.0	6.0	6.0	5.5	5.5	9.0	10.0
20.04.89.	13.0	12.0	11.5	11.5	10.5	10.0	17.0	14.0
26.04.89.	14.5	14.5	11.5	11.0	10.5	9.5	18.5	12.5

Table 5: Results of the water temperature (T_w) on different depths, air temperature (T_a) and ground temperature (T_g) measured at and around the puddle by Budinjak (temperature was measured during the capturing time between 9 a.m. and 5 p.m. hours).

DEPTH (cm)	10	20	40	50	60	80		
DATE			T_w	(°C)			T_a (°C)	T_g (°C)
25.05.88.	23.0	22.0	21.0		18.0		21.0	
10.06.88.	30.0	27.0	26.0	24.5	24.0		26.0	27.0
30.06.88.	28.2	27.9	27.5		26.0		29.0	27.0
8.09.88.	21.0	20.0	19.5		19.0		22.0	18.0
21.09.88.	16.5	16.5	16.5		16.5	16.5	15.0	16.5
28.10.88.	10.0	10.0	10.0		10.0	10.0	13.2	12.2
6.03.89.	11.5	11.0	9.5		9.0		13.0	11.0
22.05.90.	21.5	20.0	19.0	18.5			25.0	20.5
23.05.90.	24.5	23.5	22.0				24.5	22.5
24.05.90.	22.0	21.0	19.5				24.0	25.0

Table 6: Results of the water temperature (T_w) on different depths, air temperature (T_a) and ground temperature (T_g) measured at and around the puddle in Gornji Oštrc (temperature was measured during the capturing time between 9 a.m. and 5 p.m. hours).

		T.	*vulgaris*		*T.*	*carnifex*		*T.*	*alpestris*
DATE	♂	♀	ratio %	♂	♀	ratio %	♂	♀	ratio %
21.04.88	2	0	100.0: 0.0	2	15	11.8: 88.2**	37	54	40.7: 59.3
27.04.88.	11	11	50.0: 50.0	6	7	46.2: 53.8	34	18	65.4: 34.6*
28.04.88.	7	8	46.7: 53.3	9	16	32.1: 67.9	50	39	56.2: 43.8
18.05.88.	3	5	37.5: 62.5	5	3	62.5: 37.5	53	26	67.1: 32.9**
19.05.88.	7	14	33.3: 66.7	8	7	53.3: 46.7	76	51	59.8: 40.2*
26.05.88.	11	7	61.1: 38.9	7	7	50.0: 50.0	66	41	61.7: 38.3*
9.06.88.	8	11	42.1: 57.9	13	13	50.0: 50.0	144	65	68.9: 31.1***
30.06.88.	3	3	50.0: 50.0	8	1	88.9: 11.1*	31	20	60.8: 39.2
11.08.88.	0	1	0.0:100.0	1	2	33.3: 66.7	11	5	68.8: 31.2
22.09.88.	0	0		5	2	71.4: 28.6	13	11	54.2: 45.8
28.10.88.	0	0		0	0		5	11	31.3: 68.7
6.03.89.	3	0	100.0: 0.0	3	1	75.0: 25.0	20	9	69.0: 31.0*
20.04.89.	31	21	59.6: 40.4	14	15	48.3: 51.7	224	230	49.3: 50.7
TOTAL	86	81	51.5: 48.5	81	90	47.4: 52.6	764	580	56.8: 43.2***

Table 7: Number of collected male (♂) and female (♀) specimens and sex ratio (ratio %) of all three species at the puddle by Budinjak (tested by X^2 test) (* $p<0.05$, ** $p<0.01$, *** $p<0.001$).

		T.	*vulgaris*		*T.*	*carnifex*		*T.*	*alpestris*
DATE	♂	♀	ratio %	♂	♀	ratio %	♂	♀	ratio %
25.05.88.	2	3	40.0: 60.0	11	7	61.1: 38.9	24	34	29.3: 70.3
27.05.88.	0	0		0	3	0.0:100.0	4	2	66.7: 33.3
10.06.88.	1	1	50.0: 50.0	14	20	41.0: 59.0	24	13	64.4: 35.1
28.10.88.	0	0		1	2	33.3: 66.7	1	1	50.0: 50.0
6.03.89.	3	2	60.0: 40.0	1	2	33.3: 66.7	39	18	68.4: 31.5**
22.05.90.	31	34	47.7: 52.3	17	25	40.5: 59.5	31	28	52.5: 47.5
TOTAL	44	59	48.1: 51.9	44	59	42.7: 57.3	123	96	55.2: 43.8

Table 8: Number of collected male (♂) and female (♀) specimens and sex ratio (ratio %) of all three species at the puddle in Gornji Oštrc (tested by X^2 test) (* $p<0.05$, ** $p<0.01$, *** $p<0.001$).

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The Significance of Suspension Feeding for Tadpole Survival in Temporary Ponds.

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Abstract: Suspension feeding enables anuran larvae to exploit a broad range of food sources, suspended and sedimented food particles as well as periphyton. At the same time, very low concentrations, approximately equivalent to threshold concentrations of about 10 to 10,000 particles ml^{-1} are ingested. Maximal ingestion and filtering rates, threshold concentration and critical concentration are dependent upon species and larval stage. In general *Bufo calamita* larvae achieved the highest ingestion and filtering rates and also the highest threshold and critical concentrations, *Bufo bufo* larvae the lowest, and *Rana temporaria* larvae were intermediate. The early larval premetamorphic Gosner stage 28 of *Bufo calamita* and *Rana temporaria* achieved the highest and the prometamorphic G stage 40 the lowest ingestion and filtering rates and threshold and critical concentrations. Regularly in premetamorphic G stage 32 the parameters of suspension feeding were moderately greater than in G stage 40. In contrast, larval G stage 40 of *Bufo bufo* achieved the highest values of suspension feeding parameters. In general the larval feeding biology described above enables *Bufo calamita* to colonize small, new and oligotrophic waters in high density and to reduce pressure of food competition, or avoid it altogether, through exploitation of a broad spectrum of ingestible food. Furthermore, rapid development (fast growth) dependent upon high ingestion rates enables *Bufo calamita* to escape soon evaporation of the aquatic biotopes and the increasing density of predators. *Bufo bufo* larvae, in contrast, which usually inhabit permanent and nutrient rich waters, have not been selected for high and efficient exploitation of meagre food supplies. Accelerated decelopment to leave their waters has been less vital for them, instead, protective mechanisms enable this larvae to outfox their predators. The suspension feeding efficiency of *Rana temporaria* larvae is intermediate, if compared to the two larval species above in concordance with conditiones of the biotope.

Key words: suspension feeding, anuran larvae, temporary ponds, survival

INTRODUCTION

Animal populations are basically controlled by predation and competition. In general survival of anuran larvae is ruled by predation and evaporation of their temporal aquatic biotopes. Central European anuran larvae inhabit a broad range of aquatic biotopes with a clear preference of ephemeral waters. *Bufo calamita* extremely prefers small and, in some cases, oligotrophic newly formed waters for mating and spawning and thus avoid to a certain extent aquatic predators which begin to inhabit the newly formed biotopes. Larval *Bufo calamita* populations suffer under the evaporation of the small aquatic biotopes and under the increasing predation in their later developmental phase. It is hypothesized that these tadpoles have been selected to escape the drying ponds and an increasing density of predators by an accelerated development.

COLLINS (1979, *Rana catesbeiana*) showed the influence of nutrient supply and temperature on the duration of the larval period. According to TRAVIS (1983a, b, *Hyla gratiosa*) a restrictive nutrient supply not only reduces average size before and after metamorphosis, but also causes an increase in average duration of larval development. A high nutrient supply leads to rapid growth, rapid development and, therefore, early metamorphosis (HOTA and DASH, 1986; MURRAY, 1990). ADOLPH (1931, *Rana sylvatica, Rana pipiens*) views larval growth, itself determined by multifarious factors (including food availability), as decisive in determining the onset of metamorphosis. The availability of food to individual larvae is influenced by food competition. The latter intensifies relative to growth of conspecific larval density and decreasing total nutrient supply. It is therefore a contributive factor to larval size, together with total nutrient supply, the effects of interference and abiotic factors (TRAVIS, 1984; SEMLITSCH & CALDWELL, 1982, *Scaphiopus holbrooki*; WILBUR, 1977; *Rana sylvatica*). The authors above conclude that the availability of food is the main factor influencing tadpole survival in extreme ephemeral ponds. The importance of food availability is intensified by the oligotrophic situation of some of the larval biotopes of *Bufo calamita* (BREGULLA, 1988, 1986; BANKS & BEEBEE, 1987; ANDREN and NILSON, 1985a, b; BEEBEE, 1983; STRIJBOSCH, 1979). Since feeding is essential to survival of *Bufo calamita* larvae in two aspects, a high rate of food ingestion of every source at low food concentrations must be postulated.

Bufo bufo which regularly prefers to deposit its eggs in permanent and nutrient-rich waters may only accidentally occur in ephemeral biotopes or along small rivers (KUHN, 1993; MITTMANN, 1989; VIERTEL, 1976; HEUSSER, 1958, 1960;). These larvae possess protective mechanisms as schooling behaviour simulating a big animal and bitter substances in their skin; further the smashed body of an injured conspecific leads to immobility of all the other larvae, enabling them to outfox their predators (BREUER and VIERTEL, 1990; PFEIFFER, 1966; VORIS and BACON, 1966; KULZER, 1954; EIBL-EIBESFELDT, 1949). It is hypothesized, that this species has not been selected to accelerate its larval development by high ingestion rates.

Rana temporaria larvae are regularly found in both nutrient rich ephemeral and permanent waters (MITTMANN, 1989; STRIJBOSCH, 1979; SAVAGE 1961:221). An intermediate position in respect to ingestion of food is postulated.

Larger larvae are frequently better able to escape predation than smaller conspecifics (SEMLITSCH, 1990; SEMLITSCH & GIBBONS, 1988, *Hyla chrysoscelis*; CRONIN & TRAVIS, 1986, *Rana areolata*; TRAVIS, 1984, 1983a, b, *Hyla gratiosa*; CALDWELL *et al.*, 1980, *Hyla gratiosa*; HEYER *et al.*, 1975, *Physalaemus pustulosus, Leptodactylus poecilochilus, Smilisca phaeota, Bufo marinus*; CALEF, 1973, *Rana aurora*).

MATERIAL AND METHODS

Experiments measuring ingestion performed by VIERTEL (1990, 1992) describe the coulter counter method and the photometric method of measurement in detail. Definitions of the parameters and their statistical calculations are given below:

Ingestion is the process of capturing suspended particles. Ingestion rate (I or I_n) is the number of particles or the particle volume (I_v) captured per individual or per unit of biomass and time and is the gauge of actual nutrient exploitation by a suspension feeder. I_{max} is the maximal I_n and $K_{½}$ (half-saturation constant) is the initial concentration (C_i) at which half of I_{max} is reached (VIERTEL, 1992; Michaelis-Menten function; $I=I_{max}*(C_i\text{-}TC)*(K_{½}+(C_i\text{-}TC))^{-1}$).

Filtering is most often expressed as the water volume cleared of suspended particles per unit of time (HARVEY's 1937 `volume swept clear'), and is the gauge of a suspension feeder's ability to react to a nutrient suspension by increasing or lowering the water current (JORGENSEN, 1983). Filtering rate (F) is expressed as water volume per biomass of the suspension feeder and per unit of time ($F=ml*g^{-1}*30min^{-1}$). The filtering rate was calculated from I_n and C_i ($F=I*C_i^{-1}$, BERGMAN & RICHMAN, 1974; FROST, 1972). The maximal filtering rate (F_{max}) is calculated, accordingly, from I_{max} and C_i. The filtering rate is identical with pumping rate if the retention efficiency reaches 100%. The volume of water pumped per unit of time through the filter apparatus is the pumping rate. Retention efficiency is the percentage of food particles of a defined diameter removed from a given nutrient concentration (see RANDLOV & RIISGARD, 1979: RE=1- exhaled concentration divided by inhaled concentration). It is the gauge of the filtering efficiency of a filter apparatus. In the present study RE is only the percentage of particles removed from the initial concentration of silica gel particles in the experimental chamber. Therefore it is apostrophized as `retention efficiency' (`RE').

The critical concentration (CC) is the concentration of suspended nutrient above which ingestion does not increase, thus filtration peaks and then more or less decreases (FROST, 1972; MCMAHON, 1965; RIGLER, 1961). In filter-feeding zooplankton CC is termed `incipient limiting level'.

The threshold concentration (TC) is the concentration of suspended nutrient below which ingestion and filtering both cease and above which filtration increases up to CC level (FROST, 1974; MUCK & LAMPERT, 1980; SEALE & WASSERSUG, 1979).

The larval stages were diagnosed according to the normal table of GOSNER (1960)

RESULTS

Experiments with *Chlorella fusca* algae of a cell size of 5.8 µm and a cell volume of 102.2 μm^3 as suspended nutrient in various concentrations clearly demonstrated the highly different suspension feeding ability of *Bufo calamita*, *Bufo bufo* and *Rana temporaria* larvae under comparable conditions. The concentrations were measured by coulter counter technique.

Bufo calamita larvae achieved the highest `RE' values in all larval stages and concentrations (Fig. 1). Larval G stage 40 was highest at low concentrations, while G stage 28 was highest at high algal concentrations (larval G stage 28 was not tested at low concentrations). *Bufo bufo* larvae demonstrated the lowest `RE'values of the three species tested. The growth stages 28 and

32 of premetamorphosis were lowest and the prometamorphic G stage 40 was at highest (Fig. 1). *Rana temporaria* larvae achieved the second highest 'RE' values. They were lowest for larval G stages 32 and highest for G stage 28 at high algal concentrations; at low concentrations G stage 40 was at highest (Fig. 1).

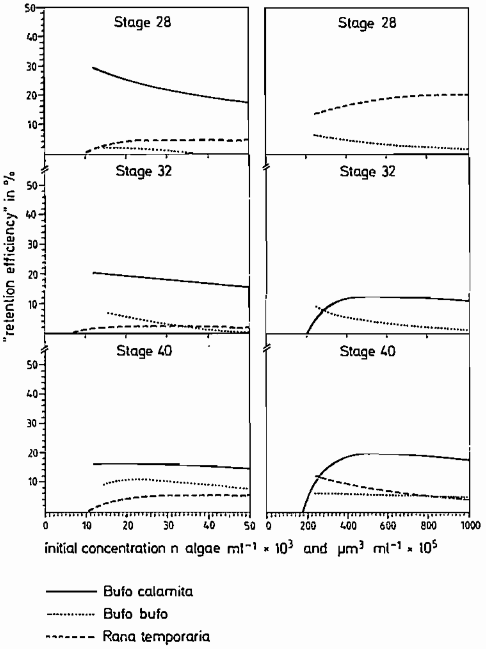

Figure 1a-c. 'Retention efficiency' plotted against initial concentration of *Chlorella fusca* algae. 'Retention efficiency' ('RE') was computed from the filtration rates ('RE'=F*800^{-1}*100%).
The curves were obtained by transforming the ingestion curves (y=(x^a-b)*x^{-1}).

The results of photometric measurement of silica gel particle concentrations (50% percentile of particle diameter: 93µm) and the calculation of I_{max}, F_{max}, TC and CC by inserting I and C_i into the Michaelis-Menten equation demonstrated a clear and different pattern for the larval species and the developmental stages studied.

The highest I_{max} was achieved by *Bufo calamita* larvae, staggered according to the larval stages; i.e., the premetamorphic growth stage 28 was highest and the prometamorphic stage 40 lowest. Premetamorphic larval G stage 32 was slightly higher than larval G stage 40. F_{max} values were comparable between the larval stages, and in general, were higher than in the two remaining species. TC and CC values were

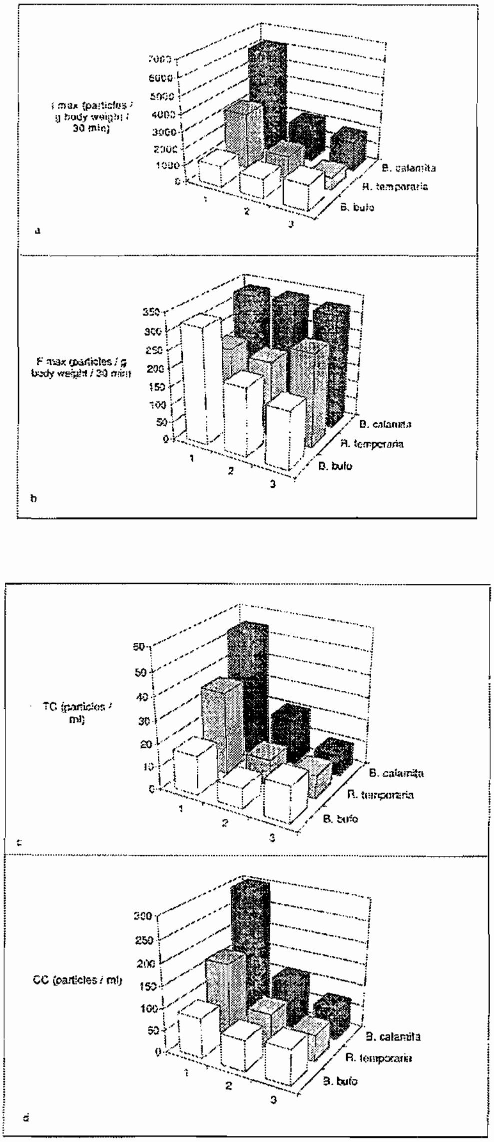

Figure 2a-d: Parameters of Michael-Menten equation. Calculated by inserting the initial concentration (C_i) and the ingestion rate (I) into the Michaelis-Menten equation $I=I_{max}*(C_i\text{-}TC)*(K_{½}+(C_i\text{-}TC))^{-1}$. Comparison of species and larval Gosner stages.

strongly staggered according to the larval stages and were higher at larval G stage 28 than in the remaining species (Figs. 2a-d).

Functional response of *Bufo bufo* larvae showed a quite different pattern. Highest I_{max} and F_{max} values were achieved in prometamorphic G stage 40 and were comparable in G stages 28 and 32. In general TC and CC were the lowest of all three species examined and were greatest at comparable values in larval G stages 28 and 40 (Figs. 2a-d).

In *Rana temporaria* larvae I_{max}, F_{max}, TC and CC values were clearly staggered according to the larval stages, i.e. the premetamorphic G stage 28 was highest and the prometamorphic G stage 40 was lowest of all species under study (Figs. 2a-d).

DISCUSSION

The different patterns of suspension feeding parameters compared well with the different life histories of the species. The results demonstrate that if the breeding site is an ephemeral and oligotrophic biotope, higher I_{max} and F_{max} values are achieved. The pressure to leave the aquatic biotope as soon as possible and with the greatest possible body length, to avoid evaporation and exposure to predators selected the larvae of *Bufo calamita* to achieve high ingestion rates at comparatively low nutrient concentrations. *Bufo bufo* larvae, usually not under pressure to leave their waters soon and endowed with protective mechanisms to oppose their predators were not selected for high suspension feeding efficiency. The intermediate suspension feeding efficiency of *Rana temporaria* larvae concorded with the intermediate life history of this species and fitted this scheme.

Two points should considered when interpreting the significance of differences in suspension feeding parameters of different larval stages: 1. Suspended nutrient is not the only food source. 2. The physiological and physical conditions of suspension feeding might not be the same for an extremely small larva as for a large larva. For the first point, it must be stated that the larval species under study scrape off periphyton from the bottom and from the surface of the vegetation and that they ingest bottom substratum (DIAZ-PANIAGUA, 1989, 1985; LÖSCHENKOHL, 1985; SAVAGE, 1952). In each case, the particles are suspended in the region of the oral disc and thus transported by the irrigation water stream and ingested. This feeding behavior explains why *Bufo calamita* is able to survive in waters whose plankton density lies below the threshold concentration and also why *Bufo bufo* larvae survive despite their low filtering efficiency. At present, the second point remains a hypothesis because no experimental evidence is available.

In general the larval species which are highly compelled to leave their waters soon, as *Bufo calamita*, or those which are under a moderate or slight pressure to leave their waters, as *Rana temporaria*, ingest more nutrient in the early premetamorphic G stage 28 than in any other stage, and in G stage 32, often moderately more than in the prometamorphic G stage 40. *Bufo bufo* larvae which are allowed to spend a longer time as larvae, achieve their highest nutrient ingestion in the prometamorphic G stage 40. High ingestion in early larval stages fits into the demand for increased growth gains leading to an accelerated development and to decreased predation due to greater body length. The higher ingestion of *Bufo bufo* larvae in prometamorphic G stage 40 seems to mirror the fact that these larvae do not need to escape drying ponds and their predators by early accelerated growth and development effected by an early high nutrient ingestion.

The above explanations of the significance of TC and CC for tadpole survival in ephemeral and oliogotrophic waters do not account for the high TC and CC values in the early larval stages of *Bufo calamita* and the more moderate values of *Rana temporaria* on the one hand and the low TC and CC values of *Bufo bufo* on the other. Low TC and CC values would be expected more from the larvae of ephemeral and oligotrophic waters than from those of permanent nutrient-rich waters. Again this contradiction can only be explained by the ability of the larvae to scrape off periphyton and to feed on bottom substratum: These larvae generate the particle concentration in the region anterior to their mouth parts, which is an effective suspension feeding strategy.

CONCLUSIONS

Bufo calamita and *Bufo bufo* larvae represent two extremely different types or strategies of survival. The ability to ingest a large amount of food at early larval stages and to exploit a broad range of food sources seems to be the key for understanding tadpole survival of *Bufo calamita* in ephemeral and oligotrophic biotopes. The ability of all larvae with oral disc and horny mouth parts to scrape off periphyton and to ingest bottom particles is the other character contributing to tadpole survival in ephemeral and oligotrophic biotopes. In general the larval feeding biology enables *Bufo calamita* to colonize small, new and oligotrophic waters at high density and to reduce pressure of food competition, or avoid it altogether, through exploitation of a broad spectrum of ingestible food. Feeding biology might enable survival of *Rana temporaria* larvae to a moderate extent in ephemeral ponds.

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Analysis of the breeding site characteristics of amphibians in a pre-alpine area (Triangolo Lariano)

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Abstract: Preference of Amphibians in the choice of breeding site were studied in Northern Italy during the years 1990 to 1992. Forty sites (ponds, canals, fountains) were examined and the species found were: *Salamandra salamandra* (Linnaeus, 1758) (12.5 % of the sites), *Triturus carnifex* (Laurenti, 1768) (52.5 %), *T. vulgaris* (Boulenger, 1882) (5 %), *Hyla arborea* (Linnaeus, 1758) (2.5 %), *Rana temporaria* (Linnaeus, 1758) (65 %), *R. dalmatina* (Bonaparte, 1840) (40 %), *R. esculenta* complex (15 %). Statistical analysis showed that the main factors influencing the presence of the species were of three different kinds: botanical (percentage of vegetational cover at the bottom, on the surface and on the edge), morphological (altitude, surface area, mean slope of the shore, etc.) and physical (temperature of the air and of the water, relative humidity). Chemical parameters of the water had little influence on the colonization of the ponds.

Keywords: Amphibians, Breeding sites, Multivariate analysis, Northern Italy.

INTRODUCTION

The decline of Amphibian populations requires more study of the ecology and biology of these Vertebrates. For correct management, each species must be known.

The aim of this research was to investigate the most important factors that determine the choice of breeding site by the Amphibians in a pre-alpine area. This study is part of wider studies carried out in Northern Italy (Lombardy) (ILDOS & ANCONA, 1992, in press a, in press b; BONETTI & ANCONA, 1993) to characterize the breeding sites of the Amphibians in different habitats and estimate the current status of these Vertebrates.

MATERIAL AND METHODS

The study area is located near Como (Lombardy, Northern Italy) and is called "Triangolo Lariano". This area is from 199 m (Como Lake) to 1686 m (S. Primo Mount) above sea level and the places examined lie between 350 m and 1435 m. This environment is characterized by hills, low mountains and a large number of springs and streams. The climate is hot rainy temperate (BELLONI, 1975).

Urban areas take up only 11.2 % of the whole territory and the remaining part is shared equally between farmland and woodland. Tourists and cattle are the main causes of disturbance.

In the years 1990 to 1992, 40 sites (30 ponds, 5 canals, 5 fountains) were studied.

Presence and reproduction of the Amphibian species were recorded and these served as the dependent variable. The independent variables were the following (short forms of each variable are enclosed in parentheses and used in table 3): surface area (area), maximum depth (depth), mean slope of the shore (slope), type of bottom (bottom), kind of geological substrate (substrate), altitude above sea level (altitude), distance from asphalted roads (asph. roads), from paths (paths), from houses (houses) and from other bodies of water (water bodies), type of body of water (type), spring and summer transparency of the water (sp./su. transparency), spring and summer percentage of vegetational cover at the bottom, on the surface and on the edge (sp./su. % bottom; sp./su. % surface; sp./su. % edge), degree of disturbance (disturbance), spring and summer temperature of the air and of the water (sp./su. air t°C; sp./su. water t°C), spring concentration of phosphates and nitrates (sp. phosphates; sp. nitrates), spring percentage oxygen saturation and red-ox potential (sp. oxygen; sp. red-ox), spring and summer concentration of sulphates and ammonium (sp./su. sulphates; sp./su. ammonium), spring and summer water hardness and pH (sp./su. hardness; sp./su. pH), spring and summer air relative humidity (sp./su. humidity), presence of fish (fish).

All data were submitted to discriminant analysis, a statistical procedure used by some investigators (BEEBEE, 1985; PAVIGNANO *et al.*,1990) for some ecological aspects of the communities of Amphibians. We used this kind of analysis to see whether or not the characteristics of the places occupied by each species differred significantly from those of non-used sites.

The small sizes of the sample of *Hyla arborea* and *Triturus vulgaris* prevented analysis of those species.

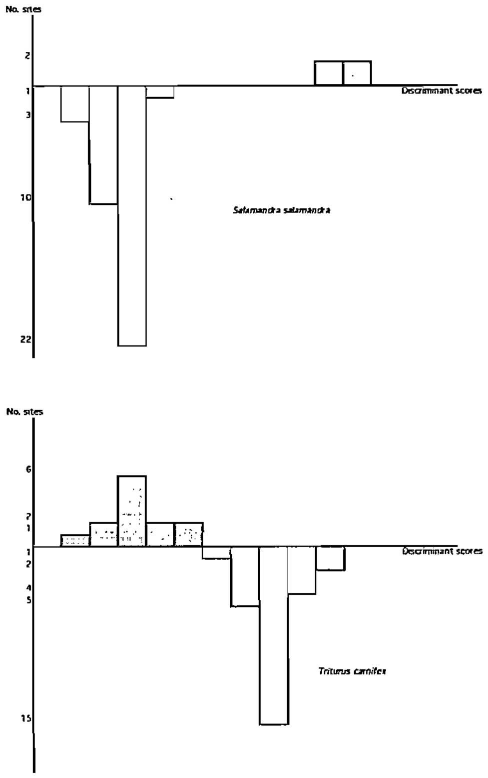

Figure 1: Discriminant grouping patterns (up *Salamandra salamandra*, down *Triturus carnifex*. Values on x-abscissa are of discriminant scores, and sites are grouped at intervals of 0.4 units. Sites without species are grouped below the discriminant score line (unshaded histograms) and those with the species are above (shaded).

First, multicolinearity test was done to remove all the variables showing a high correlation among themselves. The discriminant analysis was then done with the remaining variables. When no discriminant function could be detected under these conditions, we eliminated the variables showing little influence on distribution when canonical coefficients were examined (BEEBEE, 1985). This method was used until a discriminant function with a significant Wilks' lambda was found for all the species examined. For each discriminant function the values of the canonical correlation were calculated. The predictivity of the analysis was also calculated, to see if there was coincidence between the results obtained and those expected on the basis of the discriminant function.

To discover which variables were the main ones in determining the choice of the habitat, we calculated the correlation coefficients between each variable and the canonical variable.

RESULTS

The Amphibian species found were: *Salamandra salamandra* (12.5%), *Triturus carnifex* (52.5%), *T. vulgaris* (5%), *Hyla arborea* (2.5%), *Rana temporaria* (found in 65% of the sites), *R. dalmatina* (40%), *R. esculenta* complex (15%).

We obtained a discriminant function for each species. The results of the discriminant analysis are shown in figures 1 and 2. These histograms represent the classification of all the ponds according to their discriminant scores and to the presence/absence of the species. There was no overlap between the used and unused sites.

The values of the canonical correlation (table 1) showed good correlation (values > 0.9) effected by each discriminant function. The predictivity of the analysis was 100 % (table 2). The habitat features with the greatest influence on breeding site choice (table 3) are shown below for each species.

Salamandra salamandra prefers breeding sites located at lower altitudes, far from paths and with a steeper slope of the edges. This Amphibian was found in fountains and canals, never in ponds.

Ponds with less transparent water and with less steep slopes of the edges are preferred by *Triturus carnifex*. It colonizes places with larger surface area, lower summer water temperatures and higher nitrate content. This newt was found in ponds and canals, never in fountains (table 1).

Rana temporaria shows a preference for breeding sites located at higher altitudes, with lower air temperatures and higher relative humidity, far from houses, roads and other ponds. The Common Frog prefers places with scarce vegetation, little open water, not hard and with hotter summer water temperatures. The presence of fish limits the distribution of this Amphibian.

For *Rana dalmatina*, botanical and physical variables were important in breeding site

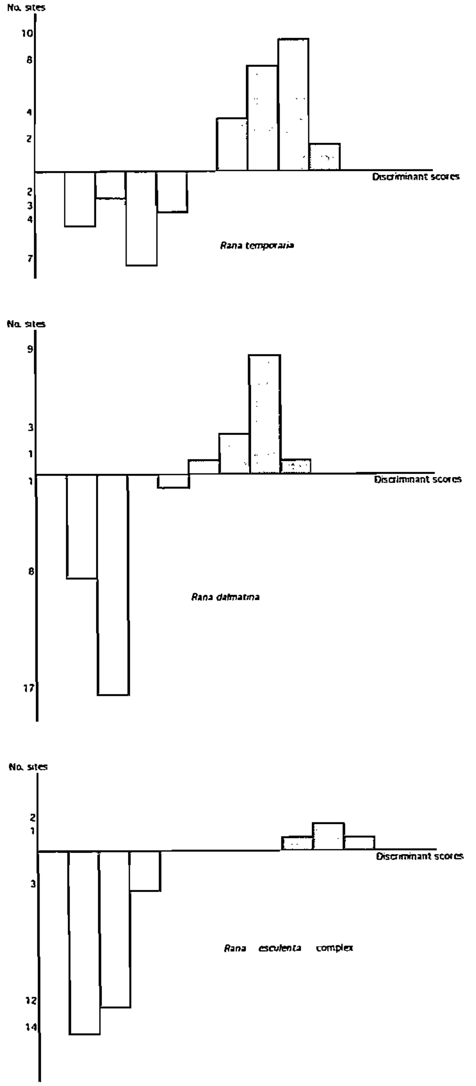

Figure 2: Discriminant grouping patterns (up *Rana temporaria*, middle *Rana dalmatina*, down *Rana esculenta complex*). Values on x-abscissa are of discriminant scores, and sites are grouped at intervals of 0.4 units. Sites without species are grouped below the discriminant score line (unshaded histograms) and those with the species are above (shaded).

selection. In fact, the Agile Frog in this area prefers bodies of water with well-developed aquatic vegetation at the bottom, on the surface and on the edge; these sites were located at lower altitudes and on more recent geological substrates (quaternary morainic deposit). This kind of soil allows only the development of xeric plants and it hosts Amphibians resistent to dehydration (POZZI, 1980). *R. dalmatina* selected sites with higher spring air and water temperatures and lower relative humidity.

Rana esculenta is found at higher water and air temperatures. The study area is rather cold for this species, being near its altitude limits. The Green Frog chooses large and deep sites, probably to have smaller daily temperature fluctuations. Those sites are rather isolated and they are suited for fishing, which can explain the positive correlation of the presence of the Green Frog with the presence of fish and with distance from other bodies of water.

DISCUSSION

The classification of the sites into two different groups according to the presence or absence of Amphibians was exact. Discriminant analysis showed the differences between the used and non-used sites for each species.

We utilized a large number of variables to characterize all the ponds as well as possible. This was necessary because of the difficulty of predicting all the factors that might influence the distribution of the Amphibians in a certain area. The variables that mainly influenced the presence of the different species were botanical (percentage of vegetational cover at the bottom, on the surface and on the edge), morphological (altitude, surface area) and physical (temperature of the air and of the water, relative humidity). The chemical characteristics of the water generally had only a small influence on the distribution of the Amphibians.

ACKNOWLEDGEMENTS

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Species Canonical	Wilks' λ	F	P	Correlation
Salamandra salamandra	0.969	0.061	3.957	0.024
Triturus carnifex	0.924	0.146	2.608	0.042
Rana temporaria	0.938	0.121	2.866	0.035
Rana dalmatina	0.970	0.060	4.055	0.022
Rana esculenta complex	0.975	0.050	4.406	0.036

Table 1. Significance of discriminant analysis applied to the classification of ponds with respect to used or unused sites by each species.

Species		Actual site numbers	Predicted numbers		% correct
			-	+	
Salamandra salamandra	-	36	36	0	100
	+	4	0	4	100
Triturus carnifex	-	27	27	0	100
	+	13	0	13	100
Rana temporaria	-	16	16	0	100
	+	24	0	24	100
Rana dalmatina	-	26	26	0	100
	+	14	0	14	100
Rana esculenta complex	-	29	29	0	100
	+	4	0	4	100

Table 2. Success rates of discriminant classifications.

Actual site numbers refer to those known to be unused (-) or used (+) by the amphibian species. Predicted numbers are shown as those expected to be unused or used, from each "actual" category, on the basis of the discriminant functions derived for each species. % correct refers to the overall number of actual used + unused sites correctly classified.

Variables	*Salamandra salamandra*	*Triturus carnifex*	*Rana temporaria*	*Rana dalmatina*	*Rana esculenta*
area		+ 0.311			+ 0.626
depth					+ 0.499
slope	+ 0.561	- 0.417			
bottom	+ 0.420	- 0.310			
substrate				- 0.468	
altitude	- 0.344		+ 0.465	- 0.398	
paths	+ 0.325		+ 0.421		
houses			+ 0.348		
water bodies					+ 0.388
type	+ 0.680	- 0.322			
sp. transparency		- 0.311	- 0.481		
sp. % bottom			- 0.472	+ 0.400	
sp. % surface			- 0.573	+ 0.331	
su. % surface			- 0.544	+ 0.438	
sp. % edge			- 0.470	+ 0.516	
su. % edge			- 0.403	+ 0.479	
disturbance			+ 0.417		
sp. air t°C			- 0.445	+ 0.553	+ 0.501
su. air t°C					+ 0.502
sp. water t°C				+ 0.347	+ 0.451
su. water t°C		- 0.399	+ 0.426		
sp. nitrates		+ 0.359			
sp. hardness			- 0.451		
sp. humidity			+ 0.421	- 0.378	
fish			- 0.372		+ 0.542

Table 3: Most important discriminating variables for amphibian species.

Significant values of the correlation coefficients between the canonical variable and the independent ones are listed for each species. + means positive correlation; - means negative correlation.

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Space occupation for egg deposition in amphibians living in plain woodland and pasture land (east of France)

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Abstract: Amphibian communities have been studied in accordance with environmental variables of the breeding sites, using multivariate analysis. The results emphasize that the opening of the surrounding pond environment is determinant for the occurence of species and for the numbers of individuals.

Key words: amphibian communities, breeding sites, clutch numbers, environmental variables, multivariate analysis.

INTRODUCTION

The present paper deals with the control state, i.e. before an environmental perturbation, of a long-term study on amphibian communities. This study was realized as part of the set of an amphibian observatory previously to the construction of a motorway which will pass through numerous wet lands. The purposes of this observatory are, firstly, to characterize qualitatively and quantitatively amphibian communities without the motorway, secondly, to point out, during ten years, the impact of the motorway on these amphibian communities.

In this long-term study, the evolution of amphibian communities is investigated in relation with the evolution of reproduction sites. Amphibians are known to be sensitive to environmental perturbations and should be therefore great biological indicators. As they are exposed to both aquatic and terrestrial pollutants, amphibians are particularly sensitive because of their highly permeable skin which can rapidly absorb toxic substances; futhermore, the egg stage is extremely susceptible to chemical pollutants, and exposure to high concentrations of certain chemicals can result in developmental abnormalities (DUELLMAN & TRUEB, 1986; BLAUSTEIN & WAKE, 1990). Habitat destruction is one of the possible cause of the decline of amphibians (BLAUSTEIN & WAKE, 1990). In our study, data on aquatic and terrestrial environment have been measured as they have been shown to be both important to obtain an accurate description of amphibian habitats (SANDERS *et al.*, 1989). The main purpose of this paper is to present the results of multivariate analysis processing on the first year of sampling.

MATERIAL AND METHODS

Study sites

The observatory is set around a future motorway section which will be located at an altitude of about 220 meters between Bourg-en-Bresse and Dôle, in the Franche-Comté region (East of France). In this region, the environment diversity and the extensive agriculture, holding potential connections between the different sectors, enable the presence and the development of several amphibian species.

Two sectors representing different ecosystem types have been chosen for this study (fig. 1):

-sector 1 (around 35 km2): "le Bois de Foulletons", which constitutes a large woodland enclosing several large ponds of different surfaces, two and two related by ditches; this sector is surrounded by several farming with small ponds.

-sector 2 (around 50 km2): "la vallée du Sevron", which is surrounded by table-lands and constituted by thick and wet grass-lands with hedges, arable agriculture and small woodland patches; this sector encloses numerous farmings, numerous small ponds of different surfaces and ditches.

Species of which the repartition area recovers the study region (according to the atlas of CASTANET & GUYETANT, 1989):

Salamandra salamandra (Ss), Triturus alpestris (Ta), *Triturus cristatus* (Tc), *Triturus helveticus* (Th), *Triturus vulgaris* (Tv), *Alytes obstetricans* (Ao), *Bombina variegata* (Bv), *Pelodytes punctatus* (Pp), *Bufo bufo* (Bb), *Bufo calamita* (Bc), *Hyla arborea* (Ha), Rana dalmatina (Rd), *"Rana esculenta"* (Re), *Rana temporaria* (Rt).

Amphibians species have been determined by visual (with the help of the guides ARNOLD & BURTON, 1978 and FRETEY, 1975) and auditory (with the help of GUYETANT & ROCHE 1987 sound track) samplings, and numbered by counting adults and clutches, from April to July 1993.

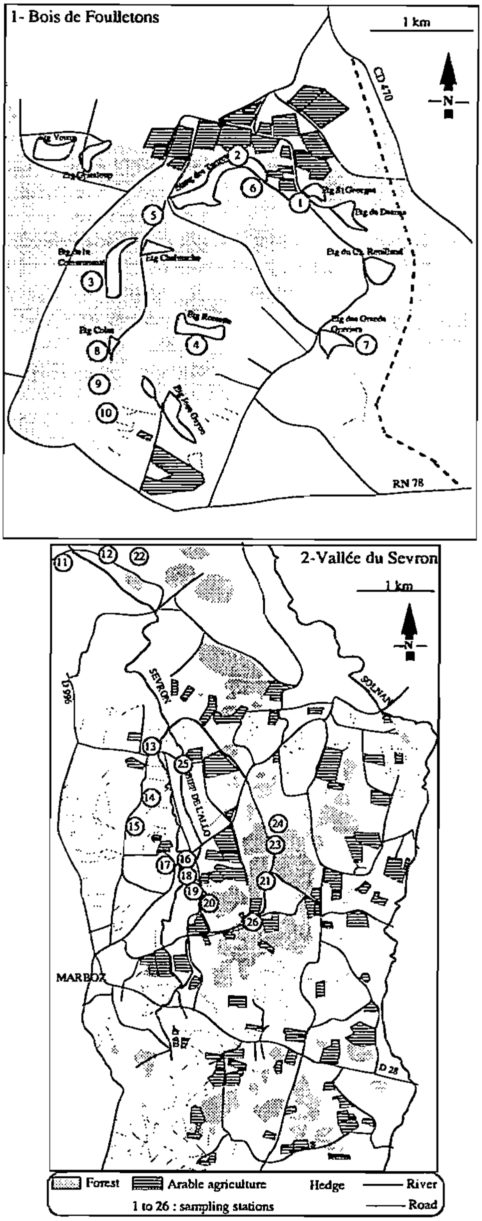

Figure 1 : maps of the two study sectors.

Environmental variables

At each sampling station, 12 environmental variables have been measured:

a) Morphometric and landscape variables:

- Mean surface of water area (SURF)
- Woodland relative surface (500 meters around) (% WOOD)
- Cultivation relative surface (500 meters around) (% CULT)
- Pasture relative surface (500 meters around) (% PAST)

b) Physical and chemical variables of water:

- Conductivity (COND)
- pH
- Dissolved oxygen (O2%)
- Nitrates (NO3)
- Ortho-phosphates (PO4)
- Sulphates (SO4)
- Chlorides (Cl)
- Bicarbonates (BIC)

Data processing

In order to get a picture of (1) the relations between the sampling stations and the environmental variables and (2) the relations between the occurence of the amphibians and the environmental variables, multivariate analysis has been applied, as in the previous studies of STRIJBOSCH, 1979a; BEEBEE, 1985 and PAVIGNANO *et al.*, 1990. The type of analysis is a principal components analysis (PCA) with supplementary variables (Macmul program, THIOULOUSE, 1989). From the ordination of the environmental variables, the PCA enables to obtain:

- the factorial map showing the typology of the sampling stations,
- the correlation circle resulting from the typology of the environmental variables,
- the factorial map showing the typology of the species abundance (supplementary variables); this factorial map will be superposed to the factorial map of the sampling stations.

RESULTS

Amphibians have been numbered at 10 sampling stations (numbers 1 to 10) in the sector 1 and at 16 sampling stations (numbers 11 to 26) in the sector 2 (Fig. 1). The species *Triturus vulgaris*, *Alytes obstetricans*, *Pelodytes punctatus* and *Bufo calamita* have never been observed in the two sectors.

Correlation circle of environmental variables (Fig. 2).

The F1 axis enables to discriminate, on the one hand, the woodlands and on the other hand, the pastures which show high conductivity and mineral salt values, surrounding the breeding sites. The F2 axis is mainly inducted by the pH and the oxygen rate.

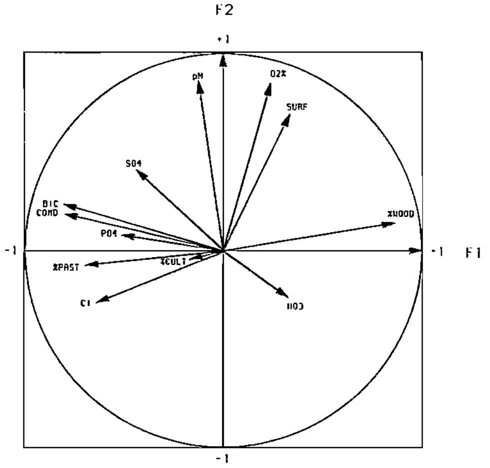

Figure 2: correlation circle of environmental variables.

Factorial map of the sampling stations (Fig. 3).

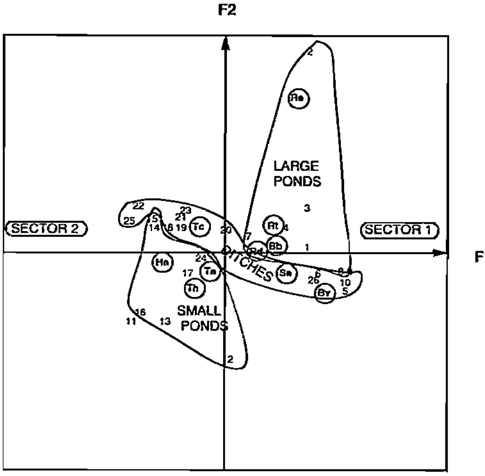

Figure 3: superposition of the factorial map of the sampling stations (numbers 1 to 26) and the factorial map of the species abundances (encircled letters). The stations corresponding respectively to large ponds, small ponds and ditches are distinguishable.

On the F1 axis, the PCA enables to discriminate the main three types of amphibian habitats which are "large ponds", "small ponds" and "ditches". The sampling stations which are constituted by large ponds are representative of the sector 1 (Bois de Foulletons), the sampling stations which are constituted by small ponds are representative of the sector 2 (vallée du Sevron) and the sampling stations which are situated in ditches and which appear between large and small ponds in the factorial map, are representative of both sectors.

Fauna variables (Fig. 3).

Species abundances, except the complex "*Rana esculenta*", mainly appear in the central part of the factorial map and thus they do not show high correlations with the axis F1 and/or F2.

The superposition of this factorial map of the fauna variables with the correlation circle of the environmental variables mainly shows the following observations: on the F1 axis, the species *Bufo bufo, Rana dalmatina, R. temporaria*, and also *Bombina variegata* and *Salamandra salamandra* show preferences for woodland occurence around the sampling station; to the opposite, the species *Triturus alpestris, T. cristatus, T. helveticus* and *Hyla arborea* prefer more open environments (pastures and cultivations), with higher conductivity, higher concentrations of mineral salts (bicarbonates, sulphates, chlorides, phosphates). On the F2 axis, the complex of the green frogs "*Rana esculenta*" are highly related to the sampling stations with large surfaces and a good oxygenation.

The superposition of the factorial map of the sampling stations with the factorial map of the species abundances (Fig. 3) shows that the species *Bufo bufo, Rana dalmatina, R. temporaria* and the complex "*Rana esculenta*" mainly breed in large ponds and are more numerous in the sector 1, when the species *Hyla arborea, Triturus alpestris and T. helveticus* mainly breed in small ponds and are more numerous in the sector 2; the species *Bombina variegata, Salamandra salamandra* and *Triturus cristatus* mainly breed in ditches and occur in the two sectors.

DISCUSSION

According to our results, space occupation for egg deposition in amphibians is mainly related to the opening of the surrounding environment. *Bufo bufo, Rana dalmatina, R. temporaria, Bombina variegata* and *Salamandra salamandra* breed in sites which are nearby woodlands, when *Triturus alpestris, T. cristatus, T. helveticus* and

Hyla arborea breed near pastures, where conductivity and concentrations of mineral salts are the highest. On the other hand, the complex "*R. esculenta*" is rather attracted by large and well oxygenated pieces of water.

Literature on amphibians has shown that the presence of woodland near the breeding site is fundamental for *R. temporaria* (HEUSSER, 1968; LOMAN, 1978; AUGERT, 1992) and *R. dalmatina*, of which the decreasing is proportionnal to the increasing of the distance between the breeding sites and the forest (LAAN & VERBOOM, 1990; HOLMEN & WEDERKINCH, 1988). As regards newts, they have been shown to breed in eutrophic places (where cattle drinks), which is probably related to the carnivorous life of the larvae (STRIJBOSCH, 1979b), when their terrestrial life is related to the occurence of woodland. As woodlands are potential habitats, pastures and fields are thought to be unsuitable for amphibians to survive longer periods than breeding and larval growth ones (STRIJBOSCH, 1979b; LAAN & VERBOOM, 1990). On the other hand, anuran have been shown to generally avoid oligotrophic and acid water; in particular, *Bufo bufo* and *B. calamita* select rather eutrophic water during their aquatic phase (STRIJBOSCH, 1979a).

When comparing the occurence of amphibian species in the two sectors under study, their diversity has been found to be higher in the sector 2 (vallée du Sevron), where 9 species were numbered, than in the sector 1 (Bois de Foulletons), where 6 species were numbered. This observation may be related to the fact that the landscape of the sector 2 shows a higher habitat diversity than in the sector 1 which is more uniformly forest. The sector 1 contains the species that mainly choose forest habitats as the common toad (*Bufo bufo*) and the common frog (*Rana temporaria*) (HEUSSER, 1968).

The sector 1, where most of the reproduction sites are large ponds, contains mainly anura, with the highest egg-laying numbers, in particular concerning the common frog (*Rana temporaria*) (20 to 200 clutches numbered according to the sampling station). However it is noticeable that frogs do not breed only in large ponds, which are more permanent water sites, but also in ditches, which are often temporarily drowned and running dry when rainfall is scarce. In other studies, *R. temporaria* has already been observed to breed in temporary ponds (ARRAYAGO & BEA, 1985).

The sector 2, with numerous small ponds and ditches, contains both anura and urodela. However, as regards the common frog (*Rana temporaria*) which was the most abundant species, the numbers of clutches per sampling station were in average lower (1 to 150 clutches numbered according to the station) while the sampled breeding sites were more numerous per km2, than in the sector 1. This might be due to the higher diversity and the lower surface of the breeding sites in this sector surrounded by an open environment. Larval survival of tadpoles is often uncertain in this sector where numerous ditches run dry before metamorphosis end.

As regards amphibian conservation, LAAN & VERBOOM (1990) have demonstrated that the probability of occurence of species in suitable patches increases with an increasing connectivity of the landscape by woodland. Such an observation should be seriously taken in consideration in the case of human constructions, as motorways. On the other hand, it should not be forgotten, according to the observations of GIBBONS (1988) and PECHMANN *et al.* (1991), that it is generally difficult in amphibians to distinguish declines resulting from human activities from natural population fluctuations without long-term data on the natural variations in population sizes.

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Choices of wintering places in a floodplain along the river Rhine by *Bufo bufo*, *Bufo calamita* and *Rana temporaria*

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Abstract: A radio-tracking method was used to determine wintering places of *Bufo bufo*, *Bufo calamita* and *Rana temporaria* in relation to inundation frequencies in a floodplain. Based on inundation frequencies three different parts were distinguished: parts which have a chance to inundate once every ten years, which have that chance once a year and last the almost permanently inundated waterbodies in the area. All *Bufo bufo* spent the winter in the area with an yearly inundation frequency. *Bufo calamita* wintered in parts which have a chance to inundate once every ten years and once a year. *Rana temporaria* wintered in the water and in the parts which have a chance to become inundated every year.

Keywords: floodplain, flooding, wintering, radio-telemetry

INTRODUCTION

Amphibians living in floodplains have to face a factor which is specific for this habitat: floodings or inundations. In the basin of the river Rhine, in which this study has been carried out, floodings can occur throughout the whole year, but they occur mostly in winter and spring. The waterlevel fluctuates up to 6 ms (CREEMERS, 1991). When floodings occur during their active period, amphibians can migrate to higher places, climb in trees or stay in the water (MARIAN, 1977; PINTAR, 1979; PINTAR, 1984). But how do they react on floodings during their "inactive" period in winter?

In a floodplain they seem to have three possibilities for wintering:

-they stay in the water

-they avoid inundation by migration to higher, dry parts of the floodplain

-they stay in lower parts of the floodplain which are mostly dry in autumn, but will then have a big chance to become flooded during winter or early spring.

The question is which of them will be chosen by the different species and if the last one is chosen will they survive a flooding during wintering.

STUDY AREA

The study area "Millingerwaard" is a semi-natural area in the basin of the river Waal, the main branch of the Rhine system in the Netherlands. It is situated close to the German border. The riverbanks are bordered by dunes and small dikes protecting the area for moderate floodings. At a varying distance between 1 and 2 km from the river the study area is enclosed by a large dike protecting the hinterland, a part of the former floodplain, for inundations.

Based on inundation frequencies three different parts were distinguished. Firstly, places with a mean inundation frequency of once every 10 years. These are two former properties of brick factories and the property of a church community. These were considered as high water free places. Secondly, parts of the study area which have a mean chance to inundate once a year. Mostly this part of the study area is "dry" when amphibians start wintering. Here old riverine willow woods are alternated by young ones on places were recently clay is dug out. Other parts of this area are in use for agricultural purposes. An old oxbowlake and temporally and permanently water carrying claypits are the waterbodies, the third component in the area. When the waterlevel in the river is rising before a flooding, the waterlevel in these bodies also rises. So when inundation occurs it will have no influence.

MATERIAL AND METHODS

A radio-tracking method (NULAND, 1981) was used for determining the sites where the amphibians wintered. In autumn 1991 two adult *Bufo bufo* and three adult *Rana temporaria* were tagged and four adult *Bufo bufo* and five adult *Bufo calamita* in autumn 1992. Except for one, all toads and frogs were taken from that part of the area which has a mean chance to inundate every year. One *Bufo calamita* was found at the edge of the property of a former brick-factory, which seldom becomes flooded. Before it started wintering it migrated to the lower part of the study area.

As soon as possible after capture transmitters were implanted surgically in the abdomen of the animals. Within two or three hours after the operation they were released at the place where they were caught in the field. In the first year of investigation the animals were followed with a hand-held receiver; in September and October every two days, during the winter once a week and just before migration started every day. An automated radio-tracking system registered the animals 24 hours a day the second year.

RESULTS

All specimens studied survived the winter and started migration in spring of the next year. Table 1 shows where the animals stayed during their inactive period in winter.

	mean chance of inundation		
	once every 10 year	once a year	water
B. calamita	3	2	-
B. bufo	-	6	-
R. temporaria	-	1	2

Table 1: Wintering places of tagged *Bufobufo*, *Bufo calamita* and *Rana temporaria*.

All *Bufo bufo* wintered in the lower part of the floodplain. One specimen spent the winter at the edge of a dune near a willow tree. All the others wintered in the border of a road through a 40 year old willow wood.

Two *Bufo calamita* wintered in the dilapidated fundamentals of an old building of a former brick factory and one in an old shed in the same area. Two specimens wintered along the border of a sandy path in the lower part of the floodplain. The vegetation at the last places was dominated by *Calamagrostis epigeios*.

One *Rana temporaria* wintered along a road through a 40 year old willow wood in the lower part of the area. Two others under water in claypits.

DISCUSSION

The results show that all species can spend their "inactive" period in the lower part of the floodplain which has a chance to inundate once a year. However, in both years of investigation the Rhine did not raise to that high levels that the study area became flooded. So the effect of inundation during the "inactive" period of amphibians remains unsolved. The question still is, will they survive. Schmid (1965) suggests for some terrestrial species that they will not survive. Then inundation during wintering will have a great influence on populations.

Amphibians which winter in the water or at places which seldom become flooded or in the hinterland of the floodplain will have to compensate the losses in the flooded part after a winter inundation. As high water free places are rare in Dutch floodplains and great parts of the hinterland are cultivated and seem to form largely unsuitable habitats, the effect on populations will be very big. This could explain low densities of amphibians in Dutch floodplains.

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Feeding of an Iberian population of *Bufo bufo* during the reproductive period

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Abstract: The feeding of *Bufo bufo* during the reproductive period has been studied in a population located 30km to the North of Barcelona (Spain). The stomach contents of 23 dead individuals were used. 52% of these showed full stomachs with contents partially digested. The numerical relative abundance index (%N), the percentage of stomachs which present a determinate prey (%P) and use intensity index of the resources (IU) are stimated. The first index shows that the most important preys are: Hymenoptera, Colleoptera, Araneida, and larvae of Lepidoptera. IU indicates that Colleoptera and Geophilomorpha are important preys. *Thaumetopoea pytiocampa* was ingested by 33% of toads. Climate conditions and the long reproductive period determines the presence of preys in the stomachs during the reproductive period.

Key words: *Bufo bufo*, breeding, feeding, Spain, Anura, Bufonidae.

INTRODUCTION

The common toad (*Bufo bufo*) is a Palearctic anuran with a wide distribution area. There are some papers on the diet of this species (PELLANTOVA 1978, GUHA *et al.* 1980, LARSEN 1984, GITTINS 1987), and general references about its feeding habits can be found in some European field guides (COTT 1936, ANGEL 1946, SMITH 1951, SAVAGE 1961, GUYETANT 1967, SALVADOR 1974, BAS-LOPEZ 1982, BEA *et al.* 1994).

Some adults of this species were found dead during research on its reproductive biology (HEUSSER 1969, WELLS 1977, GUHA *et al.* 1980, GITTINS 1987). The data about the feeding of toads during the reproductive period are scarce and some authors affirm that these amphibians do not feed at this time. On the other hand most of the published studies refer to the common toad's diet in center and north Europe. This study reports the findings of a study in dead animals found in the South of Europe.. Therefore, we considered the finding of dead animals very interesting to approach this subject.

MATERIAL AND METHODS

The studied population is located 2 Km from the coast and 30 Km North of Barcelona. This population has been widely described in previous papers (CAMPENY, 1983; CAMPENY & MONTORI, 1985; CAMPENY & MONTORI, 1988). The specimens of *Bufo bufo* were found dead mainly because of shooting during the reproductive period. February-May 1982-1983. Eight toads were collected in 1982 and fifteen in 1983. In the analysis only the stomach content were considered. The method described by RUIZ (1985), (1981, 1983) Y JOVER (1989) was used for the statistical treatment of the diet.

The estimated indices are the following: %N, %P, λ" (RUIZ & JOVER, 1983) and IU (Use intensity index of the resources)(JOVER, 1989). The latter was described by JOVER (1989) and takes into account the importance of one resource, combining in a single number: the proportion in which a resource contributes to a collective's total diet, the percentage of individuals which eat the resource and the higher or lower homogeneity of the utilization of this resource. (CARRETERO & LLORENTE, 1991). The diversity index H (Average Individual Diversity) & H_z (Added diversity) was calculated using Margalef's index, which has some advantages in relation to other index.

RESULTS

All individuals used in the study were in reproductive period and sexually active. 23 individuals were collected, 20 of them had at least one prey in the stomach contents. Of these, five showed their stomach content to be absolutely digested and unidentifiable. Three toads had the stomach empty and the remains of preys were found at the end of intestine (fragments from Myriapoda, Lepidoptera larvae, Araneida, Colleoptera, and Hymenoptera, among others). These three toads together with the five that presented unidentifiable contents were not included in the data analysis.

In the fifteen contents analyzed 106 preys from 12 different taxa were identified (x=7.07 preys/stomach, s=6.64, range between 1 and 20 preys). Six individuals had eaten moult.

Estimations of %N, %P and IU (Use index) and the average and added diversities for the analyzed sample are gatered in table 1. The %P shows that the Araneida and Lepidoptera larvae (both with 40%) are the most consumed taxa but are not very important in %N for the population. Attending to IUR index, the most important preys of *Bufo bufo* were: Himenoptera, Araneida, larvae of Lepidoptera and the adult of Colleoptera.

Prey order	%N	%P	λ"	IUR
Araneida	15.94	40.00	12.79	23.23
Opiliona	1.89	13.33	11.97	1.20
Acari	0.94	6.67	0.03	0.00
Coleoptera	12.26	33.33	5.43	14.43
Coleoptera lar.	2.83	20.00	3.14	2.86
Lepidoptera	1.89	13.33	0.18	1.20
Lepidoptera lar.	12.26	40.00	9.32	17.77
Heteroptera	3.77	20.00	3.47	3.40
Hymenoptera	34.91	26.67	21.14	27.40
Diptera	0.94	6.67	1.32	0.00
Dermaptera	0.94	6.67	0.03	0.00
Insecta unident.	2.83	13.33	3.21	1.53
Insecta (l.) un.	0.94	6.67	0.12	0.00
Iulida	0.94	6.67	0.15	0.00
Diplopoda	1.89	6.67	0.47	0.00
Geophylomorfa	4.72	33.33	27.08	6.98
Isopoda	0.94	6.67	0.15	0.00

H_i (average diversity of individual diversities) = 0.748 (se = 0.385)

H_t (total accumulate diversity through "jack-knife" estimate) = 3.133 (se = 0.343)

Table 1: Descriptor index of the diet

DISCUSSION

In general, the preys identified confirm the results obtained by other authors. (LESCURE, 1964; MAZURE, 1966; GUYETANT, 1967; MATHIAS, 1971; LIZANA *et al.*, 1990; BEA *et al.*, 1994) This authors observe that Formicidae are an important part of the diet of *Bufo bufo*, as in our studied population (Table 1). The common toad seems to be a specialist to predator of small preys, as referenced by other authors (WHEATHER, 1986, LIZANA *et al.*, 1990). In spite of this, we observe that major preys are also eaten, within this population (3 cm long for Lepidoptera larvae, 2 cm for adults of Lepidoptera and 5-13 cm for Geophilomorpha).

On the other hand, the larvae of Lepidoptera are not eaten at all (GUYETANT, 1967), or are found in the diet in a reduced number (WHEATER, 1986), while they appear with high or medium values in all food indices estimated in this study. The Myriapoda, not found in the diet of *Bufo bufo* by GUYETANT (1967) and considered accidental by LIZANA *et al.* (1990), represent 7.65% in our analysis of the diet, similar to that reported by BAS-LÓPEZ (1982) and BEA *et al.* (1994). WHEATER (1986) found a progressive increase in the percentage of Myriapoda in relation to the body size of toads, up to 19.82% in the biggest toads (>40mm).

According to MARGALEF(1977), many invertebrates are in wintering during the cold season, in temperate latitudes. However, if winters are mild, several Spiders, Miriapods, Isopods and Coleopters, among others, are still in activity. Thus, it is in this period that major deviations in the diet are observed. It would necessary to note that if the presence of Lepidoptera larvae and Miriapoda corresponds to a deviation of the kind described by MARGALEF (1977) or if those are habitual preys. We should consider that the values obtained by BAS-LÓPEZ (1982) and BEA *et al.* (1994) also refer to meridional reproductive populations.

The results obtained by PELLANTOVA (1978) in south Moravia, only for the reproductive period, show that the most important prey are Himenoptera and Formicide, which represent 38% of the diet, followed by Colleoptera (25%) and Araneida (18,5%). Miriapoda (Diplopoda + Quilopoda) and larvae of Lepidoptera are present with 2.5%. In a general sense we can consider that these results are similar to the ones obtained with the population studied in this paper.

The low presence of aquatic larvae of Coleoptera in our population indicates that the common toad normally eats on the ground, although it also eats preys from the water.

All Lepidoptera larvae eaten, except one, were *Thaumetopoea pytiocampa*. Until now we only know five vertebrate species that eat these larvae (*Upupa epops*, *Cuculus canorus*, *Clamator glandarius*, *Parus major* and *Cyanopica cyanea* -Pedro Cordero *in verbis*-). We believe that the ingestion of these larvae cannot be by chance because 33% of toads had this prey in their stomachs. On the other hand we do not believe that the death of some of them was caused by the caterpillar due to the fact that some toads had an almost completely digested this prey, together with some others that had just been consumed.

The data referred in the bibliography show that the common toad do not feed during the reproductive period. HEUSSER (1969) indicates that *Bufo bufo* starts its feeding after this period. GUHA *et al.* (1980) and GITTINGS (1987) show similar results. According to WELLS (1977) an explosive reproductor can survive all the reproductive period without feeding because the low metabolic rate recovers energy quickly when the reproduction has finished.

The reproductive period of *Bufo bufo* which normally lasts from 3 to 28 days (ANGEL, 1946; HEUSSER, 1969; SALVADOR, 1985), lasted 41 days in 1982 and 64 days in 1983 in our population. Consequently this can not be considered an explosive reproductor.

Regarding diversity indexes, the value of Shannon index of diversity obtained by LIZANA *et al.* (1986) is 1.61. This is clearly lower than our results. This may be explained by the great amount of ants eaten by the toads that Lizana studied and the low proportion of common preys and the presence of unusual preys in our toads (Miriapoda, larvae of Lepidoptera, Isopoda, Diptera, Acari and Coleoptera larvae). Nevertheless, these results should be compared with caution, since the indexes used by Lizana and ours are not the same and the number of toads analyzed is small. This diet structure may be in relation to the relative scarcity of available preys during this period.

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Reproductive effort in *Bufo bufo*

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Abstract: Clutch mass and female weight were measured of 47 females of amplexus pairs of *Bufo bufo*. As amphibian weight is highly dependent on water content of the body, data on Relative Clutch Mass of amphibians are hardly comparable with those of reptiles. It is demonstrated that populations of *Bufo bufo* at about the same place in the distribution area of the species can be very different not only in population parameters such as the mean length of males and females or the number of eggs per female but even in their life strategy.

Key words: Relative Clutch Mass, egg number, reproductive effort, *Bufo bufo*

INTRODUCTION

Reproductive effort is the amount of energy used by an organism for reproduction in comparison with the total amount of energy consumed in the period between two reproductions. Establishing the amount of consumed energy in the field is very difficult. Therefore Relative Clutch Mass (RCM) is often used as a measure for reproductive effort. RCM is the weight of all eggs (or young) of a female divided by the weight of that female. In herpetological studies RCM mostly is used in studies on reptiles. Here I will discuss the possible use of RCM in the amphibian species *Bufo bufo*.

MATERIAL AND METHODS

In spring 1993 I caught 47 amplexus pairs of *Bufo bufo* in three different localities; 33 pairs in an area with woodlands, pastures, agricultural fields and farmhouses (population 1), 9 pairs in a woodland area (population 2) and 5 pairs in a flood plain area (population 3). I took these pairs to the laboratory and let them spawn in separate aquaria.

To establish the RCM one has to measure clutch weight and female weight. Weighing the eggstrings of toads would include a great amount of yelly. Therefore I estimated the number of eggs of the eggstrings of a female by counting the eggs of at least five small pieces of the strings and weighing these small pieces and the total clutch, and I estimated the volume of the single eggs of that eggstrings by measuring the diameter of the eggs (accuracy 0.05 mm), assuming that the eggs were round. Multiplying egg number and egg volume I got the total egg volume. Taking 1 cm^3 as 1 g I got the estimate of the clutch mass used in this study. Females were weighted as soon as possible after egg-deposition, but in any case within 10 hours. For calculation of the reproductive effort I divided the weight of the clutch mass by the sum of the weight of the clutch mass and the weight of the female immediately after spawning. This sum is an estimation of the weight of the female just before spawning.

RESULTS

The number of eggs per female varied between 1859 en 6305. Figure 1A gives the egg number in relation to female length in mm. For population 2 and 3 there is an indication that egg number increases with increasing female length, but the number of observations is to small to draw firm conclusions. As far as population 1 is concerned no relation between both parameters was found. Figure 1B shows the egg diameter in relation with female length. No relation between these parameters was found. Data suggest that the population of the flood plain area is different from the other two populations in this respect. Figure 1C gives the clutch mass (total egg volume) in relation with female length. For population 2 and 3 again an indication is found that clutch mass increases with female length. For population 1 no correlation was found too.

In figure 2A I compare the length of the females with their weights immediately after spawning thereby making a distinction between females that are relatively heavy or light regarding to their length. Figure 2B shows that the clutch mass of "heavy" and "light" females is about the same. I also checked the number of eggs of both groups as well as the diameter of the eggs and did not find a difference either.

In Figure 3 I compare the total egg volume of the females with their weight immediately after spawning. Dividing clutch mass by female weight just bfore egg deposition (see methods) gives the reproductive effort. In figure 3 lines of

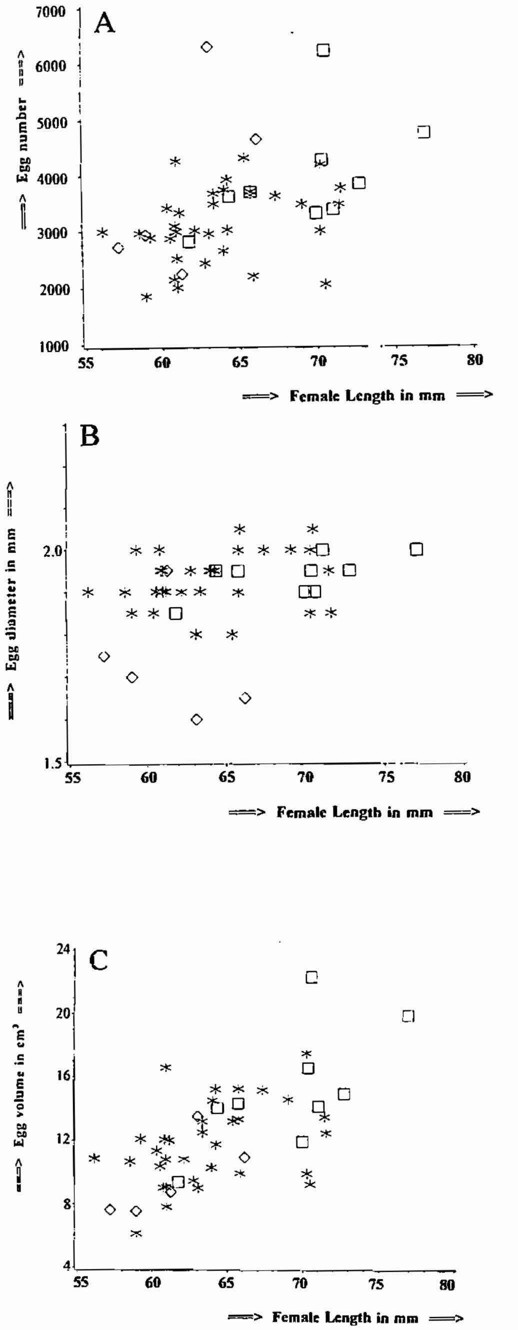

Figure 1: Relation between female length and egg number (A), egg diameter (B) and egg volume (C). Population 1 is represented by a star, population 2 by a square and population 3 by a diamond.

equal reproductive effort are given. The reproductive effort of population 2 and 3 lays between 0.2 (20%) and 0.3 (30%) and in both populations there is an indication of increasing reproductive effort with increasing female weight. In population 1 many females have a reproductive effort between 20 and 30% too but in this population a number of light females have higher values, up to 36 %.

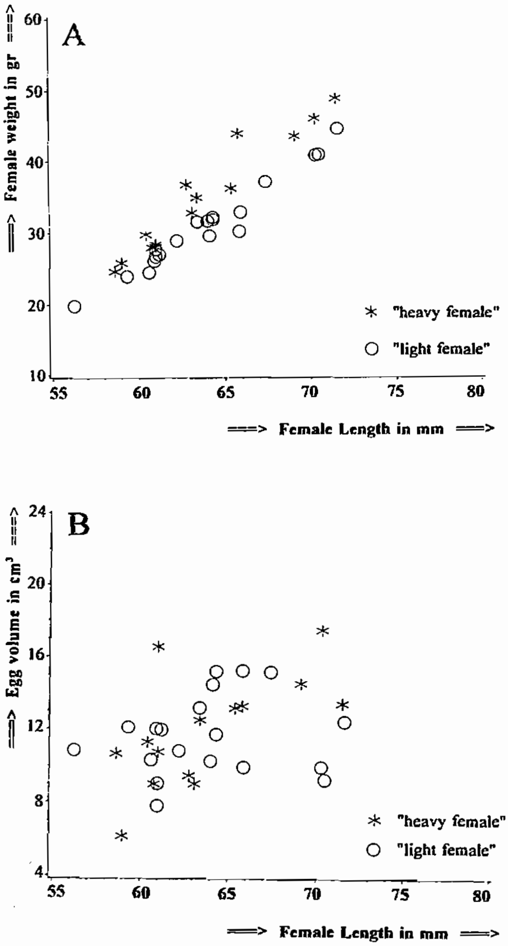

Figure 2: Relation between female length and female weight immediately after spawning (A) and egg volume (B). See text for further explanation.

DISCUSSION

For the three populations of this study no clear correlation was found between the number of eggs of the females and their length. KADEL (1977) measured two different populations. On a double logarithmic scale he found a correlation between egg number and female length and showed that differences may exist between populations. The females of my populations are smaller than those of the two populations of Kadel, but the number of eggs per female is clearly higher.

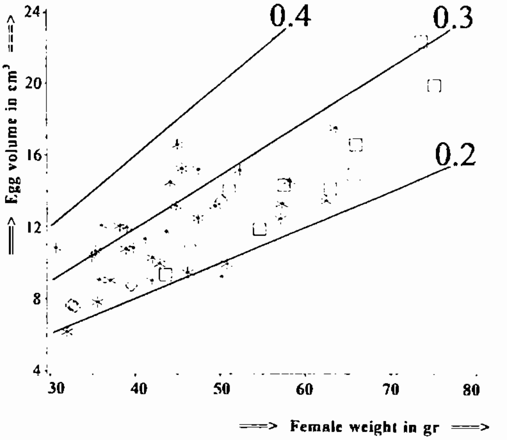

Figure 3: Relation between female weight just before spawning and egg volume. Lines of equal reproductive effort (0.2, 0.3, and 0.4) are given. See text for further explanation. Symbols as in figure 1.

GITTINS *et al.* (1984) suggest that the maximum number of eggs at a certain length increases with length and that the minimum number of eggs is independent of length. Putting the number of eggs against female length in a figure results in a filled right angled triangle; the base of this triangle is the minimum number of eggs, the hypothenuse represents the maximum number of eggs at the different lengths and the perpendicular line indicates that the largest females may have the minimum as well as the (greatest) maximum number of eggs and all values in between. The females of this study have about the same length as those of GITTINS *et al* (1984) but far more eggs; they never have the minimum number mentioned by Gittins et al. The data (figure 1A) also do not show the triangle mentioned above. In the discussion after the lecture, Kuhn suggested that this could be caused by the fact that in the different studies the toads were not caught at random regarding to their length. This is true for population 1; from this population we captured more females of the "middle" class. The fact, however, that we never measured the minimum number of eggs mentioned by Gittins, suggests that the relation between number of eggs and female length is more complex. In any case it confirms the finding of KADEL (1977) that populations may be different regarding the relation between the number of eggs and female length.

Based on the diameter of the eggs and the number of eggs per female Kadel calculated the total egg volume per female. For one of the two populations he found a relation between this total egg-volume and female length. I did find an indication for this relation in two of the three populations. Comparing my data with that of Kadel reveals that the angle of the line is almost the same, however the total egg volume per length is almost double.

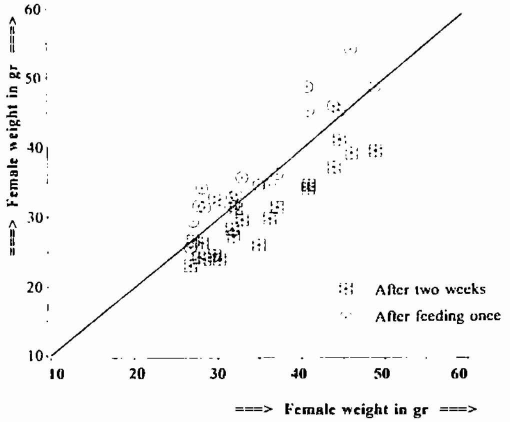

Figure 4: Relation between female weight immediately after spawning (X-axis) and (Y-axis) weight after two weeks in terraria without food (squares) and after feeding and another week in terraria (circles). Line of no change in weight is given.

In literature it is suggested that in amphibians there is a relation between the weight (at a certain length) and condition. Therefore I made a distinction between heavy and light females (figure 2A). One would expect that (for the same lengths) females in a better condition should produce higher total egg volumes. This was not found.

Reproductive effort is expressed as the ratio between clutch weight and female weight just before egg (young) deposition. Estimating the appropiate weight of the female is difficult, however. It is commonly known that the weight of amphibians fluctuates during the year (HEMMER AND KADEL, 1972) and that a part of this fluctuation may be attributed to a changing water content of the body (see for a discussion on this subject RYSER, 1989). I estimated the weight of the female just before spawning by taking the sum of the clutch weight and the weight of the female immediately after spawning, assuming that the water content of the females is about the same during this period. This assumption, however, is incorrect if female weight increases or decreases sharply within 10 hours after egg deposition. In a pilot study I kept twenty females weighted within 10 hours after egg deposition in separate terraria with free access to water and measured their weight again after two weeks: the weight decreased up to 25%. Then I gave the females food ad libitum during one day. On the average they consumed 1.5 gram. After another week I weighted the females once more: as can be seen in figure 4, the weight increased up to 45% due to water content of the body. This pilot study shows that there may be a great variation in female weight in a period of three weeks after egg deposition. This variation is due to the water content of the body that possibly is related with feeding activity. I nevertheless think that the weight data of this study may be compared with each other as I measured all females very shortly after egg deposition before they did leave the water and resumed feeding activity.

An other problem, caused by the fact that the water content of the female body fluctuates during the year, is that it remains unclear how one has to estimate the appropiate weight of amphibian females if one wants to compare the values of reproductive effort of amphibians with those found in studies on reptiles. For studies on merely amphibian species the method used will give comparable results.

In the data presented it is conspicuous that a number of light females of population 1 have relatively high values for their reproductive effort (figure 3). This could be caused by the fact that the habitat of population 1 is originally a poor sandy dune area; only the last century agricultural activities enriched some parts of this area. In poor areas r-selection will occur rather then K-selection, so a selection on producing as much young as early as possible. This hypothesis is supported by HEMELAAR (1986) who studied population 1 and 2 skelettochronologically in 1981. She found that spawning in population 1 occurred at an earlier age then in population 2.

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Aspects of the reproductive biology of female *Rana graeca* (Amphibia, Anura) in Greece

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Abstract: Some aspects of the reproductive biology of female *Rana graeca* were studied in Macedonia, Greece. The body length of mature females was found to vary between 5.45 and 7 cm, and their body weight between 19.606 and 43 gr. The minimum ovarian weight was observed in July and the maximum in March. The weight of fat bodies showed a peak in August which was followed by a decrease during Autumn. The number of eggs per female ranged between 765 and 1135, leading to the conclusion that most females deposit two egg masses.

Key words: Reproduction, Ranidae, Amphibia, Greece.

INTRODUCTION

Data on some aspects of the reproductive biology of female *Rana graeca* are presented here, as a continuation of a broader study on the geographical distribution, biology and ecology of the species in Greece (ASIMAKOPOULOS, 1992).

MATERIAL AND METHODS

The study which was carried out in 1988 and 1989 was based on observations and samples from the locality Virout, at the springs of Gramoska river, on Paiko mountain, Macedonia, Greece. In 1988, samples were taken in July, August, September and October. No sampling was carried out during the hibemation period, while an additional sample was taken in March 1989, just before oviposition. For every sample, the mature females were used for the following five measurements: body length, body weight, weight of ovaries, number of eggs, weight of fat bodies.

RESULTS AND DISCUSSION

During the study, a total of 24 mature *R. graeca* females were collected. This number is satisfactory according to the small size of the populations of the species. The small size of the populations and especially the very small number of the mature animals was the main problem we faced. Among the mature females, the smallest had a length of 5.45 cm, whereas the largest had a length of 7 cm In table 1 the mean values of the five measurements of mature females at different months are presented.

Month	N	L. X±S.D. (range)	W. X±S.D. (range)	W.o. X±S.D. (range)	n.e. X±S.D. (range)	W.f.b. X±S.D. (range)
July	3	6.1±0.1 (6.05-6.25)	32.97±1.67 (31.29-34.62)	0.55±0.002 (0.54-0.57)	1063.3±21 (1043-1085)	----
August	6	6.48±0.32 (6.15-6.9)	34.36±7 (27.26-43)	1.82±0.67 (1.81-2.73)	1018.5±97.6 (839-1135)	0.36±0.23 (0.17-0.67)
September	6	6.61±0.24 (6.4-7)	35.58±3.15 (32.4-39.97)	4.72±0.79 (3.76-5.7)	935.8±90.1 (765-1006)	0.24±0.09 (0.12-0.32)
October	6	6.01±0.46 (5.45-6.52)	30.31±8.90 (19.61-41.02)	4.66±2.03 (2.38-7.07)	840±30.6 (797-881)	0.13±0.05 (0.05-0.17)
March	3	6.13±0.3 (5.87-6.46)	30.49±7.32 (22.4-36.74)	7.37±2.87 (4.4-10.12)	892±29.8 (865-924)	0.09±0.01 (0.09-0.1)
Total	24	6.31±0.39 (5.45-7)	33.00±6.31 (19.61-43)	3.79±2.51 (0.54-10.12)	943.1±103.1 (765-1135)	0.22±0.16 (0.05-0.67)

Table 1: Mean values (X), Standart deviation (S.D.) and Ranges (In parenthesis), for body length (L.), body weight (W.), weight of ovaries (W.o.), number of eggs (n.e.) and weight os fat bodies (W.f.b), at different months, in mature females *Rana graeca* from Virout of Paiko mountain.

The ovarian weight showed a continual increase from July, when it was about 1.6% of the body weight, to March, when it reached 24.2% of the body weight approximately.

The weight of fat bodies showed a remarkable and unexpected variation. In the specimens collected in July it was negligible. In August it showed a peak, and then it was continually decreasing during September and October. In March, after hibernation and just before oviposition, the weight of the fat bodieswas very low.

The decrease of the fat bodies mass, during Autumn before hibernation, when the animals are still active and feeding, has been also reported for R. *ridibunda* (KYIIAKOPOUIOU-SKLAVOUNOU & LOUMBOURDIS, 1990; Loumbourdis and KYRIAKOPOUIOU-SKIAVOUNOU, 1991), as well as for several reptiles (GREGORY, 1982). It has been suggested that, during preparation for hibernation, the lipid content of fat bodies is transferred to the liver and body, as lipids, glycogen or proteins (LOUMBOURDIS & KYRIAKOPOUIOU-SKIAVOUNOU, 1991). However, the study of the seasonal variations of the liver and body fat is necessary before any final conclusion on the lipid cycle of *R. graeca* females is drawn.

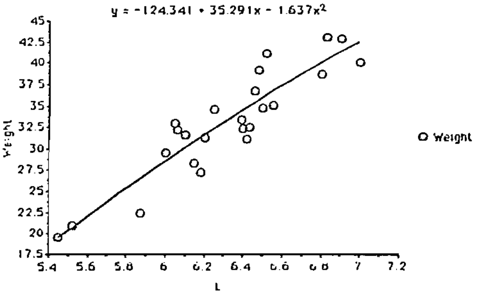

Figure1: Polynomial regression relation between body length (L.) and body weiht in mature females *Rana graeca* from Virout of Paiko mountain

The polynomial regression analysis showed a high corellation between body length and body weight ($r=0.913$, $p=0.0001$) (Figure 1). There was also a significant corellation between body length and weight of fat bodies ($r=0.695$, $p=0.0026$) (Figure 2). On the contrary, there was not found any significant relation neither between body length and weight of ovaries ($r=0.284$, $p=0.6307$), nor between body length and number of eggs ($r=0.391$, $p=0.3272$), suggesting that further search is needed on these topics.

The number of eggs per female ranged between 765 and 1135, close to those reported by BESHKOV (1970b) from Bulgaria (213-1320, usually 800-1250), but less than those reported by BRUNO (1967) from Italy (1000-3000). As it has been reported by ASIMAKOPOUIOS *et al.* (1990) and ASIMAKOPOULOS (1992), the number of eggs per egg mass in Virout of Paiko mountain, ranges between 268 and 730; so it is concluded that most females deposit their eggs in two egg masses.

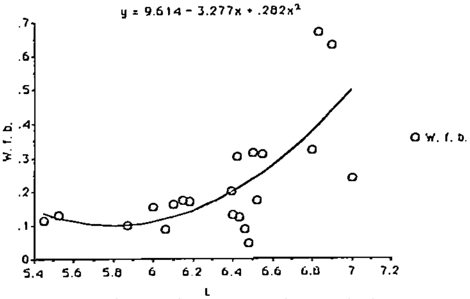

Figure 2: Polynomial regression relation between body length (L.) and weight of fat bodies (W.f.b.) in mature females *Rana graeca* from Virout of Paiko mountain

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Aspects of trophic biology in the *Rana* Klepton *esculenta* (Anura: Ranidae)

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Abstract: A preliminary study on the feeding of *Rana* klepton esculenta has been carried out in some ricefields belonging to the properties of "Cascina Galdina" and "Cascina Picchetta" in the municipal district of Cameri (Piedmont, North Italy). At present both these two sites are into the "Parco Ticino Piemontese". Through the analysis of the excreta (picked up during the autumnal season), we have been able to obtain a representative picture on the feeding of this animal during the fattening phase introductory tothe period of latency. To establish the feeding preferences in the examined biotope we considered the percentage composition of the preys and their number in the single feeds.

Key words: Trophic biology. Rana klepton esculenta. Piedmont North Italy.

INTRODUCTION

The study in object wants to put into evident the alimentary variability of *Rana* kl. *esculenta* (Amphibia, Anura) ; the probably alimentary interrelation between females and males of these species ; to take note some difference in the autumnal and spring feeding.

The work is based on dates picked in the years 1987/88, 1988/89 in two ricefields: "Cascina Galdina" and "Cascina Picchetta" ; Cameri - Novara (Piedmont - North Italy). The whole extention of the two ricefields is 117 perches (2958.94 m), periodical coltivated with maize and soya. Actually both are inside the "Parco Ticino Piemontese", but during the years of resource only "Cascina Galdina" was made part of it. They are surrended by a vegetation of rows and deciduous trees, these harpered the animal-catch.

MATERIAL AND METHODS

The two years resource, from the end of March to October, included the annual biological course of *Rana klepton esculenta*. For this work was prefered autumnal period before the hibernation. The animal-catch was made in the months of September-October (once a week) above all during the night: from 21.00 to 06.00. In total about 150 speciments were found, parted into 50 females and 50 males. They were put into vessels with damp paper on the botton and with a riddled cover for the respiration. The animals were kept under controlled stalling for 24/48 hours to check any trofic activity.

The first evacuations were noted after 12 hours, but in some cases an other one after 48 hours. The pellets, picked up and put into denatured alcohol, were studied at the Museo Civico di Storia Naturale di Milano by a binocular microscope (Table 1) with the help of experts. The used method permitted to obtain important results without damaging the animals. For the statistical survey were calculeted: 1. niche breadth (Levins'measure and Hurlbert's adaptation). 2. niche overlap (Pianka's mesaure). 3. food comparation between autumnal and spring period.

RESULT AND DISCUSSION

The table one present a very differentiated alimentation because the species live in an ambient whether aquatic or terrestral. The food is composed by a lot of invertebrates and completed with stones, vegetation, seeds. The stones helps in the food's trituration.

Food resources	Males		Females	
	A	B	A	B
Annelida	-	-	4	2.61
Arachnida	3	2.36	-	-
Cladocera	-	-	20	13.07
Collembola	-	-	-	-
Coleoptera	16	12.59	15	9.80
Diptera	5	3.93	10	6.53
Gasteropoda.	1	0.78	-	-
Hemiptera	2	1.57	5	3.26
Hymenoptera	36	28.34	37	24.18
Lepidoptera	9	7.08	14	9.15
Odonata	11	8.66	3	1.96
Plecoptera	1	0.78	1	0.65
Tricoptera	1	0.78	2	1.30
Vegetation/Stones/ /Seeds	42	33.07	42	27.45
Frog's skin.	-	-	-	-
Total	127	100	153	100

Table 1. Autumnal food composition. A=n° of specimens. B=percentage presence.

Niche breadth

LEVINS (1968) proposed the niche breadth to be estimated by measuring the uniformity of distribution of individuals among resource states and suggested that one way to mesure is:

$$B = 1 / p_i^2$$

where B = Levins' measure for niche breadth, p_i = proportion of individuals found in our using resource state i (table 1). "B" is maximum when the same number of individuals occurs in each resource state, so that the species does not descriminate amont the resource states and has the broadest possible niche. Levins'"B" is minimal when all the individuals occur in only one resource state (minimum niche breadth, maximum specialization). The range of "B" is from 1 to "n", where "n" is the total number states. It is sometimes useful to standardize niche breadth proposed the following measure for standardized niche breadth:

$$BA = (B-1)/(n-1)$$

where BA = Hurlbert's standardized niche breadth, B = Levins' measure of niche breadth, n = number of possible resource states.

Rana klepton esculenta male:

$B_M = B = 1/ \Sigma p_i M^2 = 1/0.2766 = 3.6153$ (Levins)

$BAM = (BM-1)/(n-1) = 2.6153/14 = 0.1868$ (Hurlbert)

Rana klepton esculenta female:

$BF = B = 1/ \Sigma p_i F^2 = 1/0.2926 = 3.4176$ (Levins)

$BAF = (BF-1)/(n-1) = 2.4176/14 = 0.1726$ (Hurlbert)

Niche overlap

To calculate Pianka's niche overlap suggested the following formula:

$$O1,2 = n\ \Sigma p_i 1 * p_i 2 / = \Sigma p_i 1^2 * p_i 2^2$$

where $O_{1,2}$ = Pianka's measure of niche overlap between species 1 and 2:This measure of overlap ranges from 0 (no resource in common) to 1.0 (complete overlap).

Males - Females: *Rana klepton esculenta*

$OM,F = n\ \Sigma piM * piF / = \Sigma piM^2 * piF^2 = 0.1833 / = 0.2766 * 0.2926 = 0.7230$

where M = male and F = female.

Difference between autumnal and spring food

The table 2 present the spring food of *Rana klepton esculenta*. The comparation between autumnal (table 1.) and spring (table 2.) food wants to consider the possible differences.

Some differences can be observed in the tables, but the small sample of preys does not allow a statistical analysis using the χ^2 test.

In particular we noticed the following differences: 1) in spring period, the invertebrates are present in minimal part that in autumnal period 2) in autumnal food there is not " frog's skin "

Food resource	*Rana kl. esculenta* "complex"			
	Males		Females	
	A	B	A	B
Anellida	-	-	4	5.47
Arachnida	3	4.10	1	1.36
Cladocera	-	-	-	-
Collenbola	-	-	-	-
Coleoptera	4	5.47	17	23.28
Diptera	8	10.95	5	6.84
Gasteropoda	-	-	-	-
Hemiptera	8	10.95	7	9.58
Hymenoptera	3	4.10	2	2.73
Lepidoptera	2	2.73	6	6.84
Odonata	2	2.73	-	-
Plecoptera	-	-	-	-
Tricoptera	-	-	-	-
Vegetation/Stone/ /Seeds	38	52.05	28	38.35
Total	73	100	73	100

Table 2.Spring food composition. A=n° of specimens, B=percentage presence.

CONCLUSIONS

To conclude we note how males (0.1868) and females (0.1726) results to have a similar niche breadth with intermediate values little discordant with them.

The differences of niche breadth are present also in niche overlap: males and females, making the most of different available food resource, have an almost complete overlap (0.7230).

The difference of food comparation between autumnal and spring period is possible only analysing graphics and tables but is not confirmed by the χ^2 test.

ACKNOWLEDGEMENTS

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The yearly cycle of the Jumping Frog (*Rana dalmatina*) in Sweden. A 12 year study

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Abstract: A little pond (30 x 80 m) with a spawning population of the Jumping Frog (*Rana dalmatina* was visited every day in March - May and in July - August during the year 1982 till 1993. A new map is Sweden (presented of the geographical distribution of the Jumping Frog in southeast Sweden (Ahlén, Andrén, Nilson, 1992 & Berglund, 1994). See figure 1.

Key words: *Rana dalmatina*, Sweden, reproductive cycle.

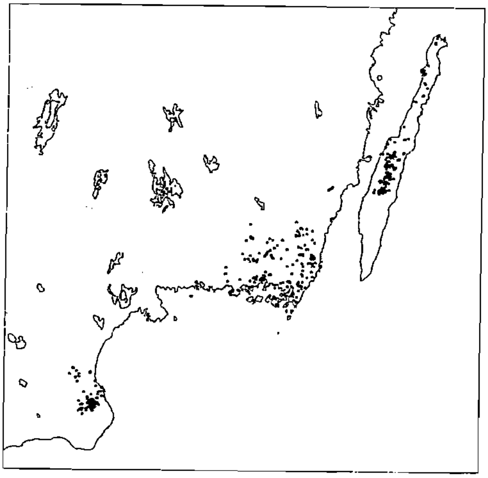

Figure 1: The geographical distribution of the Jumping frog (*Rana dalmatina*) in southeast Sweden. Each dot represents a small water, where the Jumping frog reproduces.

INTRODUCTION

This species was in Sweden first discovered near Ronneby (WESTERLUND, 1890) and later rediscovered in 1946 by Paul von Sydow at Rödeby near Karlskrona (GISLÉN & KAURI, 1959). The Jumping Frog was located in the spring of 1981 at Stora Vörta, 5 km NE off Kadskrona, in the south pond of an old brick works.

MATERIAL AND METHODS

During the years 1982-1993 daily records were made and every egg-cluster was marked with a plastic number on a flower-stick. Notations were made on a map as well as in a diary every day, every year during spring and summer so was the simple strategy!

RESULTS

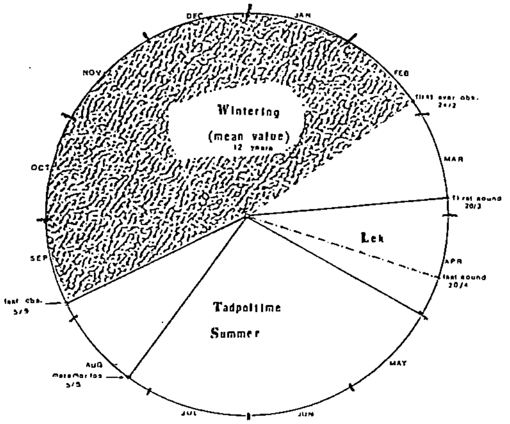

Figure 2: The mean values are represented in a circlediagram of the yearly cycle.

All the information was put together in table 1 and in the circlediagram in figure 2, where the mean values are used during the twelve years from 1982 till 1993. The duration of the spawning period can be found in table 1: N° 3, as the difference between N° 2 and N°1. The egg-cluster period (N° 6) can also be calculated as the difference between N° 5 and N° 4. The duration of the tadpole-period (N° 11) is calculated by the difference between N° 10 and N° 9. So the minimum duration of the tadpole-stage averaged 101±13 days at the 95% confidence level, which is a short time for this species. It should be stressed, however, that this figure is based on the very first observation of a fully metamophosed frog, not on the median ,date or the last observation of a tadpole (STRÖMBERG 1987, 1988).

The landscape has not changed since the investigation started and cows and horses are still pensively grazing around the pond and the groves in the east with oaks and beeches at Skarva are luckily still intact.

A.-	82	83	84	85	86	87	88	89	90	91	92	93	X
1.-	24.3	26.3	4.4	14.4	16.4	9.4	31.3	3.3	25.2	15.3	18.3	18.3	26.3
2.-	79.4	7.4	12.4	7.5	1.5	29.4	2.5	11.4	1.4	15.4	29.4	22.4	20.4
3.-	17	12	8	23	15	20	33	40	34	32	42	35	2 6
4.-	5.4	22.4	8.4	3.4	14.4	16.4	5.4	14.3	12.3	19.3	18.3	18.3	31.3
5.-	29.4	27.4	25.4	6.5	29.4	10.5	1.5	24.4	26.4	25.4	30.4	23.4	29.4
6.-	24	36	17	33	15	24	27	41	45	38	48	36	32
7.-	126	111	133	99	60	77	74	75	196	267	234	177	136
8.-	3.4	26.3	8.4	31.3	17.4	16.4	31.3	3.3	24.2	12.3	3.4	1.4	28.3
9.-	-	3.5	26.4	23.4	4.5	3.5	4.5	11.4	8.4	19.4	11.4	22.4	23.4
10.-	6.8	12.8	11.8	26.8	19.8	14.8	23.8	15.7	18.7	20.7	2.7	10.7	5.8
11.-	-	101	107	125	107	103	111	95	101	93	83	80	101

A: Year
1: First call (d & m)
2: Last call(d & m)
3: Duration of the spawning period (d)
4: First egg cluster (d & m)
5: last egg cluster (d & m)
6: Difference first/last (d)
7: Number of egg cluster
8: First adult frog seen (d & m)
9: First hatched tadpole seen (d & m)
10:First metamorphosed frog seen (d & m)
11:Duration of the tadpole-period (d)

(d & m)=(day & month) (d)=(day)

Table 1:Observations on spawning of the Jumping Frog (Rana daimatina) at Stora V6rta, Sweden, during the years 1982-1993

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Age determination by skeletochronology in low- and high-elevation populations of *Rana italica*

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Abstract: The aim of the present study was to test the validity of the skeletochronological method, based on the count of the lines of arrested growth (LAG), in *Rana italica* (Amphibia, Anura). Possible differences were detected in the histological aspect of LAG in two populations from southern Italy, living at markedly different altitudes. Skeletochronological analysis was performed on diaphyseal cross-sections of femur stained with Herlich's hematoxylin. In about 92% of the lowland population (Montecorice, Salerno, 50 m a.s.l.) and 83% of the highland one (Vesalo, Salerno, 950 m a.s.l.) the LAG were well-defined and relatively easy to count. In both populations we observed partial or total endosteal resorption of the first LAG deposited. Endosteal remodelling processes, more marked in the highland population, did not hinder accurate age determination. In our sample, male frogs from the highland population were bigger and older, in both average and absolute values, than those from the lowland one. Finally, we provide an interpretation of these differences, and stress the need for further investigations, that are already in progress, in order to provide further explanation on some details.

Key words: Skeletochronology, Rana italica, Amphibia.

INTRODUCTION

Skeletochronology has proved one of the most appropriate methods for determining individual age in amphibians (HALLIDAY & VERRELL, 1988; FRANCILLON *et al.*, 1990; LECLAIR, 1990; PATON *et al.*, 1991; CHERRY & FRANCILLON, 1992; BASTIEN & LECLAIR, 1992; DENTON & BEEBE, 1993; PLATZ & LATHROP, 1993). It consists in counting the lines of arrested growth (LAG) recorded in the skeletal tissue, chiefly in diaphyseal sections of long bones.

Recently, interpopulational comparative investigations on some amphibian species from temperate regions have pointed out that new data on the demography of these species can be obtained by this method (HEMELAAR, 1988; RYSER, 1988; DENTON & BEEBE, 1993; CAETANO & CASTANET, 1993).

The present paper aims at ascertaining the validity of this method in *Rana italica* (Amphibia, Anura), a brown frog endemic to the Italian peninsula, as well as detecting possible differences in the histological aspect of the LAG in two populations living at markedly different altitudes. Finally, skeletochronology is used in an attempt to get preliminary information on the age structure of the two populations studied.

MATHERIAL AND METHODS

Animals

We used specimens of *Rana italica* from populations living in two different localities of Cilento (Province of Salerno, Southern Italy): Montecorice, about 80 m asl and Vesalo, about 950 m asl. The skeletochronological analysis was performed on a sample of 32 males and 30 females from Montecorice and 45 males and 33 females from Vesalo, collected from November 1987 to April 1990. Body weight, to the nearest 0.1 g, and body lenght (snout-urostyle), to the nearest 0.1 mm, were recorded for each animal.

Study area

The two localities are subjected to clearly different climatic conditions owing to their different altitudes and geographic positions (GUARINO *et al.*, 1993). Montecorice is characterized by the typical Mediterranean low shrub vegetation; Vesalo is rich in beech-woods. Both areas are crossed by a stream.

Histological procedures and skeletochronological interpretation

The femurs were fixed in 70° alcohol, decalcified in 5% nitric acid for at least 1.30 h, cross-sectioned by a cabinet cryostat and stained with Erlich's hematoxylin for about 30 minutes. The sections were placed on polylisinate slides (Sigma) and mounted in Aquamount (Gurr). Several femurs were also successfully embedded in paraffin and cut using a rotatory microtome. The histological sections were analysed independently by three of us; the different data were then view together in order to obtain a single interpretation. The degree of resorption of the inner LAG was estimated by the method described by PATON *et al.* (1991). As far as the outer margin of the femur cross-section is concerned, a further LAG besides those clearly visible was attributed on the basis of the date of capture.

RESULTS

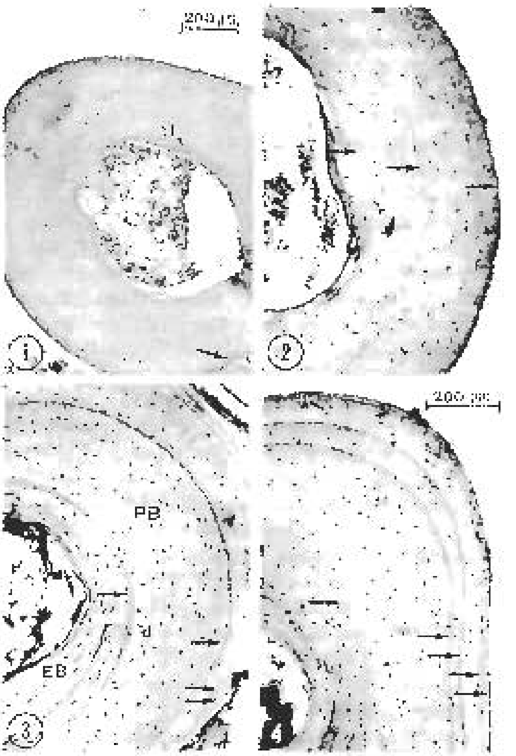
Bone histology

In adults, femur cross-sections at diaphyseal level consisted of two layers: an external layer made principally of parallel fibered bone with numerous osteocytes randomly distributed over it (periosteal bone); an internal one made of lamellar bone with few and flattened osteocytes (endosteal bone) (fig. 3). The two layers appeared sharply separated by a cementing line of resorption. In some individuals, the parallel fibered bone in the periosteal cortex was gradually replaced by the lamellar bone probably owing to a change in local osteogenesis rates. In fact, the fibrillar matrix organization of the bone is related to different rates of osteogenesis (CASTANET *et al.*, 1993). Blood vasa were arrayed radially and orthogonally to the bone shaft.

Skeletochronology

In most individuals of both populations, the LAG were well-defined and relatively easy to count (tab. 1) (figs. 1-4). The innermost LAG was generally thinner than the following ones. The difficulties encountered by us in LAG count were the following for both populations (tab. 1): a) absence of LAG in a few frogs; these animals were not taken into account in the next analysis. b) endosteal resorption, partial or total, of the first LAG deposited (figs. 1,2). The percentage of frogs affected by partial removal of the first inner LAG was markedly higher than the percentage of those affected by entire removal. In addition, endosteal remodelling processes were more marked in the mountain population (tab 1). c) presence of very close hematoxylinic lines or double LAG (*sensu* FRANCILLON *et al.*, 1990); d) presence of not well-defined hematoxylinic lines or false LAG (*sensu* CASTANET & SMIRINA, 1990) (fig. 3); this lines were acyclical and were not counted as a true LAG.

In our sample, the males from the highland population were, on the average, significantly older than the lowland ones (Student's test, t=22, df=61, P< 0.01). The oldest specimens were also found in the mountain population (fig. 5). Linear regression analysis showed that in both populations body size was significantly related to age both in males (Montecorice: r= 0.72, df=27, P<0.01; Vesalo: r=0.65, df=33, P<0.01) and females (Montecorice: r= 0.66, df=36, P<0.01; Vesalo: r= 0.78, df=26, P<0.01). In both populations and both sexes, though bigger frogs were generally older, we found individuals showing the same body length but very different ages.



Figures 1-4: Femur cross-section of *Rana italica* at diaphyseal level. Figs. 2-4 are at the same magnification. Arrows indicate LAG. Abbreviations: d= double LAG; r= partially resorbed LAG; rl= resorption line; EB= endosteal bone; PB= periosteal bone. 1) Frog from Vesalo with 1 LAG plus 1 totally resorbed. 2) Frog from Vesalo with 3 LAG the first of which partially resorbed. 3) Frog from Montecorice with 4 LAG the first of which is double. 4) Frog from Vesalo with 5 LAG.

DISCUSSION AND CONCLUSION

In amphibians from temperate regions, LAG are generally an annual phenomenon related to

	MONTECORICE			VESALO		
	♂♂	♀♀	♂♂+♀♀	♂♂	♀♀	♂♂+♀♀
TOTAL (n) 32	30	62	46	33	79	
LAG	90.6%	93.3%	91.9%	76.1%	90.9%	82.4%
NL	9.4%	6.7%	8.1%	23.9%	9.1%	17.6%
LAGpr	27.6%	39.3%	33.3%	61.0%	60.0%	57.9%
LAGtr	3.4%	7.1%	5.3%	11.4%	16.6%	13.8
LAGd	34.5%	46.4%	40.5%	34.3%	23.3%	30.7%
LAGf	34.5%	35.7%	35.1%	28.6%	36.6%	30.7%

Table 1: Percentage of individuals showing well-defined lag (lag), absence of lag (nl), partially (lagpr) or totally (lagtr) resorbed lag, double lag (lagd) and false lag (lagf).

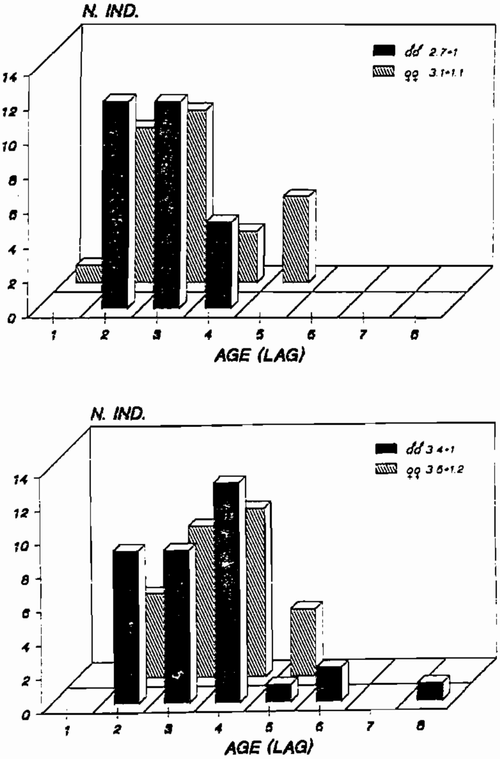


Figure 5: Frequency distribution per age classes: a) Montecorice; B) Vesalo. In each histogram mean+ standard deviation are also reported.

periods of torpor occurring during colder months (FRANCILLON, 1979; CAETANO & CASTANET, 1987; CASTANET *et al.*, 1993). We can consider this true also for *Rana italica*, where, however, the influence of other factors, such as the breeding season (February-March) (GUARINO *et al.*, 1993), cannot be ruled out. In this period, these anurans eat little or nothing, and allocate a lot of energy to reproduction; consequently, the increase in their body size is relatively little or absent. The role of the breeding season in determining the annuality of LAG is obvious in populations living in coastal areas of southern Italy, which can have continous annual activity depending on the climatic conditions (temperature in particular) of each year. An analogous explanation has been provided by Esteban (1990) for *R. iberica* and *R. temporaria*. LAG aspect does not differ in the two populations, and similar results have been obtained by HEMELAAR for *Bufo bufo* (1985). This suggests that LAG distincness is not only dependent on the length and severity of the cold period, but other factors might also be involved.

Endosteal resorption was observed in individuals of both populations without, however, hindering accurate age determination. Like in other amphibians (HEMELAAR, 1985; CAETANO & CASTANET, 1993), the degree of endosteal resorption differs between the two populations, being more marked in the mountain one. Nevertheless, it did not appear to increase with age, since it involved individuals of different age. Some authors (HEMELAAR, 1988; CAETANO & CASTANET, 1993) have showed that the processes of bone remodelling are more marked before sexual maturity is attained, then they decrease. Further investigations on the age at maturity in *R. italica* is needed to confirm this point.

In agreement with other authors (FRANCILLON-VIEILLOT *et al*, 1990; PATON *et al.*, 1991), the double LAG were counted as a single LAG. Unlike what has been reported for *Triturus marmoratus* (CAETANO & CASTANET, 1993) and *T. carnifex* (ANDREONE *et al.*, 1990), in *R. italica* double LAG are not more frequent in the highland than in the lowland population, and are aperiodical in both populations. This disparity might be the result of the absence of a rigorous estivating period in *R. italica*.

A comparison between the two populations shows that highland male frogs are bigger and older than the lowland ones. Larger body size and greater longevity are characteristics shared by temperate-zone amphibians living at high altitudes. This is the consequence of their larger body size and higher age at first reproduction (BERVEN, 1982; HEMELAAR, 1988; RYSER, 1988; CAETANO & CASTANET, 1993). Surprisingly, the differences in age structure between females of the two populations were not significant. A study is in progress, aiming at explaining the peculiar age structure of the two *R. italica* populations investigated. Finally, in *R. italica*. as in other species of amphibians (VERRELL & HALLIDAY, 1988; PLATZ & LATHROP, 1993), the size of the animal does not allow certain estimation of the individual age.

ACKNOWLEDGEMENTS

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Intra- and interpopulational comparison of temperatures selected by *Hyla labialis* (Anura)

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Abstract: The purpose of this study was to compare the selected temperatures of three populations of the tropical frog *Hyla labialis* which live at widely separated altitudes above sea level, and hence under markedly dissimilar climates. Animals were tested in a laboratory gradient from 6 to 39°C, recording the temperature of each individual's position repeatedly over a period of six hours. The preferred temperatures for each frog category were calculated as means and as continuous temperature ranges including 50% of the recorded data (R50). Larvae from all populations selected temperatures within a narrower range than did adult frogs. There was no apparent association between selected and environmental temperatures, except for the R50s of adult frogs which decreased with elevational level. Starving animals tended to select lower and recently fed animals higher temperatures. The wide range of temperatures selected by adult frogs was associated with large individual differences in weight condition and reproductive activity level. The ecological significance of some selected temperatures is discussed.

Key words: Anura, neotropic, high mountains, temperature selection, etho-physiological condition.

INTRODUCTION

Anuran amphibians thermoregulate mostly by behavioural means (HUTCHISON & DUPRE 1992), actively moving about within certain limits of their habitat's thermal mosaic (BRAGG 1964, HADFORD 1966, WOLLMUTH *et al.* 1987) or staying in places with suitable thermal conditions (SEYMOUR 1972, VALDIVIESO & TAMSITT 1974). These behaviours can also be observed in an artificial temperature gradient established in the laboratory, where larvae as well as adult frogs limit their locomotor activity to certain portions of the available space, thus revealing their preferred temperatures (BEISWENGER 1978, FLOYD 1984, LILLYWHITE *et al.* 1973, LUCAS & REYNOLDS 1967).

The intra- and interpopulational variation in temperature selection encountered among anurans has been interpreted in several ways: as adaptation to the climate of a population's habitat (FLOYD 1984, SNYDER & WEATHERS 1975, WOLLMUTH & CRAWSHAW 1988), as an anticipation to successful reproduction (FREED 1980) or proper physical performance capacity (JOHN-ALDER *et al.* 1988), as a sign of changing physiological requirements during maturation (WOLLMUTH *et al.* 1987), and as a consequence of a frog's nutritional state (LILLYWHITE *et al.* 1973). Even the intrapopulational range of selected temperatures may be quite large, suggesting a low thermoregulatory predision. However, the reliability of such an interpretation could be verified by focussing on individual frogs within a population, which may thermoregulate precisely, but each at a different body temperature (HUTCHISON & DUPRÉ 1992).

The aim of the present study was to measure the populational and individual preferred temperatures of the tropical frog species *Hyla labialis*, and look for conformities between thermal preferences, climate, behaviour, and physiology.

MATERIAL AND METHODS

The animals used in this study belonged to three different populations of *Hyla labialis*, living in the eastern chain of the Colombian Andes at widely separated altitudes (2000, 2600, and 3500 metres above sea level), and hence in different climates, but within a radius of about 50 km around the capital Santa Fé de Bogotá. All individuals arrived at the laboratory on the same day of capture. Temperature selection was measured using the technique described by LUCAS & REYNOLDS (1967). The water temperature at each individual's position in a gradient that spanned from 6°C to 39°C was recorded every 20 or 30 minutes over a 6 hour period (10:00-16:00). According to FOUQUETTE (1980), these water temperatures were regarded as the body temperatures of the experimental animals. After testing, all animals were released again at their respective capture sites.

Populational thermal preferences were obtained from 293 animals captured fortuitously between August 1987 and August 1989 (LÜDDECKE & MONJE 1989). Three animal categories were distinguished within each population: young larvae (stages 25 to 30, GOSNER 1960), advanced larvae (stages 35 to 40, GOSNER 1960), and adult frogs regardless of sex. For each category the arithmetic mean preferred temperature (MPT) was calculated, and the range of continuous temperatures containing 50% of all recorded temperatures (R50) was estimated.

Individual thermal preferences were obtained from 203 animals which I carefully chose from those encountered in the field between April 1991 and February 1992, or that were treated in the laboratory prior to testing in order to get individuals in contrasting conditions, as listed below: a. high versus low index of weight condition (IWC), which was calculated according to HEMMER & KADEL (1972) as a measure of nutritional state. Each individual was tested only once, b. starved versus recently fed. Many individuals were used three times: freshly caught, starved, and recently fed, c. reproductively active versus inactice. Males and females were considered reproductively active when encountered in amplexus or when showing characteristics that indicate reproductive readiness (males with enlarged and strongly pigmented thumb pads and vocal sac; females with enlarged oviducts or eggs visible through the ventral skin). Individuals shortly after successful mating were considered reproductively inactive. Some individuals were used twice: shortly before and after spawning in a laboratory aquarium.

Following BOWKER's (1984) example, from the recorded data I calculated for each individual the MPT and standard deviation (SD). The SD-values of all individuals belonging to the same experimental category were averaged to provide for each category a pooled SD as a measure of its thermoregulatory precision. Finally, averaging the individual MPT-values, I calculated for each category the MPT±SD. These represent the thermal preferences of the categories and the variation among the individuals composing a category.

RESULTS AND DISCUSSION

Populational data:

Table 1 shows that *Hyla labialis* from all populations selected temperatures within a wide range, but that there was an obvious preference for higher temperatures, as evident from the relative positions of the MPT-values and the R50s within the total ranges. The R50s of larvae are notoriously narrower than those of adult frogs, which means that larvae were selecting temperatures more accurately than did adult frogs. Most larvae selected temperatures within the range of available habitat water temperatures (Table 2). Larvae from different populations selected essentially the same temperatures. An interpopulational comparison reveals no correspondence of larval thermal behaviour to changing environmental water temperatures.

	Metres above sea level		
	2000	2600	3500
Adult frogs	(37)	(54)	(30)
MPT	27,0	26,0	25,8
R50	28-36	27-35	24-31
Rtot	8-38	6-37	6-35
Advanced larvae	(35)	(22)	(24)
MPT	29,3	27,9	29,1
R50	27-30	27-30	28-30
Rtot	17-32	10-33	17-32
Young larvae	(50)	-	(41)
MPT	29,0	-	28,6
R50	27-31	-	28-31
Rtot	16-35	-	18-34

Table 1. Populational thermal preferences (in °C) of adult frogs, advanced and young larvae of *Hyla labialis* from three different altitudes in the eastern chain of the Colombian Andes around Bogoti. (N): quantity of experimental animals, MPT: mean preferred temperature, R50: temperature range containing 50% of recorded data, Rtot: total range of selected temperatures.

	Metres above sea level		
	2000	2600	3500
Air			
mean	17	13	8
max.	27	22	21
Water surface			
mean	20	16	12
max.	35	32	31

Table 2. Some ambient temperatures (in °C) in the habitat of *Hyla labialis* from three different altitudes in the eastern chain of the Colombian Andes around Bogotá.

However, such a correspondence is apparent for adult frogs: animals from the warmer climate (at 2000 m altitude) preferred higher temperatures than did frogs from the colder climate. Frogs from intermediate elevations selected intermediate temperatures. This holds for the three different measures used to express thermal behaviour. Most adult frogs preferred higher temperatures than the air temperatures available in their respective habitat. The difference between selected and available temperatures was largest for highland frogs. This means that at 3500 m altitude animals would have less opportunity than at lower altitudes to encounter sites with temperatures that match

		Metres above sea level					
	Category	3500 Male	3500 Female	3500 Larva	3500 Climax	3500 Metamorph	2600 Male
Condition							
High IWC	MPT	27,3±3,5	24,1±5,1	29,9±1,4	29,4±1,2	31,1±1,4	31,2±2,1
	pSD	1,9	2,3	1,5	1,3	1,4	1,4
	(N)	(9)	(14)	(18)	(18)	(11)	(15)
Low IWC	MPT	28,2±4,4	26,1±5,2	-	-	-	-
	pSD	2,0	1,7	-	-	-	-
	(N)	(21)	(14)	-	-	-	-
Starved	MPT	-	-	30,4±1,4	28,2±3,7	28,3±1,8	29,7±4,0
	pSD	-	-	1,3	2,4	2,6	3,7
	(N)	-	-	(16)	(9)	(24)	(11)
Very starved	MPT	-	-	-	-	25,4±4,4	-
	pSD	-	-	-	-	2,3	-
	(N)	-	-	-	-	(5)	-
Recently fed	MPT	-	-	-	-	29,3±2,6	31,6±1,6
	pSD	-	-	-	-	1,6	1,1
	(N)	-	-	-	-	(11)	(6)
Reprod. inactive	MPT	30,4±2,7	26,7±4,9	-	-	-	-
	pSD	1,6	1,5	-	-	-	-
	(N)	(14)	(8)	-	-	-	-
Reprod. active	MPT	23,8±4,1	21,9±4,2	-	-	-	-
	pSD	2,7	2,5	-	-	-	-
	(N)	(9)	(10)	-	-	-	-

Table 3. Thermal behaviour of six categories of the species *Hyla labialis*, each in contrasting etho-physiological conditions. MPT: mean preferred temperature ± SD (grand mean), pSD: pooled SD, (N): quantity of experimental animals.

their thermal preferences. For *Hyla labialis* at 2600 m altitude VALDIVIESO & TAMSITT (1974) described that frogs elevated their body temperatures by basking up to 29°C, some 13 °C above the surrounding air temperature. It is likely that animals basking at 3500 m altitude can do similarly, given that SINSCH (1991) measured body temperatures of up to 32°C in basking *Bufo spinulosus* in the Peruvian highlands at 3200 m altitude. Even at 2000 m altitude adult *Hyla labialis* were often encountered basking (pers. obs.). Therefore the thermal preferences of *Hyla labialis* determined in the laboratory probably reflect the intended field body temperatures of basking frogs

Individual data:

a. High versus low IWC: among adult frogs in their natural environment, a wide range od IWC was encountered. Males as well as females with a high IWC selected somewhat lower temperatures than did individuals with a low IWC (Table 3). This tendency was more pronounced in females (two degrees C difference) than in males (about one degree C difference). The thermoregulatory precision of these frogs was intermediate (pooled SD ranged from 1,7 to 2,3) with respect to the entire range for all experimental groups (pooled SD ranged from 1,1 to 3,7).

As a low IWC supposedly signals a deficient nutritional state, this result seems unexpected at first glance. it probably means that low-IWC frogs are not necessarily starved and that high-IWC frogs have not necessarily fed recently. Therefore, the IWC apparently is not a reliable predictor of thermal behaviour. This failure suggests that there may exist other, more proximate factors related to the thermal behaviour of *Hyla labialis*.

b. Starved versus recently fed: as almost no larva or metamorphic frogiet with a low IWC could be encountered in the field, individuals were treated in the laboratory prior to testing by starving them for a couple of weeks. Starved individuals, with the exception of larvae, selected lower temperatures than freshly caught ones (Table 3). Also, and again with the exception of larvae, starved individuals reduced their thermoregulatory precision, this actually being the main treatment effect. Usually the starvation effect was small: MPT dropped one or two degrees C below that of freshly caught individuals. Only after a long starvation period was there an appreciable decrease in MPT: from initially 31,1°C to 25,4°C in juveniles starved for 40 days. It is unlikely that starvation periods of that duration are suffered by frogs under natural conditions, as this species does not hibernate or estivate. Frogs recently fed after a period of starvation, on the other hand, immediately chose higher temperatures again and notoriously improved their thermoregulatory precision (Table 3). Even severly starved frogs did not select the lowest temperatures available in the gradient, although this would have reduced their energy expenditure. A possible reason for this behaviour may be that at low body temperatures the frog's physical performance capacity would be insufficient for subdueing prey or escaping from predators. The physical performance breadth of *Hyla labialis* is actually being measured (NAVAS, pers. comm.).

c. Reproductively active versus inactive: all reproductively active males had a low, and all such females a high IWC. Both sexes selected the lowest of all temperatures and both showed a diminished thermoregulatory precision. Remarkably, reproductively active males with low IWC selected much lower temperatures than inactive males with low IWC. On the other hand, immediately after spawning individuals of both sexes, even without having fed, raised their MPT to values noticeably above those found for low-IWC individuals of unknown reproductive state.

These results indicate a strong impact of reproductive behaviour on thermal behaviour. The drop in MPT of reproductively active animals may not be coincidential (e. g. due to more locomotor activity in the gradient), but actually a life history trait: when spawning in a thermal gradient gradient in the laboratory, *Hyla labialis* deposited its eggs in places with low temperatures (LÜDDECKE, pers. obs.). Moreover, I found that on sunny days the maximum temperature of egg masses in a pond at 3 500 m altitude was 25°C in open water and 28°C inside aquatic vegetation. These values are below the highest environmental water temperatures (Table 2), and well below the critical thermal maximum of 33°C for embryonic development and survival (LÜDDECKE & MONJE 1989). Hence the similar thermal preferences of reproductively active *Hyla labialis* males and females may serve to spawn in places with a proper temperature range for embryonic development, as also occurs in *Rana sphenocephala* (CALDWELL 1986).

The postreproductive preference for high temperatures, on the other hand, may facilitate certain physiological functions: during the prolonged diving bouts employed for spawning (LÜDDECKE, pers. obs.) the frogs eventually accumulate lactate in their tissues. Lactate accumulation has been detected by BENNETT & HOUCK (1983) in courting *Desnmnathus ochrophaeus* salamanders. In addition, GATTEN (1987) and LINDINGER *et al.* (1987) found that it takes frogs and toads several hours to completely metabolize accumulated lactate. It remains to be shown whether this occurs in *Hyla labialis*, too. Certainly, all the individually determined preferred temperatures fell within the populationally determined ones, most even fitted into the populational R50s, with the exception of the MPTs of reproductively active frogs. The fact that the MPTs of frogs in certain ethophysiological conditions can divert from those of the entire population, has to be kept in mind when studying the thermal biology of a species: presenting a single MPT for an entire adult population may give a distorted view, particularly if the sampling of the experimental animals is done during a breeding season, as is likely to occur, because that is when they are easiest to encounter. Thermal preferences calculated from data obtained from such animals would, of course, depend on the proportion of reproductively active to inactive individuals in the sample.

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Mating behaviour in *Hyla arborea*, I. Density movement and residency in breeding site

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Abstract: The mating behaviour of *Hyla arborea* was studied in a few breeding ponds in southern Bohemia from 1986 to 1988. The numbers of breeding frogs in the various ponds fluctuated markedly both during the breeding period and in the individual seasons. The breeding periods in the different ponds were neither synchronous nor the same length (maximum 54 nights). The frog's breeding activity and the choice of the males' calling sites depended on the temperature of the water and the air, on changes in the water level and on the development of the vegetation in the breeding pond. The lower air temperature threshold for calling was 2-1°C. New individuals of both sexes entered the water during the whole of the breeding period. No significant correlation was found between the time when the males entered the water and their size. In two studied aggregations the individual males remained in the pond for an average of 15.4 nights (range 1-47) and 10.1 nights (range 1-27). Here the cumulative sex ratio amounted to 4.4 : 1 and 2.7 : 1, with males in the majority. Males which remained in the pond for more than five nights during the breeding period exhibited three types of spatial attachment to a particular place: permanent (the longest distances between calling sites were 2.5-10 m), temporary and zero. The occupied ranges overlapped and did not have the character of long-term defended territories. Except for just a few very exceptional cases, calling males maintained a minimum distance of 0.5 m.

Key words: *Hyla arborea*, breeding, mating behaviour.

INTRODUCTION

Despite of the number of publications on the breeding phenology of Hyla arborea as well as on its calling and spatial activity in the breeding site (available data are summarized inter alia by KOWALEWSKI 1974, SCHNEIDER 1977, BORGULA 1990, TESTER 1990, GIACOMA *et al.* 1993, SCHNEIDER 1993) very little is known about an exact course of the breeding of individual frogs during their whole breeding period. Therefore, the aim of this paper was to get concrete data on density fluctuation, movement and residency of individually marked tree frogs in a definite breeding place.

MATERIAL AND METHODS

The mating behaviour of *H. arborea* was studied in a few breeding ponds in southern Bohemia from 1980 to 1988. Detailed studies were then conducted in two neighbouring ponds denoted A (longest distance across, about 100 m) and B (about 280 m) in 1986 and 1987. In order to monitor the movements of frogs in the breeding places the two ponds were divided, by means of regular grids of marks, into sectors 5 by 5 m (pond A) or by 4 by 3 m (pond B). Here all breeding tree frogs were captured, measured, marked individually by toe-clipping and recaptured in successive nights of the breeding period. The character of the calling site and the place of individual calling males in the pond were noted (except of brief irregular intervals) for each night of the investigation.

RESULTS

The numbers of breeding tree frogs in the various ponds fluctuated markedly both during the breeding periods (sensu WELLS 1977) and in the individual seasons. The breeding periods in

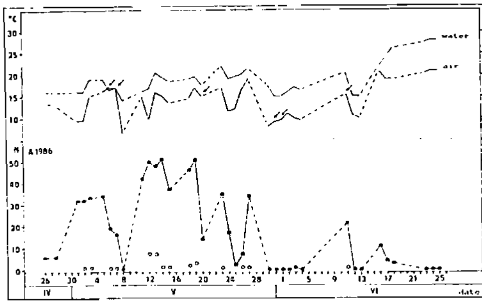

Figure1: Course of reproduction in *H. arborea* and variation in the temperature of air and water in pond A-1986 (temperatures measured at the onset of vocalization). Closed rings, number of males in pond: open rings, number of females in the pond; arrows indicate windy days; dashed line indicates breaks in observations.

Type of calling site	Total		t<10°C			t>10°C		
(Pond A-1986)	N	%	N	%		N	%	
Shore-shelter	89	20.2	8	13.8		81	21.1	
Shore-freely	26	5.9	1	1.7		25	6.5	
Water edge-shelter	57	12.9	20	34.5		37	9.6	
Water edge-freely	28	6.3	4	6.9	84.5	24	6.3	64.3
vegetation in open water	211	47.7	25	43.1		186	48.4	
above-ground sites (up to 1.75 m)	31	7.0	0	0.0		31	8.1	
Total	442	100	58	100		384	100	

Table 1: Basic types of calling sites of male H. arborea in pond A-1986 and differences in the frequency of their selection at air temperatures above and below 10°C.

Type of calling site	Total		t<10°C		t>10°C	
(Pond A-1987)	N	%	N	%	N	%
Shore-shelter	7	7.8	2	4.7	5	10.6
Shore-freely	2	2.2	0	0	2	4.3
Water edge-shelter	0	0	0	0	0	0
Water edge-freely	0	0	0	0	0	0
vegetation in open water	79	87.8	41	95.3	38	80.8
above-ground sites (up to 1.75 m)	2	2.2	0	0	2	4.3
Total	90	100	43	100	47	100

Table 2: Basic types of calling sites of male H. arborea in pond A-1986 and differences in the frequency of their selection at air temperatures above and below 10°C.

the different ponds were neither synchronous nor the same length (several days to about two months). The breeding season lasted approximately three months (1986: 22.4. to 22.7., 1987: 9.4. to 15.7., 1988: 24.4. - ?). The frogs' breeding and calling activity and the choice of the males' calling sites depended mainly on water and air temperature (see Figures. 1 and 2).

The lowest air temperature observed at the onset of vocalization was 2°C, subsequently dropping to 1°C (at water temperature 14.5-14.0°C).

At low air temperatures the males regulated their body temperature by plunging into water (see Tables. 1 and 2).

Besides low temperatures, the activity of tree frogs in their breeding place was negatively affected by windy weather (Figures. 1 and 2), development of aquatic vegetation in the ponds (both during the breeding periods and in the course of several successive years), and variation in the water level in the ponds (both natural - drying up of periodical pools, and artificial - draining of ponds as a result of their fishery management).

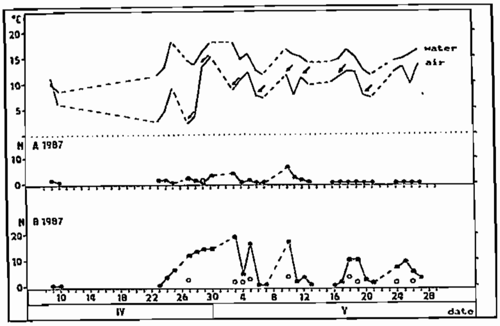

Figure 2: Course of reproduction in H. arborea and variation in the temperature of air and water in ponds A-1987 and B-1987. Explanations as in Figure. l.

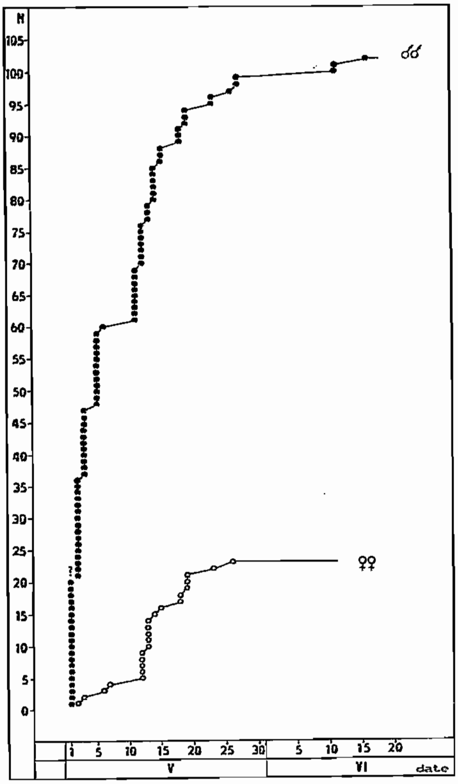

Fig.3. New males (closed rings) and females (open rings), arrival rate in pond A-1986.

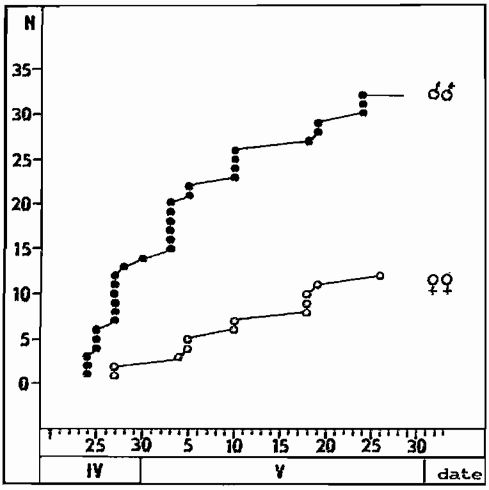

Figure 4: New males (closed rings) and females (open rings) arrival rate in pond B-1987.

Figures. 1 and 2 show the concrete course of breeding in ponds A and B. In pond A (in 1986) the breeding period lasted at least 54 nights (including breaks caused by oscillations in weather conditions) and involved a total of 102 males and 23 females. The cumulative sex ratio in that pond was 4.4 : 1 in favour of males (the operative sex ratio, however, was at least 6.9 : l). Next year the breeding period in that pond lasted only 16 days and the pond was visited by 9 males and 1 female. The breeding period in pond B-1987 lasted at least 34 nights, and 32 males and 12 females participated in reproduction. Here the cumulative sex ratio was 2.7 males : 1 female (the operative sex ratio was at least 3.3 : 1).

New individuals entered the pond practically throughout the breeding period. Their arrival was not uniform, that of the females culminating with a considerable delay against the males (see Figures. 3 and 4). No significant correlation was found between the time when the males entered water and their size.

In pond A-1986 the males stayed for an average of 15.4 nights (median /M/=13.5, range 1-47), 25.5 % of them staying for over 20 nights (Figures. 5 and 7). In pond B-1987 they stayed for an average of 10.1 nights (M=8.0, range 1-27) and only 11.1 % of them stayed for over 20 nights (Figures. 6 and 7). However, some of the males were observed to visit other choruses in the course of breeding (see Figures. 5 and 6). The females stayed in the water generally for one night only, during which time oviposition took place immediately. However, several females were present in the water for a second

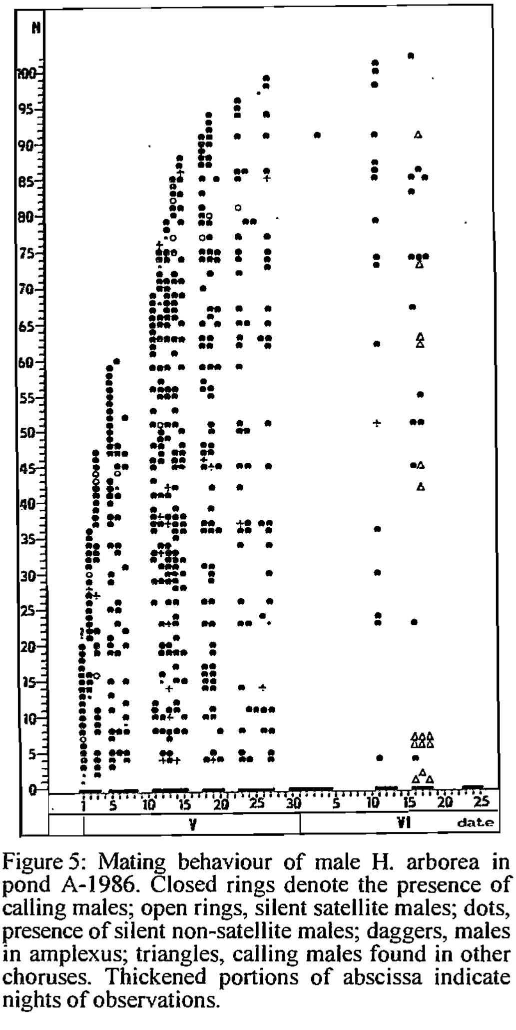

Figure 5: Mating behaviour of male H. arborea in pond A-1986. Closed rings denote the presence of calling males; open rings, silent satellite males; dots, presence of silent non-satellite males; daggers, males in amplexus; triangles, calling males found in other choruses. Thickened portions of abscissa indicate nights of observations.

time, with intervals of 1, 5, 6, 9, 21 and 23 days between the two visits (in two cases even a second clutch of eggs was found).

Males which remained in the pond for more than five nights during the breeding periods exhibited three types of spatial attachment to a particular place: (1) permanent (the longest distances between calling sites were 2.5-10 m; on occasional evenings some of the males may have occurred even outside this preferred area), (2) temporary, and (3) zero. The concrete situation in pond A-1986 was as follows: of the 102 males that visited it, 66.7 % stayed for more than 5 nights. Of these, 39.7 % belonged to group 1 and the distances between their calling sites on any two consecutive evenings were: M=1.5 m, mean /x-/=4.2 m, range 0.0-23.0 m; N=30. The second group comprised 33.8 % males and the distances between their calling sites on any two consecutive evenings were: M=2.5 m, x-=10.4 m, range 0.0-97.0 m; N=29. Group 3 comprised 26.5 % males and the distances between their calling sites were: M=37.0 m, x-=37.1 m, range 0.5-99.0 m; N=12. In pond B-1987 the mutual ratio of the three groups was distinctly different. Of the 27 individually marked males, only 14 (51.9 %) stayed in the pond for more than 5 nights. Only 14.3 % of these males belonged to group 1, 21.4 % to group 2 and 64.3 % to group 3. The number of observations was too small to evaluate the distances of their calling sites on consecutive evenings. In both ponds the occupied ranges overlapped or they were occupied within also by males from groups 2 and 3 and did not have the character of long-term defended territories. The distances between calling sites (N=442), maintained by the vocalizing males, did not decrease below 0.7-0.5 m even at the highest densities of the chorus. In a few exceptional cases, the vocalizing males were closer to each other (0.40, 0.30, 0.26, 0.20, 0.11 and 0.10 m).

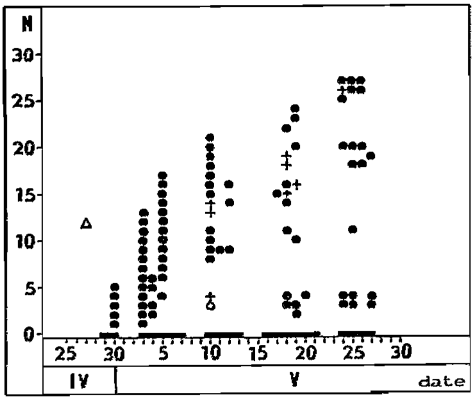

Figure 6. Mating behaviour of male *H. arborea* in pond B-1987. Explanation as in Figure 5.

Tables. I and 2 show the basic types of calling sites of H. arborea and frequencies of their occupation. The selection of calling sites was affected, besides actual weather conditions, by marked competition pressure exerted by a numerous population of Rana esculenta complex due to which the tree frogs were displaced from deeper parts of the ponds grown with aquatic

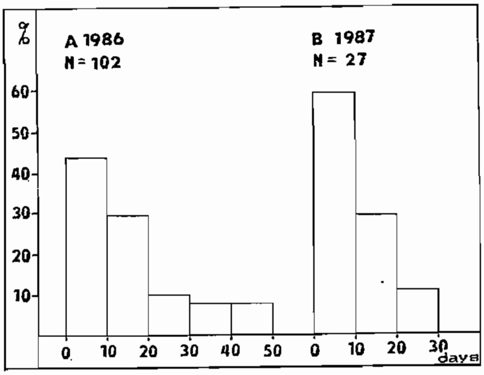

Figure 7. Male residency in breeding ponds A-1986 and B-1987.

vegetation. A big female R. esculenta complex was observed swallowing a vocalizing male tree frog, and individual tree frogs were found with characteristic scars caused by frog-bites.

Discussion

Concrete data on the duration of the breeding season of *H. arborea* have been summarized by Kowalewski (1974), Borgula (1990), Tester (1990) and Giacoma *et al.* (1993). Our data from southern Bohemia are in general agreement with the range of the published data, except that in that region the breeding season may be more extended into the summer season. The temperature regimen in a breeding site is the major factor determining the onset of the breeding period and the breeding and calling activity in *H. arborea.* According to various authors (Schneider 1967, 1971, 1977; Christaller 1984; Borgula 1990), 8-5°C is the lower air temperature threshold for calling. Tester (1990) reports exceptional cases of still lower temperatures (which, however, he did not measure directly in the breeding site). Our data document the males' capability of behaviourally regulating their body temperature and vocalizing even at air temperatures of 1°C. Their tolerance of low air temperatures seems to decrease somewhat in the course of the breeding season (see Figures. 1 and 2), probably in connection with the gradual drop of the frogs' sexual activity towards the end of the breeding season. The rather short stay of individual male H. arborea in the ponds under study (compared to the total length of the breeding period), the same as the gradual arrival of the frogs in water indicate that to understand the reproductive system in H. arborea and to evaluate correctly their numbers in individual breeding sites, one must also know the duration of the reproduction activity of the individual frogs. Apparently, the average time spent by the males in a pond may even be much shorter (5.3 days) than shown by our observations (see Giacoma *et al.* 1993). In view of the observed movements of some of the breeding males from one pond to another, however, it is obvious that the mating activity of at least some males is longer and less frequently interrupted than what would be suggested by the results of their observations made at just one pond (see Figure. 5). This, then, would better agree with the general conclusion of Wells (1977) that individual males of frogs with prolonged breeding are present at the breeding area for a substantial portion of the breeding period.

Evaluation of the spatial occupation of a pond by vocalizing males brings to the fore the question of their territoriality. Wells (1977) concludes that in many temperate-zone hylids it is difficult to separate territorial behaviour from the behaviour aimed at maintaining certain individual distances between males. Here the aggressive behaviour of the males is not immediately bound on the defence of a permanent territory, and maintaining minimum individual distances between vocalizing males may play a more important part in the system of their reproduction than the occupation of a permanent calling site. Similarly, our own results show that sites occupied by a part of the males for rather long periods of time did not have the character of defended territories. It appears that the males occupied the permanent sites rather in dependence on their diurnal sites and the sheltering possibilities on the shore. The possibility of better orientation of the frogs on distinctly developed banks may also have played a part. This is suggested by the differences observed in the representation of males with permanent spatial residency between ponds A and B. In pond B where the vegetation obliterated the division line between shore and water surface the shore utilization and the representation of spatially permanent males was disproportionately lower. The distances between vocalizing males, mutually maintained by means of their territorial voice, were beyond the limit reported for this species (0.30 m; Schneider 1967) save for quite exceptional cases.

The frequent selection of calling sites in various sheltered places was probably correlated with the predation pressure exerted by the population of *Rana esculenta* complex. These frogs were often observed watching motionless in proximity of the calling male tree frogs or their shelters, and one may expect that they oriented themselves by their calls. Phono-orientation in certain frog "predator-prey" systems has been described e.g. by JAEGER (1976), KLUGE (1981) and PERRILL & MAGIER (1988).

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Mating behaviour in *Hyla arborea*. II. Mate selection and male mating success

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Abstract: The mating tactics and mating success of *Hyla arborea* males were investigated in two breeding ponds in southern Bohemia in 1986 and 1987. Satellite behaviour, as an alternative male mating tactics, was demonstrated. The total proportion of satellite associations in the two breeding aggregations was 2.6 % and 1.3 % (on individual nights it averaged 2.0 % and 0.7 %, with a range of 0-8.7 % and 0-7.1 % respectively). The snout-vent length of satellite males was significantly smaller than that of dominant males and also of a sample of all the calling males in the pond. Satellite behaviour was not observed in choruses numbering fewer than 17 calling males. No size-assortative pairing was demonstrated. The average reproductive success of the males was 20.6 % and 29.6 %. Male mating success was not dependent either on the males' body size or on the character of their spatial attachment in the breeding pond during the breeding period. In one case, male mating success showed a positive correlation to the length of male residency in the breeding pond.

Key words: *Hyla arborea*, mate selection, mating success.

INTRODUCTION

Tree frogs (Hylidae) are a very good object for behavioral studies. Therefore the various aspects of the anuran mating behaviour have been still investigated in many hylid species. However, the existing studies dealing with mate selection, male mating tactics and male mating success have been made mostly on different species of American tree frogs. Little research have been carried out on palearctic hylids. Among them, *Hyla arborea* appears to be the best studied species (see e.g. MORAVEC 1987, DE ORENSE ET TEJEDO-MADUEÑO 1990, FRIEDL ET KLUMP 1992, GIACOMA *et al.* 1993), nevertheless other more comprehensive studies would be needed for a better understanding of its general mating system. In this paper some additional data on mate selection and male mating success in *H. arborea* are presented and discussed.

MATERIAL AND METHODS

The mating tactics and mating success of male *H. arborea* were investigated during two breeding periods (1986 and 1987) in two small neighbouring breeding ponds denoted A and B in southern Bohemia (see methods in MORAVEC 1987 and in the part I. in this volume). In individual nights the observations were started at least one hour after the start of calling of the males and each male association was observed for at least one minute. The studies were led with the aid of hand and head lamps, which did not appear to affect the behaviour of the frogs.

Satellite behaviour (sexual parasitism), as an alternative male mating tactics, was demonstrated. The total proportion of satellite associations was 2.6% in pond A-1986 (N=460 calling sites investigated) and 1.3% in pond B-1987 (N=151). On individual nights it averaged 2.0% (range 0.0-8.7) in A-1986 and 0.7% (range 0.0-7.1) in B-1987.

In the breeding pond A-1986 the average (x-) snout-vent length (SVL) of the satellite males was significantly smaller (x-=38.1 mm, SD=2.8, N=11) both in comparison with the dominant males (x-=41.4 mm, SD=1.5, N=10; t=3.45, df=19, P<005) and with the sample of all adult males in the pond (x-=39.9 mm, SD=2.7, N=85; t=2.05, df=94, P<0.025) (see also MORAVEC 1987). The small number of satellite associations found in pond B-1987 does not permit any closer size analysis. However, data obtained on another 12 satellite associations in ponds in the neighbourhood and during the same period confirm the validity of the results obtained. Here the satellite males were also significantly smaller than the dominant ones (t=4.18, df=23, P<0.005), and no significant differences were found in the SVL either between the satellite males from pond A-1986 and the pooled sample from other ponds (x-=38.1 mm, SD=2.7, N=12; t=0.58, df=21, P>>0.05) or between the dominant males from the two samples (pooled sample: x-=41.9 mm, SD=1.7, N=12; t=1.28, df=21, P>0.05). Both in the breeding pond A-1986 and B-1987 the satellite behaviour was not observed in choruses numbering fewer than 17 calling males.

The problem of whether the female mate choice is observed under natural conditions was examined from two points of view: 1) whether

Locality	SVL (mm)	N	Ampl	P'	χ^2 Ampl. x P'
A-1986	33-36	10	1	2.3	$\chi^2 = 0.91$
	37-40	38	9	9.0	$0.25<P<0.5$
	41-45	41	11	9.7	
B-1987	33-36	2	0	0.6	$\chi^2 = 0.74$
	37-40	4	1	1.2	$0.25<P<0.5$
	41-45	21	7	6.2	

Table 1. Comparison of the mating success of male *H. arborea* in different size classes in ponds A and B. AMPL.= number of females won; P'= predicted probability of winning a female.

Locality	Type of spatial attatchment	N	Ampl	P'	χ^2 Ampl. x P'
A-1986	permanent	27	7	7.9	$\chi^2 = 0.74$
	temporary	23	6	6.8	$0.25<P<0.5$
	zero	18	7	5.3	
B-1987	permanent	2	1	1.0	$\chi^2 = 0.22$
	temporary	3	2	1.5	$0.25<P<0.25$
	zero	9	4	4.5	

Table 2. Comparison of the mating success of male *H. arborea* in relation to the type of their spatial attachment in ponds A and B. For other explanations see Table 1.

Locality	Days in pond	N	Ampl	P'	χ^2 Ampl. x P'
A-1986	0-10	45	1	9.3	$\chi^2 = 24.76$
	11-20	31	6	6.4	$P < 0.999$
	21-30	10	5	2.1	
	31-40	8	3	1.6	
	41-50	8	6	1.6	
B-1987	0-10	16	2	4.7	$\chi^2 = 4.38$
	11-20	8	5	2.4	$0.75<P<0.90$
	21-30	3	1	0.9	

Table 3. Comparison of the mating success of male *H. arborea* in relation to the number of days spent in ponds A and B. For other explanations see Table 1.

the potential female mate choice can be detected in the SVL of the successful (amplectant) males, and 2) whether there is size-assortative pairing in *H. arborea*.

(1) Comparing the mean SVLs of successful and unsuccessful males in locality A-1986 revealed no statistically significant differences (successful males: x-=40.5 mm, SD=2.3, N=21; unsuccessful ones: x-=39.7 mm, SD=2.8, N=73; t=1.20, df=92, P>0.05). In locality B=1987 the difference between the mean SVLs of the two groups of males was somewhat more marked but even here it was not distinctly significant (successful males: x-=42.5 mm, SD=1.5, N=8; unsuccessful ones: x-=40.8 mm, SD=2.5, N=19; t=1.72, df=25, P = about 0.05). The correlation between SVL and successful mating of males is illustrated in Table 1, from which is obvious that a comparison of the numbers of actually observed amplexes with the expected probabilities of mating in different size classes does not exhibit any statistically significant difference in any of the localities under study.

(2) Nor did the analysis of the SVLs of males and females found in 52 amplexes in the ponds under study during 1986 to 1988 confirm the presence of any correlation between the sizes of the amplectant partners (r=0.131).

The mating success of male *H. arborea* can be suitably evaluated on the basis of amplexes attained with females, as in the case of the present species each amplectant male will fertilize, under normal conditions, the clutch of eggs of respective female (the data obtained on the number of amplexes attained in localities A-1986 and B-1987 are shown in Figures 5 and 6 in the part I,see MORAVEC, this volume). The mean success of males was 20.6 % (A-1986) and about 29.6 % (B-1987) but some of the males may have attained multiple success.

Of the potential factors that may have affected the individual mating success of male *H. arborea*, we studied the following: A) spatial attachment of males in the breeding pond during the breeding period (see part I.), and B) length of male residency in the breeding pond. (A) The presence or absence of the spatial attachment to a particular part of the breeding pond did not show any significant influence on their mating success either in the pond A or in the pond B. (B) In locality A-1986 the mating success of males increased significantly with the duration of their stay in the pond (Table 3), and both these criteria showed here a positive correlation (r=0.432, N=102, P<0.01). In locality B-1987, however, no such correlation was demonstrated.

DISCUSSION

Altogether three basic hypotheses have been suggested to explain the function of the satellite behaviour (WELLS 1977a, FORESTER ET LYKENS 1986, PERRILL & MAGIER 1988): 1) silent satellite males wait for the vacation of vantage calling sites occupied by vocalizing dominant males, 2) satellite males directly seize females attracted by the vocalizing males, 3) sexual parasitism is a protection against predators orienting by the calls of the vocalizing frogs.
(1) and (2): These two hypotheses are not mutually exclusive. The validity of the former one has been confirmed in the case of *Hyla versicolor* (Fellers 1979). In a number of hylid frogs, incl. *H. arborea*, however, the validity of the second hypothesis has also been demonstrated (e.g. PERRILL *et al.* 1978, MYIAMOTO & CANE 1980, PERRILL 1984, FORESTER & CZARNOWSKY 1985, ROBLE 1985, MORAVEC 1987). (3) In our case its validity cannot be excluded in regard of the observed predation of Rana esculenta complex on calling males of *H. arborea* (see part I.).

The fact that smaller males adopt the satellite behaviour more frequently than bigger ones (see also GERHARDT, 1982, PERRILL *et al.* 1982, FORESTER & LYKENS 1986) make it possible to understand sexual parasitism as an alternative strategy of the smaller males in winning a female. The following may be the causes of selecting this mating tactic by the smaller males: A) Small males must economize their energy more than bigger ones because of the high energetic demands of vocalization (see TAIGEN & WELLS 1985). This can also serve to explain the observed presence of bigger but injured males among the satellite ones (MORAVEC 1991). B) Reproductively active males compete for suitable calling sites and, in more numerous aggregations, the smaller and weaker males are expelled to suboptimal sites. Satellite behaviour then is a form of compensation for this disadvantage (see FORESTER & LYKENS 1986). C) The advertisement calls of smaller males are less attractive for females than the calls of bigger ones. The generally accepted theory states that the bigger size of the males reflects their greater fitness. Experimental results show that females (and probably also males) can obtain information on the male's quality through differences in the acoustic parameters of its advertisement call, being a function of the size of the male's body (RYAN 1980, 1983; DOHERTY & GERHARDT 1984; FORESTER & CZARNOWSKY 1985; ROBERTSON 1986; LYKENS & FORESTER 1987). By potential selecting bigger males, then, the females could enable them to transmit their higher quality to the progeny (LANDE 1981, KIRKPATRICK 1982, ARNOLD 1983). According to LYKENS & FORESTER (1987), however, female *Pseudacris crucifer* do not distinguish the age of males but prefer only the most readily perceivable frequencies of their calls that vary approximately in the middle of their perceivable range. This eliminates male age classes whose vocal frequencies lie at the limits of the preferred range (the few oldest and the most numerous youngest males).

It follows from the observations described above that, as a result of the female choice, bigger males should be more successful breeders. Such situation has been confirmed in certain species of frogs (for a review see SULLIVAN 1983: Table IV)). In other cases, on contrary, it has not been demonstrated (e.g. WELLS 1977a, PERRILL 1984, FORESTER & CZARNOWSKI 1985). In the case of *H. arborea*, on the one hand, it has been observed that bigger males have a higher probability of mating (see GIACOMA *et al.* 1993) but, on the other, the results obtained by FRIEDL & KLUMP (1992) as well as my own ones do not confirm the higher mating success of the bigger males. This fact can be explained as follows: i) The result of the female mate choice in frog species with a prolonged breeding period can be influenced by alternative male mating tactics (e.g. by mentioned sexual parasitism), which make even small males successful (see VERRELL 1983, WELLS & SCHWARTZ 1984, FORESTER & CZARNOWSKY 1985). Thus the estimation of the effect of female mate choice by analysing the sizes of amplectant and non-amplectant males can be misleading. ii) In the case of some hylid frogs the ultimate effect of the female mate choice can be affected by certain characteristics of male vocalization that are not correlated with their SVL and, at the same time, are preferred in female phonotaxis. For example, higher intensity and length of calls or increasing calling rate as a result of various social interactions (e.g. WELLS & SCHWARTZ 1984, FORESTER & CZARNOWSKY 1985, WELLS & TAIGEN 1986, etc.). My own observation confirm that the presence of a female has a stimulating effect on the intensity and duration of male vocalization in *H. arborea* (MORAVEC 1991). Thus, one may expect that social interactions can affect the result of female mate choice even in this species. iii) In contrast to experimental conditions under which the selective choice of bigger males has been studied as a rule, in natural choruses the female choice seems to be more complex (see e.g. DOHERTY & GERHARTDT 1984). Some individual differences in the frog's calls can also be obliterated by the muffling effect of vegetation and weather conditions, acoustic interference, etc. Thus, the choice of bigger and qualitatively better males in natural choruses seems to take place, first of all at low densities of the calling males (RYAN 1983, WELLS & SCHWARTZ 1984, FORESTER & CZARNOWSKY 1985). Since even sexual parasitism occurs at higher densities of calling males only, one may add that its negative impact on the mating success of bigger males is minimal in scanty choruses. Thus, if fitter males are only selected in scanty choruses, the question is also posed whether such selection can be of any significance for the prosperity of a population. A certain explanation may be seen in that the positive effect of the mentioned selection is essential in stimulating the growth and viability of just the scanty, week population. Since size-assortative pairing in *H. arborea* has not been demonstrated in our case (similarly also FRIEDL & KLUMP 1992), one may refute the hypothesis stating that females select such mates that would suit them best by their size in order to secure optimum egg fertilization (see LICHT 1976). This is also suggested by the observation that in *H. arborea* both partners can compensate for their size disproportionality behaviourally in the course of fertilization (see MORAVEC 1989).

In the males of many frog species with prolonged breeding, efforts to attain mating success are connected with vocalization competition, maintaining minimum mutual distances, or even defence of individual territories (see WELLS 1977b). In our case, in which no defence of true territories has been demonstrated, it is obvious that the duration of the males's attachment to permanent sites in the pond has no effect on their mating success.

Often a positive correlation is reported between the mating success of male frogs and the length of their stay in the pond (e.g. KLUGE 1981, RYAN 1983, SULIVAN 1985, inter alia). However, FRIEDL & KLUMP (1992) found no such correlation in *H. arborea*. In our case, this correlation was only confirmed in pond A-1986. In general, however, the presence of such a correlation in *H. arborea* seems to be logical as, evidently, the more nights a male will vocalize in a pond the higher its chance to win one of the successively coming females is.

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Thermal ecology of a population of *Testudo hermanni* in the Ebro Delta (NE Spain)

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Abstract: The thermal relations were analyzed in an introduced population of Hermann's Tortoise (*Testudo hermanni*) sited in a sandy area of the Ebro Delta (NE Spain). Usual techniques in thermal ecology were used. Body, air and substrate temperatures of 242 animals were recorded. Results define this species as heliotherm. The body temperatures were low (mean 27.30) with a relatively narrow range (13.40-37.20). The relation between substrate and body temperatures followed a logarithmic curve. Intraspecific and seasonal differences were registered but no evidence of thermal inertia was detected in the largest animals. Conversely, only the juveniles showed capability of thermoregulation. The adult females reached lower body temperatures than males at the same environmental temperatures. During winter, the active adults showed thermal compensation indicated by higher temperature residuals than those in other seasons. These findings are discussed in comparison to other populations taking into account the thermal environment and the species constraints.

Key words: Thermal ecology, thermoregulation, Chelonia, *Testudo hermanni*.

INTRODUCTION

Terrestrial chelonians can be interesting objects for studies on thermal ecology. First, their bodies are large and hemispherical (MEEK & AVERY, 1988) and, second, their movements are slow in comparison to other reptiles (see JAYES & ALEXANDER, 1980; MEEK & AVERY, 1988). So, on one hand, the low surface/volume relation could provide large species with thermoregulation mediated by thermal inertia. On the other hand, their low mobility would not allow them accurate behavioural themoregulation (MEEK, 1984) and the proximity of shade would be important for preventing overheating (MEEK & AVERY, 1988).

The present paper deals with these thermal traits in an introduced population of Hermann's Tortoise (*Testudo hermanni*) in NE Spain, their intraspecific variation and the possible environmental influences.

T. hermanni is a Mediterranean species of medium size which ranges the Balkan Peninsula, Greece, Italy and some Mediterranean islands (ARNOLD & BURTON, 1978). In the Iberian Peninsula, only the relictual population of the Alberes (NE Spain-SE France) can be considered native at present (FRETEY, 1987; CHEYLAN, 1984; LLORENTE *et al.*, 1995). The thermal ecology of this species has been previously investigated (CHERCHI, 1956-1960; CHERCHI *et al.*, 1958; MEEK & INSKEEP, 1981; MEEK, 1984; PULFORD *et al.*, 1984; MARAGOU & VALAKOS, 1992) and other members of the same genus have also been studied (BANNIKOV, 1951; CLOUDSLEY-THOMPSON, 1974; LAMBERT, 1981; MEEK & JAYES, 1982; MARAGOU & VALAKOS, 1992).

MATERIAL AND METHODS

The population studied was introduced in the Parc Natural del Delta de l'Ebre (delta of the Ebro river, UTM 31TCE09) in 1987-1988 (BERTOLERO, 1991), in a group of sand islets of the Alfacs Peninsula with 5.8 Ha of total surface. 44 individuals were set free and bred successfully (BERTOLERO, 1991). The population is now estimated at more than 200.

The space around the islets lacks vegetation and it is covered or not by the sea depending on the season. When it is dry the tortoises can pass from one islet to another. The vegetation is composed of psammophile and halophile plants (CURCÓ, 1991) and a wood of White Pine (*Pinus halepensis*) which was set up in 1940-50. The climate is littoral Mediterranean, with a long dry season in summer (mean annual rainfall=548 mm, mean temperature=16.6°C; see PANAREDA & NUET, 1973).

Sampling was carried out in 1992 on sunny days without strong wind. The visits to the area depended on the transport possibilities, so it was impossible to carry out uniform prospection. Animals were active between February and November (see also BERTOLERO, 1991). Standard techniques in thermal ecology (AVERY, 1982) were used on 242 animals. Cloacal temperature (TB), air temperature (TA) 10 cm above the ground and substrate temperature (TS) in the place of sight, were recorded. All three were measured in shade using a digital thermometer with a K type thermocouple (resolution 0.1°C, reading time 1'). Every tortoise was also weighed in the field with a dynamometer and its carapace length was measured.

Three work classes were distinguished: adult males (carapace length: 126-157mm), adult females (151-195mm) and juveniles (34-138mm). For the study by seasons, data were grouped as follow:

Winter = December + January + February
Spring = March + April + May
Summer = June + July + August
Autumn = September + October + November

The analysis of covariances (ANCOVA, SOKAL & ROHLF, 1981) was used to compare the TB values among groups correcting the effect of environmental temperatures.

RESULTS

The descriptive statistics of TB, TA and TS are shown in Table 1, considering the pooled population and the classes. Intraspecific variation was detected. Body temperatures of the females were lower than those of the other two classes and this difference remained when TB was corrected for TA (ANCOVA, F = 4.86, $p < 0.01$, for the adjusted means) and TS (ANCOVA, F = 5.51, $p < 0.01$, for the adjusted means). There was no significant correlation of TB either with carapace length (R = 0.27, $p > 0.05$) or with body weight (R = 0.27, $p > 0.05$).

Class	N	X	S	CV	lim(95%)	range
Pooled						
TB	242	27.30	3.25	11.89	±0.41	13.40-37.20
TA	242	21.89	3.97	18.14	±0.50	11.60-41.10
TS	242	26.81	7.30	27.23	±0.92	12.30-55.00
Males						
TB	45	27.61	5.15	18.65	±1.50	16.00-34.30
TA	45	21.65	4.19	19.35	±1.22	15.40-30.60
TS	45	26.08	7.30	27.99	±2.13	16.00-45.60
Females						
TB	74	24.89	5.20	20.89	±1.18	13.40-33.60
TA	74	20.26	3.95	19.50	±0.90	11.60-29.00
TS	74	24.16	6.78	28.06	±1.54	12.30-39.60
Juveniles						
TB	123	28.64	4.52	15.78	±0.80	17.00-37.20
TA	123	22.96	3.84	16.72	±0.68	15.30-41.10
TS	123	28.68	7.21	25.14	±1.27	15.00-55.00

Table 1: Temperatures of *Testudo hermanni* (Ebro Delta). TB = body temperature, TA = air temperature, TS = substrate temperature, N = sample, X = mean, S = standard deviation, CV = coeficient of variation.

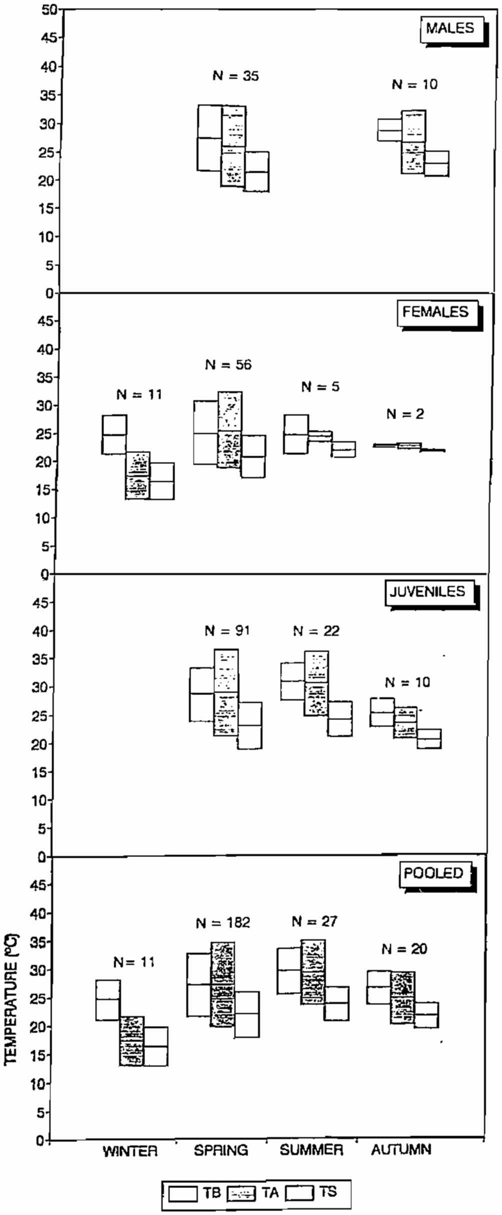

Figure 1: Seasonal variation of body (TB) and air (TA) or substrate (TS) temperatures. Horizontal lines represent mean and standard deviation intervals respectively.

The general pattern of seasonal variation consisted of higher values in summer than in the rest of year (see Figure 1). This was true for the pooled population but, considering the classes, only the differences in females were found to be significant. So, TB-TA (but not TB-TS) residuals of females were higher in winter than in the rest of the year (ANCOVA, $F = 4.60$, $p < 0.01$, for the adjusted means).

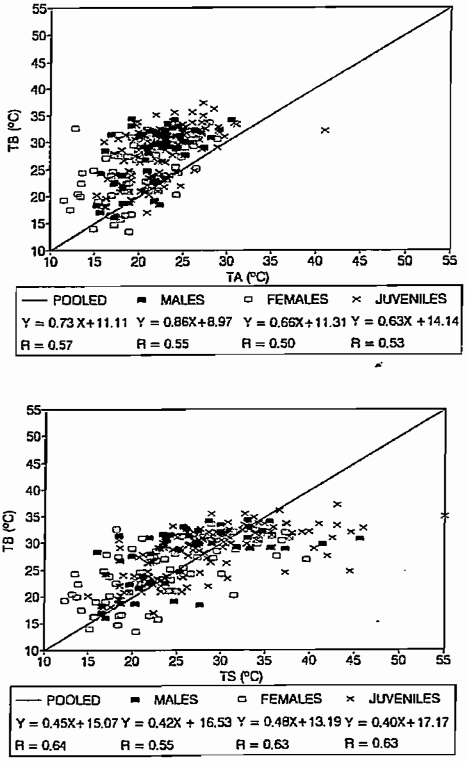

Figure 2: Relations between body (TB) and air (TA) or substrate (TS) temperatures.

The thermal relations between TB and TA or TS (Figure 2) had significant regressions in all the cases. The slopes were different (t test, $p < 0.01$) from 0 (perfect thermoregulation) and 1(absolute thermoconformity). The only exception was the TB-TA slope of males, which was not different from 1. In females, it was different only at $p < 0.05$ level.

The TB-TA slope was higher than TB-TS slope in all the cases (parallelism tests, $p < 0.01$). No differences were found in TB-TA and TB-TS slopes either by classes or by seasons in any class.

Finally, in order to improve the adjustment in the TB-TS correlation, several non-linear regression were attempted. The logarithmic regression provided a higher percentage of explained variance (R^2) than linear regression (Figure 3). This was not the case of the TB-TA regression.

DISCUSSION AND CONCLUSIONS

The body temperatures of *Testudo hermanni* varied between the usual limits in terrestrial chelonians and were intermediate between those of the congeneric species *T. graeca* and *T. marginata* (see MEEK, 1984; MARAGOU & VALAKOS, 1992). The moderately high values were very similar to those obtained by MARAGOU & VALAKOS (1992) who studied a population throughout the year in Greece. Other studies (MEEK, 1984; PULFORD *et al.*, 1984), carried out in spring or summer, registered higher body temperatures than these. This constancy shows the conservative character of the TB mean (AVERY, 1982) and seems to indicate a certain degree of thermal control (see below).

The intraspecific differences have been observed for the first time in this species. They cannot be due to the availability of different environmental temperatures since TB residuals (and not TB) were used. So, females reached lower body temperatures than the rest in the same thermal environment. The explanations based on the temperature for the development of the eggs in the reproductive season and/or the searching for egg sites, would need confirmation. For instance, sexual differences in the activity pattern have been detected in September (HAILEY *et al.*, 1984).

Considering the seasonal variation, the animals found in winter (all of them were females in February) reached suboptimal temperatures (lower the selected temperatures) but show evidence of thermal compensation. Although TB was the lowest one of the year, the TB-TA residual was the largest.

The slope of the regression line points out how much TB depends on the environmental temperatures, TA or TS (see HUEY & SLATKIN, 1976). The body temperature of *T. hermmani* depends more on the air than on the substrate. This confirms this species as heliotherm (AVERY, 1979) as other authors have recorded (MEEK, 1984; MARAGOU & VALAKOS, 1992). However, evidence of thermoregulation can be seen here in juveniles (and, to a lesser extent, in females) but this has not been detected by other authors.

Some models (POUGH, 1980; STEVENSON,

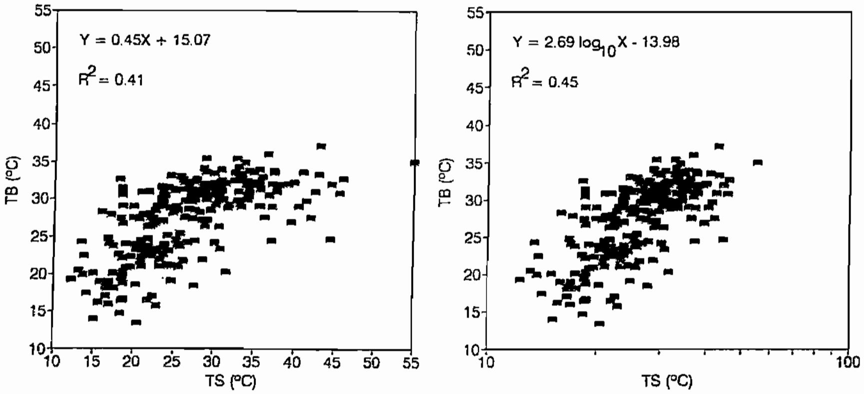

Figure 3: Linear (left) and logarithmic (right) regressions between body (TB) and substrate (TS) temperatures.

1985) predict a thermal inertia effect in ectotherms larger than 0.1-1 Kg of body mass as *T. hermanni*. However, this effect has not been detected here (see also MEEK, 1984). Probably, this is due to the more accurate behavioural thermoregulation in juveniles than in the adults (see results). Juveniles can compensate their low surface/volume relation with more availability of shade sites because of their small size. A conservative activity of juveniles at midday and in the evening, has also been reported (see HAILEY *et al.*, 1984; and LAMBERT, 1981 for *T. graeca*).

The maximum voluntary body temperatures detected here (37.20°C) is the highest detected in this species. In this kind of habitats with a reduced vegetal coverage, the sand can accumulate a great amount of caloric energy during the day (CLOULDSLEY-THOMPSON, 1991). This implies a danger of overheating and can increase TB to near the critical thermal maximum (39-42°C, see CHERCHI, 1956) and may reduce the summer activity. Tortoises are partially able to compensate for this process. While TS is lower than 28-30°C the tortoises behave as thermoconformers but above these values thermoregulation takes place (see Figure 3).

The importance of the habitat in the activity of this species has been stated previously (HAILEY *et al.*, 1984; PULFORD *et al.*, 1984). Some authors (CHELAZZI & CALZOLAI, 1986) have also shown that thermoregulatory efficiency depends on habitat familiarity. This has been suggested to be important in introduced animals (MEEK & AVERY, 1988). In this case, five years after the introduction, animals seem to be acclimatized, since thermoregulation is even more efficient than in other localities studied (see references). The environmental conditions in summer (drought, overheating) in an extreme habitat allow *T. hermanni* in the Ebro Delta to show its capability of thermoregulation (juveniles) or its thermoconformity for high temperatures (adults). The importance of studying extreme thermal environments should be considered before defining the species constraints.

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Thermal and temporal patterns of two Mediterranean Lacertidae

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Abstract: The thermal and the temporal dimensions of the niche were analyzed for two lacertid lizards, the Large Psammodromus (*Psammodromus algirus*) and the Fringe-toed Lizard (*Acanthodactylus erythrurus*), in a dune ecosystem in NE Spain. Uniform sampling was carried out in order to obtain comparative results. 134/79 cloacal temperatures and 202/116 observations were recorded for each species, respectively. Both species proved to be heliotherms with similar degree of thermoregulation. However, *A. erythrurus* showed higher body temperatures than *P. algirus* because of its use of higher environmental temperatures. Annual differences were also recorded but only the second species showed intraspecific variation. In relation to these results, the annual activity was different between species and size classes. Only the immature specimens of *P. algirus* were active all year and the rest of animals showed a winter diapause period. The daily activity of *P. algirus* lasted from 3-4 hours in winter to 12 hours in summer. In contrast, the daily range of *A. erythrurus* was 2-3 hours shorter. In summer, the pattern of population activity was bimodal in *A. erythrurus* but not in *P. algirus*. During the rest of the year the daily activity was unimodal in all cases. Thermoregulatory behaviour was mainly observed at low air temperatures and this was very rare in *A. erythrurus*. The thermal environment, the physiological constraints but also the non-thermal selective pressures could explain the different temporal strategies of the lizards in this locality.

Key words: Thermal ecology, activity, Lacertidae, *Psammodromus algirus*, *Acanthodactylus erythrurus*.

INTRODUCTION

Psammodromus algirus (Large Psammodromus) and *Acanthodactylus erythrurus* (Fringe-toed Lizard) are two medium-sized lacertids living together in many open Mediterranean areas of Iberian Peninsula and NE Africa (BARBADILLO, 1987). *P. algirus* is more North-spread than *A. erythrurus*, also reaching the SE of France (FRETEY, 1987).

This study is a part of a wider work carried out in a zone of coastal dunes where these species are especially abundant (SEVA, 1984). The thermal and temporal dimensions of the niche of these species have been previously studied in continental areas (POLLO & PÉREZ-MELLADO, 1989).

This study attempts to analyse these features in a warm climate zone, deducing the thermal and activity patterns of every species and determining the inter-intraspecific variation.

MATERIAL AND METHODS

The study area was a mosaic habitat of dunes and moist zones covered by psammophile and halophile vegetation in the delta of the Ebro river (see description in CARRETERO & LLORENTE, 1993b). The climate is littoral Mediterranean, with a long dry season in summer (mean annual rainfall = 548 mm, mean temperature = 16.6°C; see PANAREDA & NUET, 1973).

Psammodromus algirus and *Acanthodactylus erythrurus* are the only saurian species living in this area (abundance ratio: 2:1). Only two size classes (adult and immature) were distinguished for each species since both reached their sexual maturity mostly at the first year of age (unpublished data). See CARRETERO & LLORENTE (1993b) and CARRETERO & LLORENTE (in press) for the limit sizes of the classes.

A monthly sampling was carried out from March 1988 until February 1989 on 100% sunny days without strong wind. A surface of 0.35 Ha (35x100 m) was surveyed in the search for active lizards along all the period of activity (from 6 to 18 hours GMT). A 30 minute transect was done every hour between hour+15' and hour+45'. Prospection effort was kept constant for hours and months (TELLERÍA, 1986).

Usual techniques in thermal ecology were used (AVERY, 1982) with captured animals. Cloacal temperature (TB) in shade was measured no more than 15-20 seconds after the first observation. Air temperature (TA) 50 cm above the ground and substrate temperature (TS) in the place of first sight, were recorded, both also in shade. These temperatures were taken using a digital thermometer with a K type thermocouple (resolution 0.1°C, reading time 1'). Additionally, relative humidity was registered using a metal-band analogic hygrometer with an

	N	X	S	CV	lim(95%)	range
P. algirus						
pooled						
TB	134	31.44	4.27	13.58	±0.72	18.7-41.0
TA	134	22.49	5.06	22.49	±0.86	12.0-33.8
TS	134	26.81	7.62	28.43	±1.29	11.3-45.6
males						
TB	17	31.59	5.93	18.78	±2.82	18.7-41.0
TA	17	22.69	5.51	24.28	±2.62	15.0-33.8
TS	17	28.28	7.69	27.19	±3.65	18.2-42.1
females						
TB	22	34.66	2.57	7.42	±1.07	30.1-38.5
TA	22	25.29	4.54	17.97	±1.90	15.8-32.9
TS	22	29.06	5.78	19.90	±2.42	20.9-45.6
immatures						
TB	95	30.67	3.91	12.76	±0.79	22.6-37.8
TA	95	21.80	4.91	22.52	±0.99	12.0-31.2
TS	95	26.02	7.90	30.37	±1.59	11.3-44.9
A. erythrurus						
pooled						
TB	79	33.10	3.25	9.81	±0.72	24.4-39.4
TA	79	24.09	3.77	15.66	±0.83	14.0-30.6
TS	79	28.72	5.81	20.23	±1.28	18.9-43.2
males						
TB	13	34.29	2.74	7.99	±1.49	30.7-38.6
TA	13	24.33	3.46	14.22	±1.88	17.1-27.7
TS	13	30.94	8.11	26.21	±4.41	18.9-43.2
females						
TB	6	32.05	3.18	9.93	±2.55	29.1-37.6
TA	6	23.63	3.66	15.49	±2.93	19.1-29.7
TS	6	25.68	4.47	17.41	±3.58	18.9-31.6
immatures						
TB	60	33.10	3.33	10.07	±0.84	24.4-39.4
TA	60	24.09	3.90	16.19	±0.99	14.0-30.6
TS	60	28.72	5.24	18.26	±1.33	19.1-41.3

Table 1: Temperatures of the Lacertidae from the Ebro Delta. TB = body temperature. TA = air temperature. TS = substrate temperature. N = sample. X = mean. S = standard deviation. CV = coeficient of variation.

error of ±1%.

The analysis of covariances, ANCOVA (SOKAL & ROHLF, 1981) was used to compare the TB values among groups correcting the effect of environmental temperatures.

Moreover, the number of lizards seen (included those not captured), their class, the hour and the month of the location were registered. Intersexual variation in activity patterns was not considered since sex determination was sure only in captured adults. It should be cosidered that the activity results obtained here derive from two different origins: the total number of lizards and their presence above ground. Besides, individual activity may differ from population.

The behaviour of the individuals observed was divided into two types:

1- Active animals (including locomotion, defence, hunting or breeding).

2- "Basking" animals (in heliothermia).

For the study by seasons, months were grouped as follow:

Winter = December + January + February
Spring = March + April + May
Summer = June + July + August
Autumn = September + October + November

The class "adults" included captured males and females plus non-captured animals which could not be sexed but whose body sizes were large enough.

RESULTS

Temperatures

The descriptive statistics of TB, TA and TS for the captured animals (134 *P. algirus* and 79 *A. erythrurus*), considering the pooled population and the classes, are shown in Table 1. Some interspecific variation was recorded. The TB values adjusted for TS were higher in *A. erythrurus* than in *P. algirus*

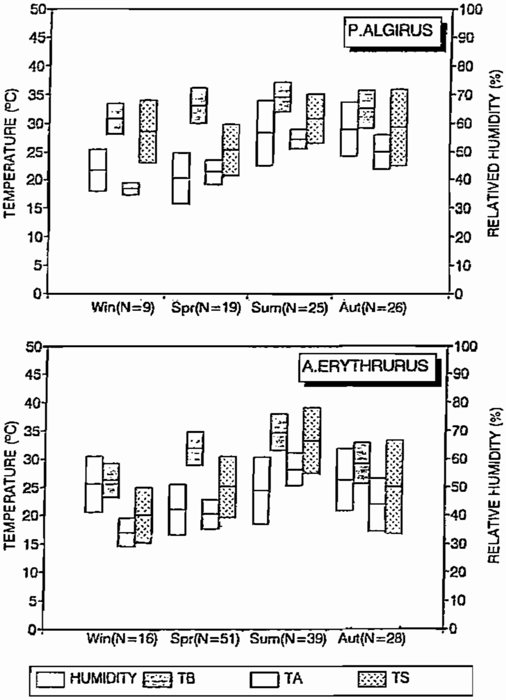

Figure 1: Seasonal variation of the temperatures and the humidity in *P. algirus* (above) and *A. erythrurus* (below) of the Ebro Delta. Horizontal lines represent mean and standard deviation intervals respectively.

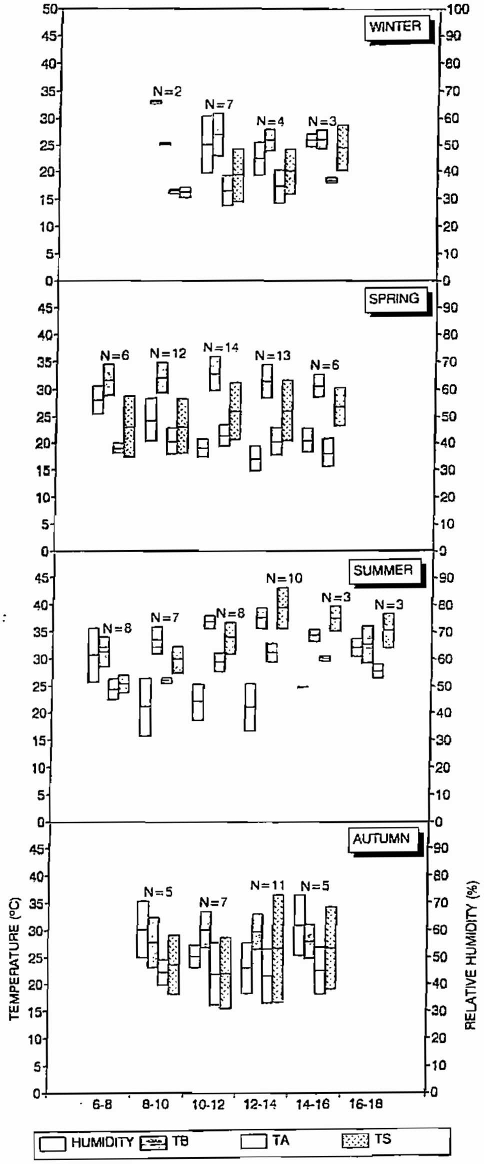

Figure 2: Daily variation of the temperatures and the humidity in *P. algirus*. Data accumulated by seasons and two-hour intervals. Horizontal lines represent mean and standard deviation intervals respectively.

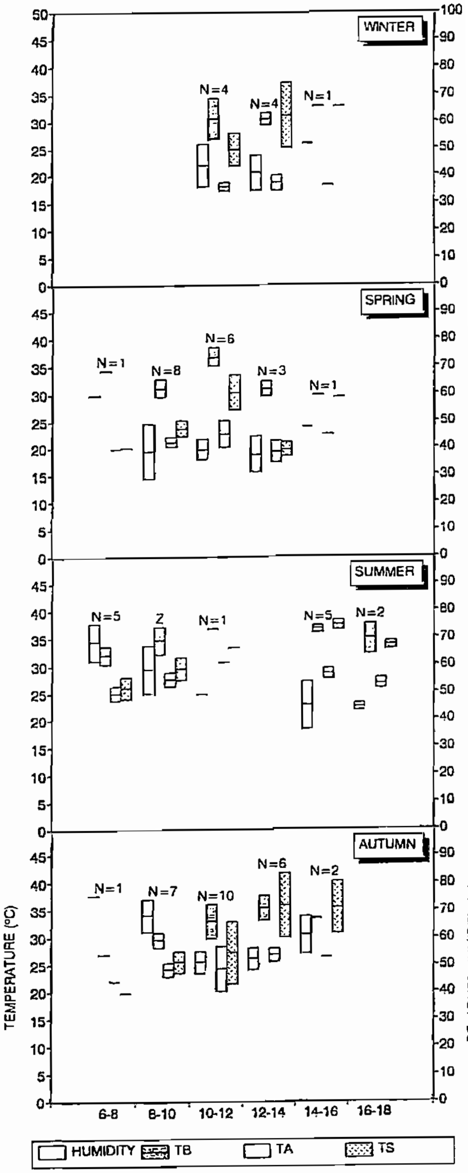

Figure 3: Daily variation of the temperatures ar the humidity in *A. erythrurus*. Data accumulated b seasons and two-hour intervals. Horizontal lin represent mean and standard deviation interva respectively.

(ANCOVA, F = 5.089, $p < 0.05$, for the adjusted means to TS). Nevertheless, the adjusted values for TA did not show differences.

The only intraspecific variation detected was that *P. algirus* females had significantly higher TBs than immatures (ANCOVA, F = 3.95, $p < 0.01$, for the adjusted means to TA) o r males (ANCOVA, F = 8.50, $p < 0.01$, for the adjusted means to TS). No differences were found in *A. erythrurus*.

The general pattern of annual variation consisted of higher values in summer than in the rest of year (see Figure 1). The increasing order of temperatures was TB, TS and TA. TS approached TB in summer. *P. algirus* showed higher TB-TA residuals in spring than in the rest of the year (ANCOVA, F = 19.45, $p < 0.01$, for the adjusted means, no slope differences). If we considered the TB-TS residuals, the pair winter-autumn was higher than the pair spring-summer (ANCOVA, F = 14.00, $p < 0.01$, for the adjusted means; no slope differences). In all cases, *A. erythrurus* showed a higher TB value in winter than in the rest of the year. Moreover, it was found that, adjusting for TS, the spring value was higher than summer one (ANCOVA, F = 8.79, $p < 0.01$, adjusted means for TA; ANCOVA, F = 5.52, $p < 0.01$, adjusted means for TS; no slope differences). No differences in environmental humidity were found among species or seasons.

The daily variations of TB, TA, TS and relative humidity are represented in Figures 2 (*P. algirus*) and 3 (*A. erythrurus*), grouped in periods of two hours. In both cases TB was the higher temperature and followed the TA values with a maximum at midday. TS increased during the day reaching its maximum at the end of the afternoon. In summer, this allowed TS to surpass TC. The humidity tended to be higher at the begining and at the end of the day.

Nevertheless, the differences in temperature were not statistically significant. However, some differences in humidity were detected corresponding to low values at midday. It was observed for *P. algirus* (one way ANOVA, $F_{spring} = 20.66$, $F_{summer} = 7.09$; $p < 0.01$) and for *A. erythrurus* (one way ANOVA, $F_{summer} = 5.76$, $F_{autumn} = 15.32$; $p < 0.01$).

The thermal relations between TB and TA (Table 2) had significant regressions in all the cases except the *A. erythrurus* females. All the slopes were different (t test, $p < 0.01$) from 0 (perfect thermoregulation) and 1 (absolute thermoconformity). All the correlations between TB and TS (Table 2) were also significant (slope

	N	R	SIG. R	regression line
P. algirus				
pooled	134	0.74	**	TC = 0.62 TA + 17.30
		0.68	**	TC = 0.38 TS + 21.13
males	17	0.65	*	TC = 0.70 TA + 15.61
		0.81	**	TC = 0.63 TS + 13.76
females	22	0.65	**	TC = 0.37 TA + 25.25
		0.63	**	TC = 0.28 TS + 26.46
immatures	95	0.76	**	TC = 0.61 TA + 17.32
		0.67	**	TC = 0.33 TS + 22.00
A. erythrurus				
pooled	77	0.53	**	TC = 0.46 TA + 21.97
		0.34	**	TC = 0.34 TS + 23.16
males	11	0.71	**	TC = 0.56 TA + 20.47
		0.75	**	TC = 0.25 TS + 26.40
females	6	0.52	n.s.	
		0.85	*	TC = 0.60 TS + 16.38
immatures	60	0.43	**	TC = 0.43 TA + 22.37
		0.57	**	TC = 0.36 TS + 22.51

Table 2: Correlations and regression lines between body (TB) and air (TA) or substrate (TS) temperatures in the two species of the Lacertidae from the Ebro Delta (* $p < 0.05$, ** $p < 0.01$, n.s. not significant).

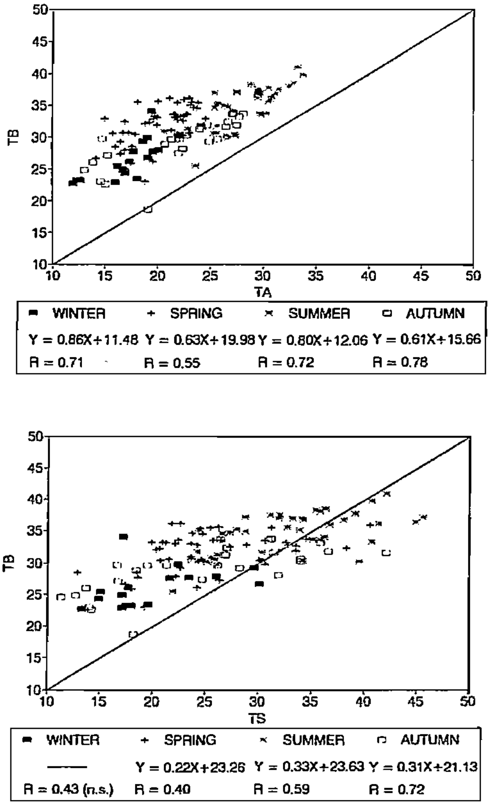

Figure 4: Seasonal variation in the TB-TA (above) and TC-TS (below) relations of *P. algirus*.

different from 0 and 1, t test, $p < 0.01$) in both species and their classes.

The TB-TA slope was higher than TB-TS one in *P. algirus* but not in *A. erythrurus* (parallelism test, $p < 0.01$ when significant). The comparison of the TB-TA (and TB-TS) slopes between both species did not detect significant differences (parallelism tests). Generally, the value of the coefficient of correlation R was higher in the TB-TA regression than in the TB-TS one for the same group. The only intraspecific difference in slope was found in *P. algirus*. The males of this species had lower slope of TC-TS regression than the rest of classes (parallelism test, $p < 0.01$).

The analysis of the slope variation among seasons for the two species (Figures 4 and 5) did not show significant differences. The TB-TA slopes of winter and summer in *P. algirus* and those of summer and autumn in *A. erythrurus* were not significantly different from 1 (t test, $p < 0.01$).

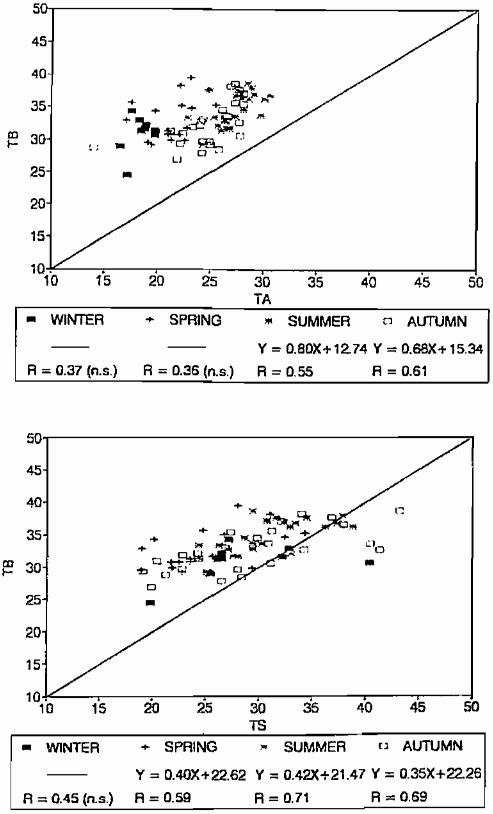

Figure 5: Seasonal variation in the TB-TA (above) and TC-TS (below) relations of *A. erythrurus*.

Activity

202 *P. algirus* and 100 *A. erythrurus* were observed (including captures). The patterns of annual activity of both species were clearly different (Figure 6). *P. algirus* had two equal maxima in spring and autumn. It was found during all the year although the activity decreased in winter. However, *A. erythrurus* interrupted its activity in January and their maxima were in late spring-summer and late summer-autumn. The second one was higher than the first.

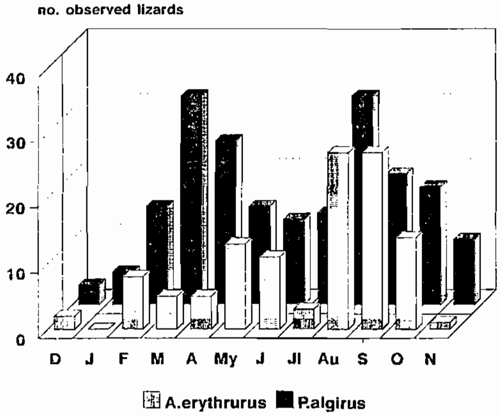

Figure 6: Annual activity of the whole populations of the two Lacertidae.

When this pooled activity was divided by classes, several intraspecific differences appeared. No sex differences were considered because of the small sample.

The adults of *P. algirus* (Figure 7) showed a clear diapause period in winter which lasted form November to February. In March, immediately after the diapause, the adults were mostly males. Immatures were active in winter when weather conditions were suitable and they were absent in mid summer.

Both classes of *A. erythrurus* (Figure 8) had a winter diapause but they did not have equal activity. So, immatures had three more months of activity than adults. These were very scarce in mid summer.

The pattern and the range of daily activity were also different among seasons and between species (Figures 9 and 10).

The total number of active individuals of *P. algirus* (Figure 9) followed a very restricted unimodal distribution (3-4 hours) in winter. In spring and autumn, the pattern was also unimodal but it was wider than in winter. The aestival activity becames more uniform and was

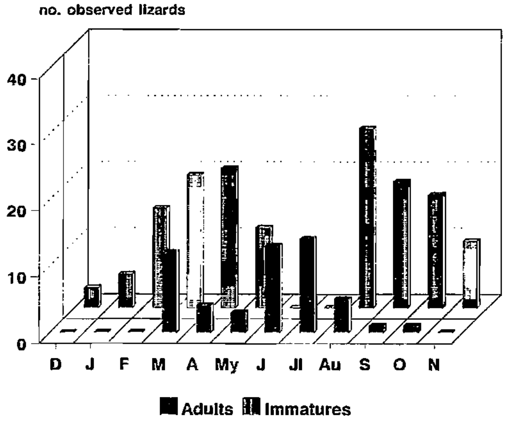

Figure 7: Annual activity of the classes of *P. algirus*.

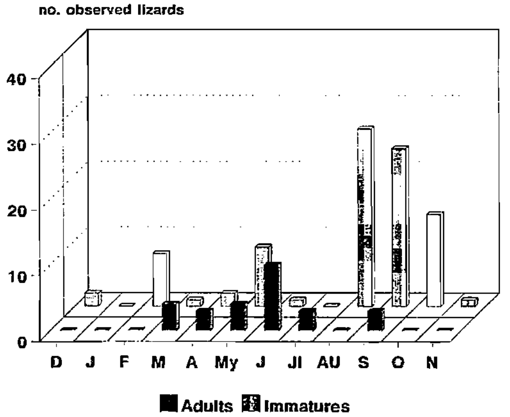

Figure 8: Annual activity of the classes of *A. erythrurus*.

slightly bimodal. The "basking" animals appeared in the coldest hours, i.e. all day in winter and the beginning and at the end of the activity in summer. In spring and autumn, the distribution of these animals followed a bimodal curve with a minimum at midday.

A. erythrurus (Figure 10) showed a more conspicuous pattern with a narrower range than *P. algirus*. Only few individuals were seen in winter at midday. The pattern was unimodal in spring and autumn and strongly bimodal in summer. The activity in the afternoon was always lower than in the morning. The individuals in heliothermia were scarce and appeared at the beginning or the end of the activity in spring and autumn.

The same results divided by classes (Figures 9 and 10) showed only slight differences. When the sample was sufficient, immatures of both species showed more uniform patterns of activity than adults. Generally, the immatures were also the first lizards seen in the morning.

DISCUSSION AND CONCLUSIONS

The first remarkable feature in the results is the high degree of thermophilia in *Acanthodactylus erythrurus*, typical of this genus (AVERY, 1982). This is especially remarkable in comparision to *Psammodromus algirus* at the same site. This difference could be associated with the Northern range of distribution in every species (see BARBADILLO, 1987) consequence of a cost-benefit optimization of energy and the reproductive success (SAINT-GIRONS & SAINT-GIRONS, 1956). SEVA (1984) points out that this trait is determinant for the latitudinal variation in the abundance ratio between these two species in coastal sandy areas.

Working in central Spain, POLLO & PÉREZ-MELLADO (1989) also find differences between these two species but other authors (SEVA & ESCARRÉ, 1980; SEVA 1982) do not find them in the coast of SE Spain (see also CARRETERO & LLORENTE, 1991). The cause of this disparity should be attributed to the environment more than to endogenous factors (MALHOTRA & THORPE, 1993). In the localities near the Northern boundary of *A. erythrurus* (Central Spain, NE Spain) the TB of this species remains constant in comparison to the Southern localities (BUSACK, 1976; SEVA, 1982, 1984; POUGH & BUSACK, 1978) but reduces its activity range. In contrast, *P. algirus* decrease its TB and remains active. So, the increment of TB to the South in this species is a consequence of the availability of different environmental temperatures (see PIANKA, 1970).

The high TB found in the females of *P. algirus* have already been registered (POLLO & PÉREZ-MELLADO, 1989; see also CARRETERO & LLORENTE, 1991). Other reproductive factors different from pregnancy (BEUCHAT, 1986; HEULIN, 1987; BRAÑA, 1993) may be involved in this marked difference, which is independent of the thermal environment. The small size effect detected here (see CARRETERO & LLORENTE, 1993a, for the discussion of an example) may be in relation to the high environmental temperatures.

Considering now the annual variation (HUEY & PIANKA, 1977), the high relative values of TB

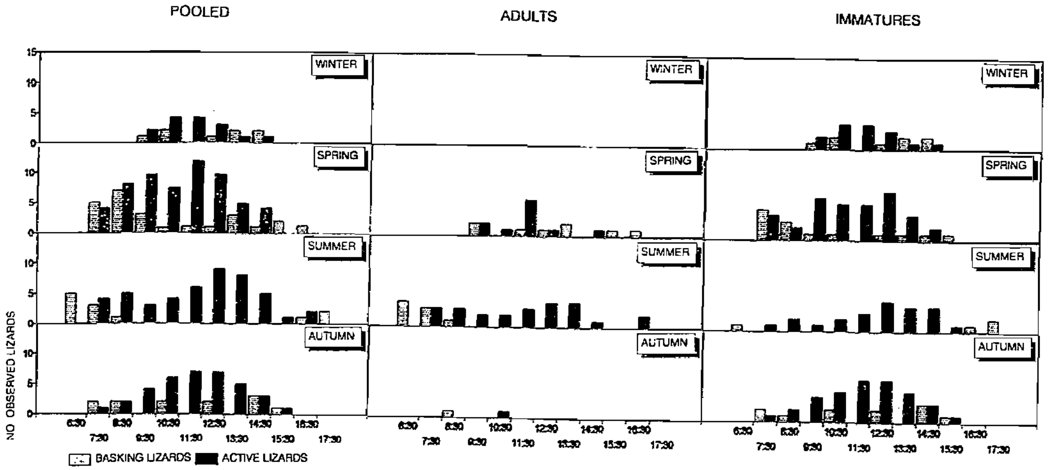

Figure 9: Daily activity considering seasons and classes in *P. algirus*.

in winter should be interpreted as a compensation for the low environmental temperatures (especially in the substrate), but below the selected TB. The situation in summer is inverse. The surprisingly high TB in spring, higher than in autumn, could correspond to a longer period devoted to heliothermia (see activity) during reproduction.

The daily variation of TB confirms the dependence of the environment in the ectotherm species. In the hours with low environmental temperatures, TB decrease but thermal compensation mediated by insolation increase (see activity). At midday, Tb is the highest, near the environmental temperatures, and insolation behaviour is scarce.

As in the deserts, sand can accummulate a great amount of caloric energy during the day (CLOUDSLEY-THOMPSON, 1991) and is an important source of heat for both species in the late afternoon when TA decrease. So, some individuals, especially *P. algirus*, have been observed at the end of the day in tigmothermia with low air temperatures. This fact can also decrease the number of active animals in summer (Figures 6, 7 and 8).

Humidity is a factor afecting the activity of reptiles (BRADSHAW, 1986; HEATWOLE & TAYLOR, 1987; CLOUDSLEY-THOMPSON, 1991) including the Mediterranean Lacertidae (SEVA, 1982; CARRETERO & LLORENTE, 1993a). Although the sample is small, the results indicate the importance of daily variations. In summer, midday is not only the hottest period but also the driest. The humidity could be a stimilus to maintain the activity in unfavourable temperatures, especially at the begining of day when dew is drunk (HEATWOLE & TAYLOR, 1987). Conversely, the water loss is an associated cost to activity when the temperatures are favourable in helio- and eurythermal reptiles (BOWKER, 1993).

The slope of the regression line points out how much TB depends on the environmental temperatures, TA or TS (see HUEY & SLATKIN, 1976). In the case of *P. algirus*, TA, or the solar radiation, is the main (not the only) influence of TB. This confirms this species as heliotherm (AVERY, 1979). However, TB of *A. erythrurus* depends equally on the air and substrate temperatures. In both species but especially in the second one, tigmothermia appears as another compatible behaviour particularly in open areas (PÉREZ-MELLADO, 1992). In this case of *A. erythrurus*, a special tactic is followed (SEVA, 1988), increasing TB gaining heat from substrate inside the burrow before going out and reducing the vulnerability during the basking period and the water loss (see also NAGY, 1991). However, the heat conductance from the substrate does not have a significant role in areas with a dense vegetal coverage as the Mediterranean forests, where *P. algirus* appears to be strictly heliotherm (CARRASCAL & DÍAZ, 1989; DÍAZ, 1991, 1992).

The thermoregulatory efficiency showed by both species was similar. This contrast with the results of POLLO & PÉREZ-MELLADO (1989) who consider *A. erythrurus* to be more independent of TA and a good thermoregulator.

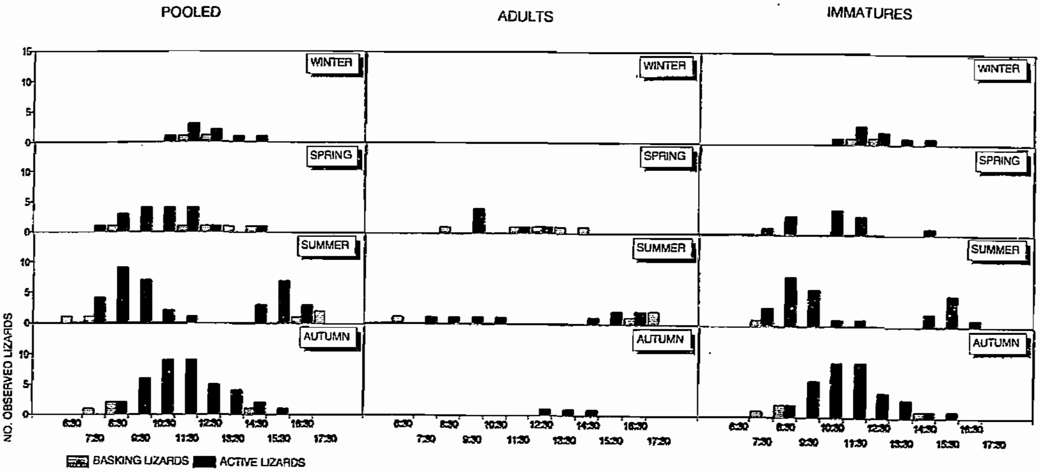

Figure 10: Daily activity considering seasons and classes in *A. erythrurus*.

Other studies corroborate these conclusions in the genus *Acanthodactylus* (BUSACK, 1975; POUGH & BUSACK, 1978; PÉREZ-MELLADO, 1992). However, other authors (Seva, 1984; CARRETERO & LLORENTE, 1991) even indicate *P. algirus* in the Mediterranean coast to be the more efficient than *A. erythrurus*. It is not necessary to invoke physiological differences to explain these changes. The buffer effect of the sea in the temperatures, prevent coastal areas from reaching extreme temperatures. Consequently, no high temperatures are reached and lizards are not found in hipothermia (see results) as in the continental climate. When the thermal stress decrease it is not strange that the high thermoregulatory potentiality does not become apparent (see MALHOTRA & THORPE, 1993). Nevertheless, *A. erythrurus* is inactive at low environmental temperatures while *P. algirus* remains active devoting most of its time to insolation (see results).

The slight tendency in the adults to thermoregulate more than immatures could be explained in terms of ethological or physiological differences (see CARRETERO & LLORENTE, 1993a). The ecological consequence is that immatures are more thermoconformers (and generally these TBs are lower) than adults. In comparison to adults, the growing lizards invest more in seaching for food than in thermoregulation (see AVERY, 1984; CASTILLA & BAUWENS, 1991). It has been demonstrated (LEE, 1980) that thermoregulatory efficiency depends on the physiological considition of the animal (fat reserves) adjusting to a cost/benefit model (HUEY & SLATKIN, 1976; HUEY, 1982). Annual results show than a long-term thermoregulation may take also place (see DAMME *et al.*, 1987; CARRETERO & LLORENTE, 1993a). A different range of body temperatures is the main way of thermal modulation in the lizard species of the Ebro Delta (AVERY, 1978; BOWKER *et al.*, 1986; HERTZ, 1992). It has been considered to be more conservative than the variation of thermoregulatory efficiency (AVERY, 1982; GILLIS, 1991). The latter is more dependent on the environment and both species present changes at geographical level.

The activity of the diurnal lizards in temperate regions is highly dependent on temperature since favourable periods are scarce (GRANT & DUNHAM, 1988). However, it should always be considered as a result of a compromise between positive (feeding, drinking, reproduction) and negative (predation, competition) pressures (HUEY, 1982; PIANKA, 1986; HEATWOLE & TAYLOR, 1987). Compared to other temperate regions, Mediterranean ecosystems are warm enough allow different strategies of activity in the same environment. This is an example. *Psammodromus algirus* remains active all the year explointing the scarce resources in winter (CARRETERO & LLORENTE, 1993b). This has been recorded on the Mediterranean coast and in the South of Spain (AMORES *et al.*, 1980; SEVA, 1984; MELLADO & OLMEDO, 1987; CARRETERO & LLORENTE, 1991). However, this species shows a diapause period in zones under the continental influence (MELLADO *et al.*, 1975; PÉREZ-MELLADO, 1982, POLLO & PÉREZ-MELLADO, 1989) in association with extreme temperatures in winter. Immatures are usually the only individuals active in this season, while adults remain inactive following a more conservative strategy (maybe under physiological control). The early appearance of males seems to be general in those members of the familiy Laceridae which have a winter diapause (NULAND & STRIJBOSCH, 1981; NICHOLSON & SPELLERBERG, 1989; SALVADOR, 1987) and it is thought to be in relation to territorial marking. The larger number of individuals was found, as expected, in the periods of reproduction and new-births. The postreproductive decrese in summer has been explained as a way of escaping predation (ROSE, 1981; ETHERIDGE & WIT, 1993) and avoiding a dry, resource scarcity period (FUENTES, 1976). *Acanthodactylus erythrurus* follows a more conservative strategy, especially marked in the adults. In the Northern boundary of its range, the activity patterns must be adjusted to the environmental constraints. So, the diapause has been detected in other Northern areas (POLLO & PÉREZ-MELLADO, 1989) but not in warmer localities (BUSACK, 1976; BUSACK & JACKSIC, 1982; SEVA, 1984). Nevertheless, the interruption of activity is longer in this species, as a consequence of physiological limits because the Fringe-toed lizard stops feeding at low temperatures (BUSACK, 1976). Its high voluntary temperatures could also produce energetic and hydric deficits that could only be balanced when there is abundance of food (Bowker, 1993; ETHERIDGE & WIT, 1993). The interpretation on the maximum of activity is the same as in the former species with a slight delay in the first one. The patterns of daily activity registered here are those typical of the diurnal lizards in warm-temperate zones (AVERY, 1978; ADOLPH & PORTER, 1993): bimodal in the warm season and unimodal the rest of the year. Thermoregulation appears in the coldest hours (see DÍAZ, 1991, 1992; CARRETERO & LLORENTE, 1993b). However, two comments have to be made.

1-The activity of *P. algirus* is not clearly bimodal possibly due to its capacity to use shaded microhabitats of plants (see Seva, 1984; CASAS-ANDREU & GURRIOLA-HIDALGO, 1993) if the summer temperatures are moderate. However, a marked bimodal pattern appears in the places where the summer is very hot (CARRASCAL & DÍAZ, 1989; POLLO & PÉREZ-MELLADO, 1989).

2-Basking was rarely observed in *A. erythrurus* because thermoregulation begins inside the burrow (see above). The behaviour is not rare in desert lizards (MCGINNIS & DICKSON, 1967) and confirms the conservative condition of this species.

The activity pattern of inmmatures in comparison to adults seems to be general (see CARRETERO & LLORENTE, 1993b for an extensive discussion). Their high surface/volume index in comparison to adults (HAILEY, 1982) and some ethological constraints can produce the same result. As seen above, immatures behave as opportunists and increase the range of activity devoting less time to thermoregulation and more to feeding. When growing is not so important both are inverse (AVERY, 1984; CASTILLA & BAUWENS, 1991). Other possibilities such as the minimization of competence or predation of the conespecific adults (MELLADO *et al.*, 1975; ROSE, 1981) cannot be ruled out *a priori*.

Hence, not only a thermal explaination, but also a general energetic model of costs and benefits should explain the activity strategies of lizards in Ebro Delta. This model should consider lizards under pressure from two opposite forces: on one hand, the energy demands for maintenance, growing and reproduction; on the other hand, the biotic (competence, predation) and abiotic constraints (STEPHENS & KREBS, 1986).

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Age and size stucture of some populations of the lizards *Lacerta agilis boemica* and *L. strigata* from Eastern North Caucasus

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Abstract: Adult and subadult specimens (N=155) from a high-mountain population of *L. agilis* and a foothill population of *L. agilis* and sympatric *L. strigata* from Daghestan were measured for snout-vent length and aged by counting annual layers in their femoral bones. In the foothill populations about 60% of adult males and 80% of adult females were 2-yr-old animals while lizards surviving more than 3 winterings made up 0-20%. Age composition of the high-mountain population was substantially different, being skewed to older age groups. Maximum age for the samples studied was 6-7 years. In L.agilis the adult females from the high-mountain population were on an average larger than those from the foohill population that can be largely attributed to the differences in age composition.

Key words: *Lacerta agilis*, *Lacerta strigata*, population age structure, growth rate.

INTRODUCTION

The technique for age determination in amphibians and reptiles based on counting annual layers in their bones has been successfully used in many herpetological studies (see references in CASTANET, SMIRINA, 1990). However for European lizards, data on the population age structure are still scarce, particularly for Caucasian taxa. This preliminary paper presents data on age and size composition of three populations of *Lacerta agilis* and a related sympatric species, *L. strigata* from the eastern North Caucasus.

L. agilis is a widespread species distributed over much of the temperate zone of the Palearctic; in the eastern North Caucasus it is presented by a distinct subspecies, *L. a. boemica* (BISCHOFF, 1988). *L.strigata* inhabits the eastern Caucasus with adjacent parts of Turkey and Iran (DAREVSKIJ, 1984).

MATERIAL AND METHODS

Samples from the following three *Lacerta* populations were examined: I- *L .agilis*, Kuli (high-mountain Daghestan, 42 00'N, 47 15'E), 1900 m above sea level; II- *L. agilis*, Sergokala, (foothill Daghestan, 42 30'N, 47 40'E), 600 m a.s.l.; III- *L. strigata*, Sergokala, the same collection site as for *L .agilis* (II). Sample I was collected during August 1989 and July 1990, and samples II and III during 11-14 June 1982.

In total, 155 specimens were used in this study. All the lizards were measured for snout-vent length (SVL). The adult specimens (N=81) and a few largest subadults were examined foraging by counting numbers of visible resting lines (LAGs) on transversal sections of the middle part of the femur diaphysis. Diameters of annuli were measured with ocular-micrometer under a light microscope. As the contours of the bone sections deviate from a circle, means of the minimal and maximal diameters of every annulus (measured in three sections from every specimen) were used to estimate an annual increment of bone width.

For nearly all the femurs examined, LAGs were well defined providing precise age estimation. Only two femurs gave some difficulties in age estimation but the possible error did not exceed 1 year.

RESULTS

Age and size structure

Age distributions in the samples of adults from the three lizard populations are presented in Fig.1. It shows clearly that the high-mountain *L . agilis* population differs from both the foothill populations by a large proportion of older animals. For the females, the ratio of 2-yr-old animals to 3-and-more years old animals differs significantly between samples I and II (X^2=12.97, df=1, P<0.001) and between samples I and III (X^2=10.59, df=1, P<0.01).

Size (SVL) distributions of the samples studied are shown in Fig.2. It exhibits a rather clear separation of the yearlings from the adults (2-and-more years old lizards) for SVL in the samples of *L. agilis* and sympatric *L. strigata*

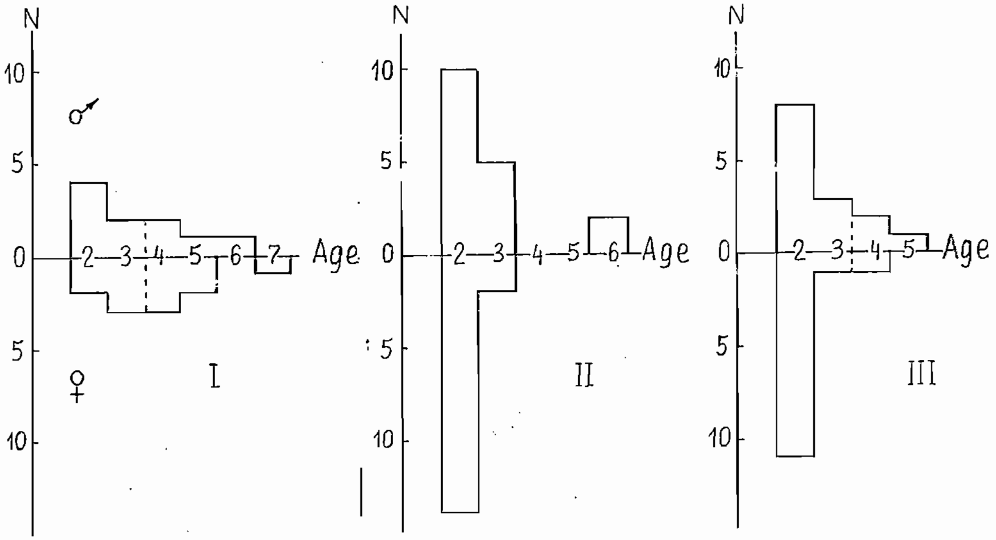

Figure 1: Age distributions for the samples of adults from three Lacerta populations.

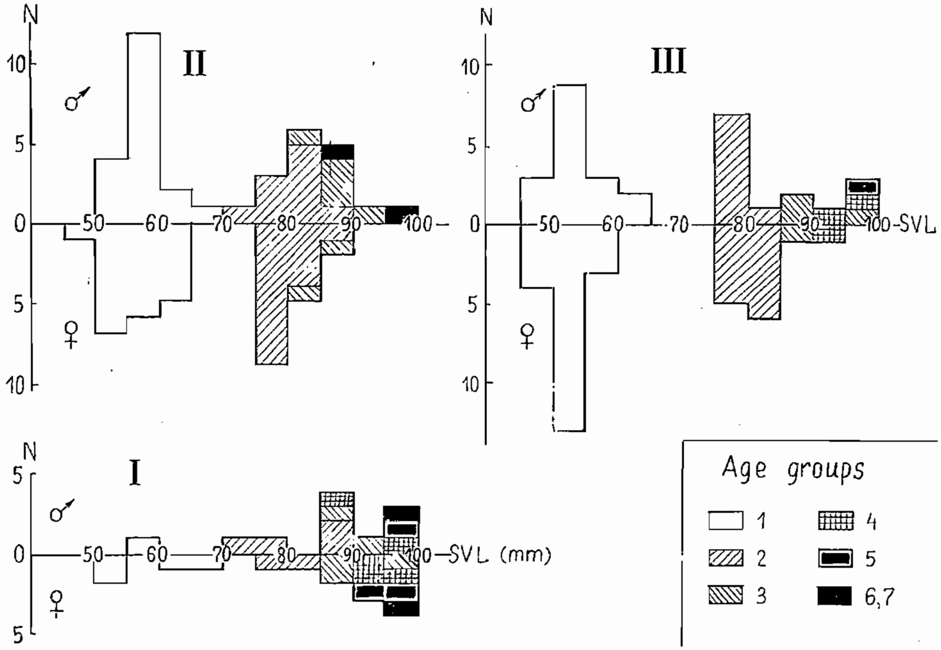

Figure2: Size (SVL) distributions for the samples from three Lacerta populations. (Age groups are indicated by different shadings).

collected in early summer (11-14 June 1982).Fig.2 also indicates a relatively small proportion of yearlings in the high-mountain *L. agilis* population, as compared to the foothill populations. The high-mountain population is also characterized by a relatively high percent of larger animals (L>90mm)(Fig.2); when the sexes are combined, this difference is significant at $P< 0.001$ ($X^2=15.07$, df=1) between samples I and II, and at $P<0.01$ ($X^2=7.08$, df=1) between samples I and III.

	L. agilis Kuli	*L. agilis* Sergokala	*L. strigata.* Sergokala
♂	0.882 (n=10)	0.879 (n=17)	0.975 (n=14)
♀	0.972 (n=11)	0.806 (n=16)	0.852 (n=13)

Table 1. Correlation (r) between SVL and femur diaphysis diameter.

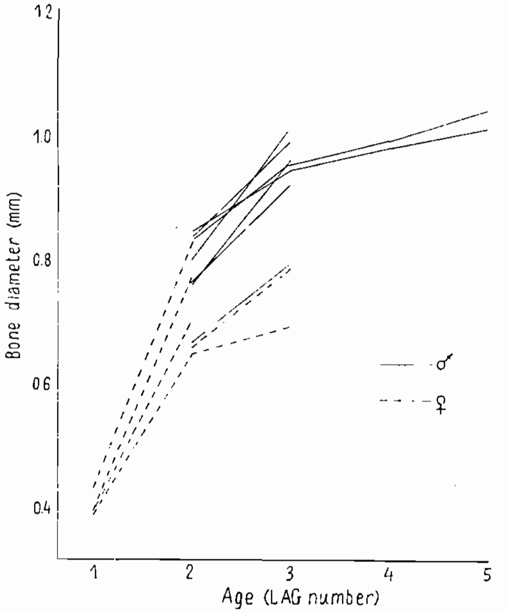

Figure 3: Annual increases of thickness of the femoral bone in individual specimens of *L. agilis* from Kuli.

Relationship between bone diameter and SVL

Correlation between diameter of the femur at the diaphysis level and the SVL is consistently higher than 0.8 (Table 1). Consequently, results for the growth rate of the femur thickness can serve as a rather good estimation of the body growth rate. Such a high correlation between the tubular bones thickness and SVL has also been demonstrated within several taxa of the lacertid genus *Gallotia* (CASTANET, BAEZ, 1991). Similar result was also obtained for a toad, *Bufo bufo* (SMIRINA, 1983) and a frog, *Rana temporaria* (RYSEN, 1988 in CASTANET, BAEZ, 1991).

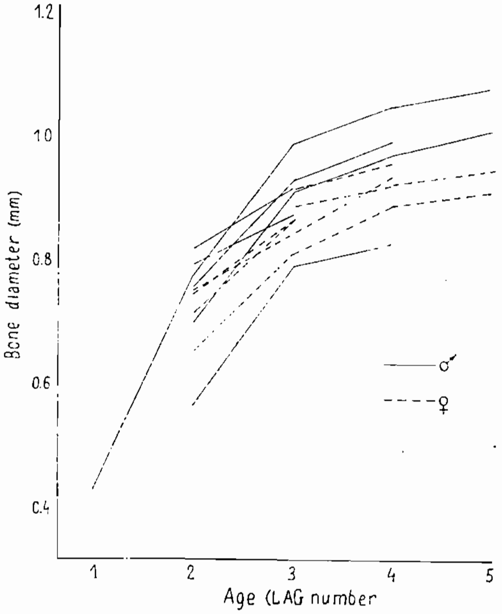

Figure 4: Annual increases of thickness of the femoral bone in individual specimens of *L. agilis* from Sergokala

Growth rate

Individual growth rates as expressed by annual increments of femoral bone thickness in the three *Lacerta* populations are presented in Fig.3, 4, and 5. As can be seen from these figures,individual growth curves show no clear differences between the populations studied. These data suggest that a higher proportion of larger adults in the high-mountain *L. agilis* population (Fig.2) can be attributed rather to its high longevity than to differences in growth rate.

According to our data, males tend to grow somewhat faster than females. Annual growth increments are largest for the period between 1st and 2nd winter. Then growth becomes slower, especially in lizards surviving 3-and-more winterings (Fig.3-5).

In all three populations under study many individuals had double resting lines. For females such individuals were more frequent in *L. agilis* than in *L. strigata* ($p<0.05$; $X^2=4.9$ and 6.2 for

Subspecies	age (number of survived winterins)					
localities and samples	2	3	4	5	6-7	8-11
L. a. boemica						
(1) Kuli, highland Daghestan, N=21	28.6	23.8	23.8	14.3	9.5	-
(2)Sergokala, foothill Daghes.,N=33	72.7	18.2	-	-	6.1	-
L.a. chersonensis						
(3) Uman, Ukraine, N=42	66.7	23.8	9.5	-	-	-
L.a. exigua						
(4) Locality 1, N=282	46.5	40.7	9.9	2.9	-	-
(5) Locality 2, N=267	38.2	44.2	13.1	4.5	-	-
L. a.. agilis						
(6) Nijmegen, Netherlands, N=498	38.0	29.5	13.5	8.6	6.4	4.0

Table 2: Age frequency distribution (%) in the samples of adult specimens (sexes combined) from different populations of *Lacerta agilis*. Sources of data: (1,2) ours, (3) SMIRINA, 1974, (4) TURUTINA, 1977, (5) STRIJBOSCH & CREEMERS, 1988. Method: (1-4) bone layers counting, (5) a long-term mark-recapture study.

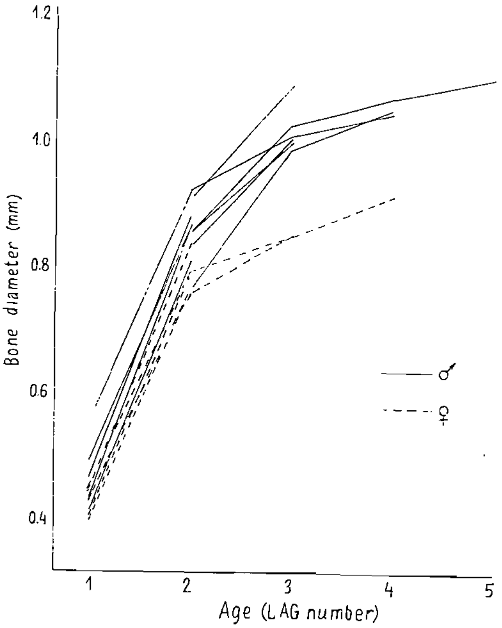

Figure 5: Annual increases of thickness of the femoral bone in individual specimens of *L. strigata* from Sergokala.

comparisons between samples I and III, and between samples II and III, respectively).

DISCUSSION

Higher mean longevity and body size found in the high-mountain *L. agilis* population as compared to both the foothill populations of *L. agilis* and *L. strigata* is in accordance with data on *L. strigata* from Armenia where high-mountain and foothill populations have been contrasted (MELKUMYAN, 1983; LEDENTSOV, MELKUMYAN, 1987). This trend was also shown for high-altitude or northern populations of other lizards (BALLINGER, 1979), newts (e.g. CAETANO, CASTANET, 1993), anurans (e.g. ESTEBAN, 1990; ISHCHENKO, LEDENTSOV, 1993). For body size, however, the opposite tendency (larger SVL at a lower altitude) was also reported for a frog (Rana macrocnemis) and a lizard (*Stellio caucasius*) (LEDENTSOV, MELKUMYAN, 1987).

Table 2 contrasts our data on the age

composition of the North Caucasian populations of *L. agilis* with the data available from a few other *L. agilis* populations. As can be seen from Table 2, a common feature of the presented age compositions is that most of adults are 2 or 3 years old animals, individuals older than 5 years being rather rare. It seems notable that besides the high- mountain Daghestan population the population from the Netherlands is also characterized by a higher proportion of older individuals (Table 2).

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Seasonal activity of the sand lizard (*Lacerta agilis*) and the common lizard (*Lacerta vivipara*) in an experimental outdoor enclosure

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Abstract: A five year field study in a mixed population of Lacerta agilis and *Lacerta vivipara* showed that year-to-year variation in annual activity decreases with increasing age of the lizards. There is a tendency for seasonal time sharing between adults and juveniles. A high degree of interspecific congruence in the presence of both species during the months of main activity could be observed in adults.

Key words: temperate lizards, seasonal activity, intraspecific, interspecific and year-to-year variation

INTRODUCTION

Although a great deal of field research on the two widespread lizards *Lacerta agilis* and *L. vivipara* has been carried out in the last 10-15 years only few quantitative data on seasonal activity of the two species are available (SAINT GIRONS 1976, NULAND & STRIJBOSCH 1981, NICHOLSON & SPELLERBERG 1989, HERMANS 1992, LENDERS 1992).

A five year study (from 1983 to 1987) on field biology of *L. agilis* and *L. vivipara* in a population introduced experimentally into a large outdoor enclosure (GLANDT 1987, 1988, 1991) gave quantitative data on the annual rhythm of activity, which are presented here.

MATERIALS AND METHODS

The semi-natural field enclosure of about 4000 square metres is situated at Metelen/Germany (7°07'/52°09'). It has a dense grass dominated vegetation interspersed with patches of bare ground and with single bushes and low trees (for details see GLANDT 1988 and 1991).

A total of 1905 sightings were made during the five year study: 1168 *Lacerta agilis* sightings (juveniles 218, subadults 295, adults 655) and 737 *Lacerta vivipara* sightings (juveniles 231, subadults 149, adults 357). Juveniles were defined as specimens in the year of hatching, adults as specimens which were at least in their third year of life and with fully developed secondary sexual characters (all *L. agilis*, several *L. vivipa*ra) and corresponding body length (most *L. vivipara*), respectively. Subadults were defined as specimens in the second year of life.

Because adults and subadults were not captured (in order to avoid too much disturbance), sex recognition in *L. vivipara* was not possible in several cases. Therefore only in adult *L. agilis*, in which sex recognition is very easy without capture, are females and males considered separately.

In most cases the sightings were made when lizards were "basking" or "sitting in the sun", but some were made when animals were "moving around" (for definition of these types of behaviour see HOUSE *et al.* 1980).

RESULTS

The data obtained are summarized in figure 1. In adult males of *L. agilis* high activity was observed from April to June with only small differences from year to year. Activity then decreased markedly. High activity from April to August could be observed in adult females of *L. agilis*, with a tendency to low levels in September.

In adults of *L. vivipara* the main activity period lasted from May to July. Activity then decreased markedly.

Juveniles of both species could be observed mainly in August and September (with a few last observations in October). The monthly levels of activity differed considerably from year to year. Subadults of both species also showed great year-to-year variation in seasonal activity.

Of the three age groups distinguished, only in the adults was a clear pattern of rhythmical activity recognizable. The younger the animals, the greater was the year-to-year variation of seasonal activity.

DISCUSSION

As a generalization the activity of the adults of both species is very low in September and - with the exception of female *L. agilis*- in August. On the other hand these two months are

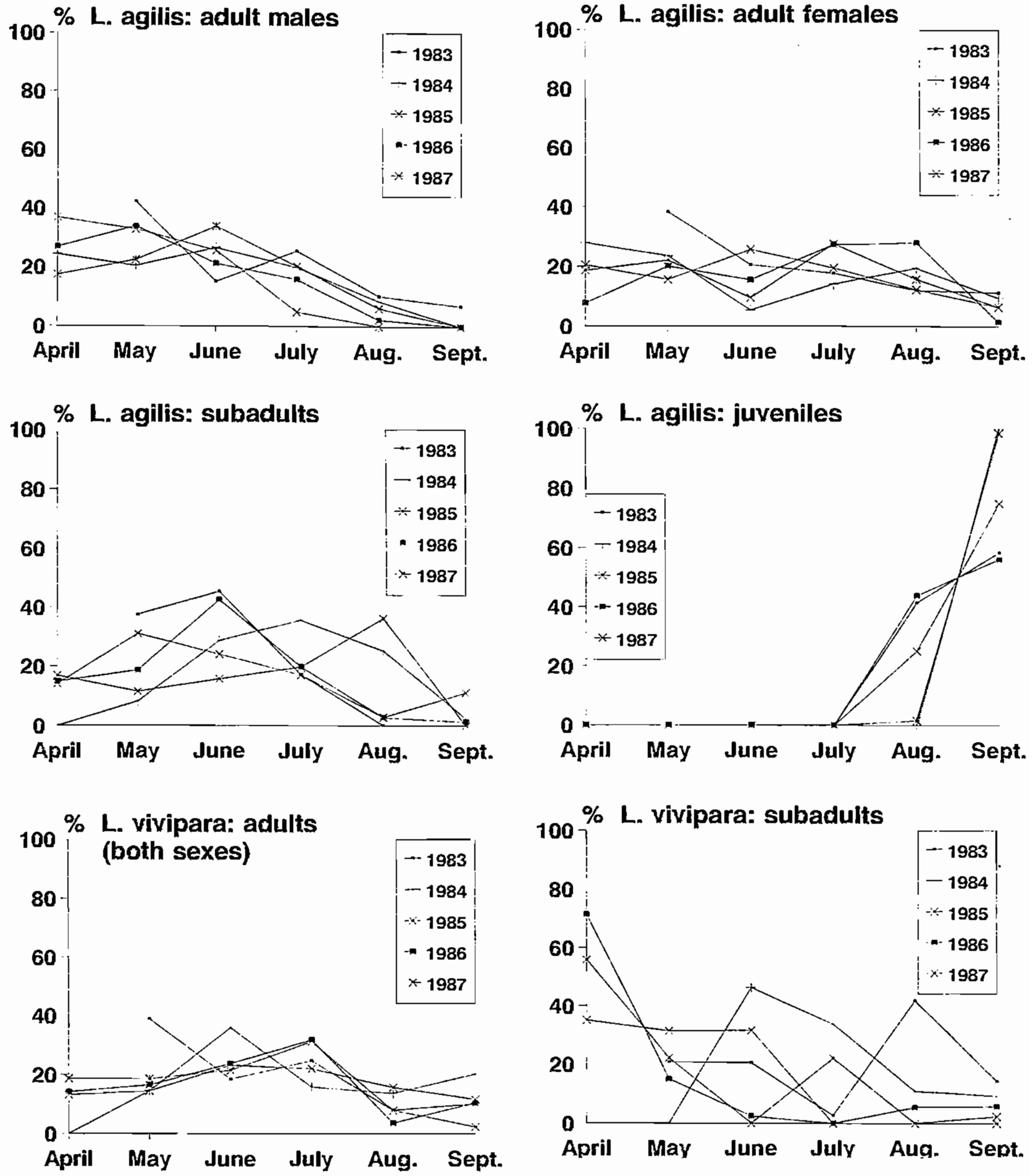

Figure1: Seasonal frequencies of the sightings of *L. agilis* and *L. vivipara* in the Metelen outdoor enclosure during five consecutive years (1983-1987). The graphics show `lizards seen per man hour' for each year separately expressed as a percentage.

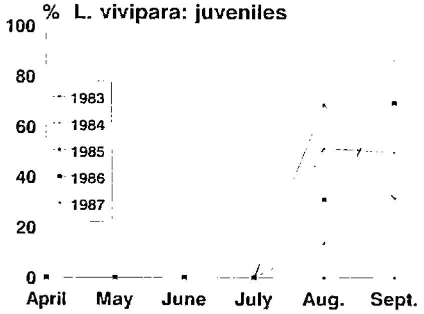

Figure1 (cont.): Seasonal frequencies of the sightings *L. vivipara* juveniles

the main time of presence (after birth and hatching, respectively) of the juveniles. Therefore I suppose that the seasonal time sharing or "seasonal allochrony" (PAULISSEN 1988) between adults and juveniles evolved in order to avoid cannibalism and interspecific predation, respectively.

A further important result is the observation that year-to-year variation of annual activity becomes lower during growth, being smallest in adult males of *L. agilis*. It may therefore be concluded that after the attainment of sexual maturity seasonal activity seems more determined by an endogenous mechanism than by the annual weather conditions (for evidence of an endogenous seasonal rhythm in the iguanid lizard Sceloporus virgatus see STEBBINS 1963).

Finally an important result is the observation of a high degree of interspecific congruence in the presence of adults of both species from May to July. In addition to the high niche overlap of microhabitat selection, especially in preferred vegetation structure (GLANDT 1991), and the quite similiar course of adult population number during the investigation period (GLANDT 1988), this fact underlines the obviously low importance of interspecific competition between the two species. SAINT GIRONS (1976) comes to the same conclusion with respect to the interspecific relation between *L. agilis* and L. viridis.

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Population structure and displacements in *Lacerta vivipara*

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Abstract: In an isolated ridge of inland dunes with a patchy habitat structure for lizards the migration rate in a metapopulation of *Lacerta vivipara* was studied. One of every three lizards proved to migrate at least once in its life. Most of these migrations occurred within one patch of suitable habitat, but at least 10% of the animals also traversed unsuitable habitats. In all cases emigration and immigration were balanced. Migration occurred also frequently in the older age classes. In all migration types the males formed the dominant group.

Key words: *Lacerta vivipara*, migration, metapopulation, genetic exchange

INTRODUCTION

Lacerta vivipara has the biggest distribution area of all European lizards. Moreover, in large parts of this area it is the only reptile species present, especially in the western and northernmost parts. In the more southern parts it nearly always restricts itself to high mountain ridges, where there are no or only few other reptile species. Obviously, this species can survive where it is too hard to live for other lizards. These regions mostly have only small patches of suitable lizard habitat. More than most other lizard species *L. vivipara* therefore often lives in small populations, separated from others by large areas of lizard-unfriendly terrain. Based on these facts one might suppose a great migration capacity in this species.

We studied population structure and displacements in this species in an isolated ridge of inland dunes near Nijmegen in the Netherlands. This area has two habitat types suitable for this species, viz. moist heather vegetations around moorland pools in the dune valleys and dry, open dune tops (STRIJBOSCH 1988). Patches of these habitats are present scattered over the whole ridge, separated by the waters of the pools, by dense woods or intensely used cultivated lands.

MATERIAL AND METHODS

In a pilot study we mapped all places where the lizards were present in the whole dune ridge. In total we found 44 more or less isolated places with lizards, with a mean nearest neighbour distance of 195 m (range 25 - 925 m). Based on this map we selected an area of 40 ha for a more detailed study. All terrain types were present there, suitable ones as well as unsuitable ones, and also 5 small populations of common lizards, at various distances from each other (Figure 1).

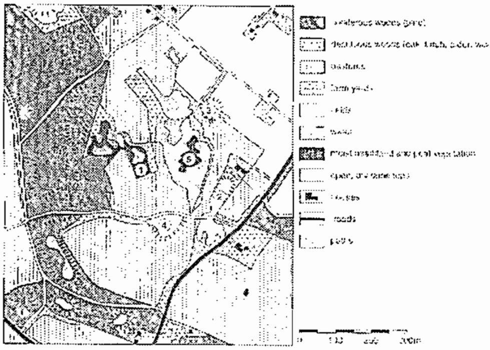

Figure 1: Study area with subpopulations 1-5.

Here we call them subpopulations, because we found a certain exchange of animals between them. Two of them (1 and 2) had fairly high numbers of lizards in the various years of study, the others only low numbers. In the course of 6 years we made 4685 captures there, involving 1048 individually marked lizards. Now special attention is paid to those captures, which reveal real displacements of an individual lizard. We speak of a real displacement when one animal is captured at two or more places at an interjacent distance of at least 25 to 30 m, i.e. two times the diameter of the average home range size of this species in this area. In his study into movement patterns of this lizard in France, also MASSOT (1992) took 30 m as an indication of displacement. All distances given here are distances covered as the crow flies.

As the age of a migrating specimen we took its age when captured for the first time after the displacement.

RESULTS

Based on the movement patterns found the lizards could be classified into five separate groups, viz.:

1) stay-at-homes:

these animals were captured only within the borders of their own home range, so they stayed within one habitat type as well as within one subpopulation. Using the number of animals captured more than once (i.e. 748), the relative number of stay-at-homes was 69%, so, crudely spoken, a good two thirds of the total population. However, it should be borne in mind, that this is a maximal figure, viz. when the animals did migrate after their last capture, this displacement is ignored by this method.

2) movers:

these animals moved over relatively larger distances, but still stayed in one habitat type and in one subpopulation. Within the biggest subpopulation (= sp 1) such displacements were seen in about 19% of the animals, viz. 18.7% of the animals of the southeastern part moved to the more or less separated northwestern part, while the reverse was observed in 19.0% of the animals. These figures should be seen as minimal, because they are calculated on only those animals, which were captured more than once. As subpopulation 1 is by far the biggest subpopulation we assume that this figure of nearly 20% is a good estimation for the whole population.

When moving sometimes small barriers must be traversed, for instance a broad, sandy path. We studied this moving type in subpopulation 2, which is separated in two parts by such a path. Here we found 25 of such displacements, involving 18 individual lizards (1 juvenile, 6 subadults, 6 females and 12 males; some of the males showed more movings per individual). Distances covered varied between 25 - 80 m, with an average value of 47 m. Figure 2a shows these movings per age class, sex and month. From this it is obvious, that moving occurred more in adults than in younger animals and more in males than in females. Furthermore the males especially moved in the month of April, i.e. early in the mating season.

3) vagabonds:

Vagabonds are animals, which migrate temporarily to small, only moderately lizard-friendly patches outside the living space of their own subpopulation, hereby traversing lizard-unfriendly habitats. This is an intermediate group between the movers and the following class, the real settlers, viz. like the movers they remain members of one subpopulation, but like the settlers they migrate through lizard-unfriendly terrain. The distances covered varied between 25 -230 m, with a mean of 80 m (n=24). Now it should be borne in mind that distance alone is not the only point, because also the degree of unsuitability of the terrains covered is strongly determining. Vagabondism was found 24 times, involving 16 individual lizards, i.e. a minimum of 2.1% of the population of animals caught more than once; in these there is one male, that travelled 6 times. Figure 2b shows, that in the males this type of displacements mainly took place in the month of May, i.e. late in the mating time and clearly later than the movings.

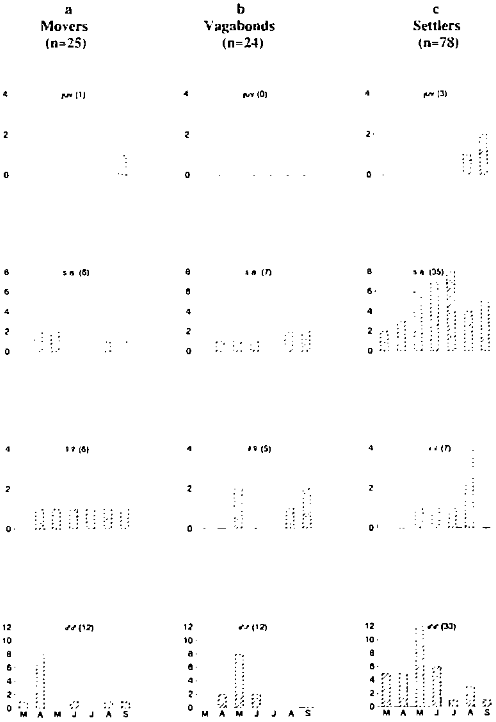

Figure 2: Numbers of migrators per age class, sex and month.

4) settlers:

these animals moved from one subpopulation to an other, hereby traversing considerable barriers of lizard-unfriendly habitats, formed by waters, intensely trodden pastures or dense woods. In some cases they later did return to their original subpopulation. In this type of

	1	2	3	4	5
2	201				
3	213	154			
4	166	60	243		
5	202	102	82	-	
total	185	125	175	118	122

Table 1: Mean distances (in m) covered by settlers between the various subpopulations.

migration it comes to genetic exchanges between separated subpopulations. For the subpopulations apart it means emigration and immigration.

This type of migration was found 78 times, involving 65 individual lizards, i.e. 8.7% of the animals caught more than once. The distances covered varied from 50 - 300 m, with a mean value of 161 m; table 1 gives the mean distances covered by the displacements between the different subpopulations. Figure 2c shows that also this displacement type occurs more in males than in females and that in the males it again has a peak in the month of May (= late in the mating season). More detailed analysis of the age of these males revealed, that especially the fourth calendar year animals, so the older males, took part in this migration type. In the females there seems to be a preference for the parturition period, but our figures are too small to prove this; yet we saw a female of subpopulation 1, that, being pregnant, migrated to subpopulation 4, where it gave birth to a clutch of young; some days later it remigrated to subpopulation 1.

The settler type of migration also occurred frequently in the younger age classes; here it means real dispersal movements, occurring especially in the second calendar year animals, when they grow to sexual adulthood. In our study area the clusters of juveniles disperse especially in late spring and early summer of their second calendar year, when also the external sexual characteristics become visible. This in contrast with the dispersal data of MASSOT (1992) from France.

Table 2 gives a survey of the numbers of emigrants and immigrants. It is clear, that these numbers are neatly balanced in each subpopulation. Table 3 shows the exchange percentages for each subpopulation. So, crudely spoken, in subpopulation 1 one out of 8 animals ever had contacts with those of another subpopulation, for sp 2 this was one out of 6, for sp 3 one out of 4 and for sp 4 even one of every two animals.

		Number of immigrants					
		1	2	3	4	5	Total
Number of emigrants	1	-	11	2	6	0	19
	2	5	-	6	13	2	26
	3	2	6	-	1	1	10
	4	12	8	2	-	0	22
	5	1	0	0	0	-	1
	Total	20	25	10	20	3	78

Table 2: Numbers of emigrants and immigrants in the various subpopulations.

	1	2	3	4	5
1	-	5.6	4.6	20.0	10.0
2	5.0	-	14.8	23.3	20.0
3	1.3	4.2	-	3.3	10.0
4	5.6	7.3	3.7	-	-.-
5	0.3	0.7	1.2	-.-	-
total	12.2	17.8	24.6	46.6	40.0

Table 3: Relative frequency of contacts between the various subpolations. S = sum total, giving the minimal percentage of settlers in each subpopulation.

5)long distance pioneers:

This class contains those animals, which left the population studied or immigrated from other places. Both cases are extremely hard to detect, so our figure for this class should be seen as an absolute minimum. Emigration was recorded 3 times in the course of 6 years of study, in all 3 cases to little pieces of lizard-friendly habitat in the southwestern part of the total study area (cf. Figure 1). All three emigrants were young males, two second calendar year animals and one in its third calendar year. The distances covered all exceeded 250 m. We suppose also immigration occurred, because sometimes we found totally unmarked adult specimens at places, where already for years all animals had been caught and marked. In the course of this study we captured and marked a total of 60 individual lizards in the three little pieces of lizard-friendly habitat in the southwestern corner of the study area, but none of these were ever seen in subpopulations 1-5.

So the relative proportion of real emigrants minimally will be 0.4%. When in this class emigration and immigration are balanced too, the class of long distance pioneers will contain some 1% of the animals, so, crudely spoken, only one out of every 100 animals.

DISCUSSION AND CONCLUSIONS

With regard to other studies on this species (e.g. BAUWENS & THOEN 1981; HEULIN 1984; LECOMTE 1992; MASSOT 1992) our relatively low numbers of migrating younger animals are striking. This difference can be attributed partly to the method used, viz. we always determined the age of migrators just after the finding of a displacement. Thus a number of the displacements found possibly took place actually at an earlier date, i.e. at a younger age. Yet, we think this is not sufficient to explain all the difference found. Furthermore, most migrators in our study already had a relatively high age before they showed their migration.

It is striking, that in all displacement types the males form the dominant group. Furthermore it is clear, that the moving type of displacements takes place earlier in the season than what is called vagabondism and settling here. In this species the males actively search for females in the mating season and therefore searching for mates maybe forms a motivation for a good part of the displacements found in spring. One might hypothesize that the males start the search for females in their direct surroundings, leading to a peak of movings in the first part of the mating season, i.e. in April. After this period they may have to cover longer distances to find still willing females, making them vagabonds or even settlers in the later phases of the mating season, i.e. in May. In this also the higher temperatures in May compared to those of April can play a role, allowing the lizards to cover larger distances.

Looking at table 3 one might suppose, that subpopulations 3 - 5 exist by the grace of the bigger ones (1 and 2). This supposition is strengthened by huge fluctuations in numbers in these small subpopulations in the various years of study.

Quantifying the presence of long distance pioneers proved to be the most difficult part of this research. We spent a lot of investigation effort searching for marked specimens in the surroundings of the population studied. But then these animals provide for possible contacts between all the distant populations in the whole dune ridge. Therefore, here it is better to speak of one big population (metapopulation), which is strengthened when comparing the data of table 1 with the nearest neighbour distance found in the pilot study (195 m).

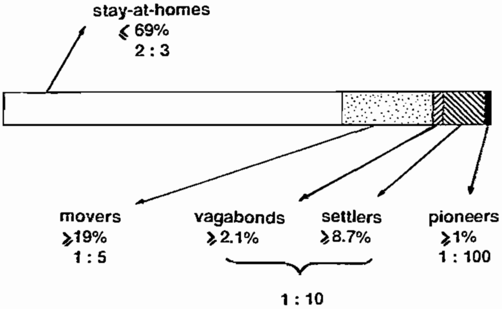

Figure 3: Population structure.

Figure 3 shows the structure of the population studied. From this one may conclude, that here maximally 2 out of every 3 animals live a more or less stationary life, that minimally 1 of every 5 animals ever moves to other places within one patch of suitable habitat, thus providing genetic exchanges within one subpopulation, that at least one of every 10 animals migrates or tries to migrate through less suitable environments, and that at least one of every 100 animals participates in real long distance dispersal. In all these displacements the males obviously play the principal part. Also in the younger age classes the number of participating males is dominant, despite the fact that searching for potential mates will not play a role here.

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Estimation of body reserves in living snakes using a Body Condition Index (BCI)

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Abstract: Using a body condition index (BCI), we show that the level of body reserves such as the mass of fat bodies and the mass of the liver can be estimated with a good precision in nongravid female *Vipera aspis* and *Coluber viridiflavus*. Wild population of adult female *Vipera aspis* include potentially reproductive females with abundant body reserves (BCI=0.70), intermediate non-reproductive females with moderate body reserves (0.52¾BCI<0.70), and emaciated females with hardly any body reserves left (BCI<0.52). The mean BCI of postparturient females is 0.52, indicating that almost all body reserves are used during reproduction. To investigate the relationship between BCI and body reserves in these three categories of individuals, we have carried out statistical analyses on 72 dissected female *V. aspis* which died accidentally. The mass of the fat bodies, the mass of the liver, and the mass of the carcass show significant differences among the three groups. In potentially reproductive females, BCI correlates strongly with the mass of the liver. In intermediate non-reproductive females, BCI correlates strongly with the mass of the fat bodies. In emaciated females, BCI correlates with the mass of the carcass, and to a lesser extent with the mass of the fat bodies. These results suggest that after parturition, a female need to go through 3 stages of body reserves recovery in order to reproduce.

Key words: Body reserves, Fat bodies, Liver, Reproduction, snakes.

INTRODUCTION

In many reptiles, maternal body reserves are known to influence reproductive frequency and/or fecundity (SAINT GIRONS 1957; MENDEZ DE LA CRUZ *et al.* 1988; BROWN 1991; ALDRIDGE 1979; DILLER & WALLACE 1984; SAINT GIRONS & DUGUY 1992). Trained herpetologists are able to evaluate the body condition of snakes subjectively, and can often predict if a female is fat or thin. However, to discriminate between the respective influences on reproduction of body reserves and other variables such as body length (BLEM 1982) or food availability (SEIGEL & FORD 1991), it is necessary to evaluate the mass of the fat bodies and the mass of the liver with a good precision in living snakes. Since 1990, inspired by a paper from LELOUP (1976), we have tested a Body Condition Index (BCI) in living snakes during the annual cycle (NAULLEAU 1992; BONNET *et al.* 1992, 1994; BONNET & NAULLEAU 1994). In *Vipera aspis* there is a reproductive threshold necessary for the induction of vitellogenesis: almost all reproductive females have a BCI=than 0.70 at the beginning of vitellogenesis (BONNET *et al.* 1992, 1994). In *Vipera aspis*, vitellogenesis starts soon after the end of hibernation in early march (BONNET *et al.* 1992, 1994). The growth of the follicles is then rapid (SAINT GIRONS & DUGUY 1992) until ovulation in first 2 weeks of June (NAULLEAU & BIDAUT 1981). Vitellogenesis is a very costly process and almost all maternal reserves are invested into the oocytes (SAINT GIRONS & DUGUY 1992). Pregnancy is long in this species and births occur from late August to October with a peak in September (SAINT GIRONS 1957). After parturition the mean BCI is 0.52 (BONNET & NAULLEAU 1994; SAINT GIRONS comm. pers.). Reproductive females cannot reproduce the following year as body reserves are too low for a further vitellogenesis (SAINT GIRONS 1957; DUGUY 1963). Thus, at the beginning of vitellogenesis which takes place after hibernation, wild population of *V. aspis* involves three classes of individuals: potentially reproductive females (high BCI=0.70), intermediate non-reproductive females (0.52¾BCI<0.70), and thin females (low BCI, with a BCI<0.52). The aim of this study was to verify if the BCI is a good indicator of body reserves in each of the 3 categories of females, and more particularly if different relationships between maternal reserves and BCI levels exist within and among the 3 groups.

MATERIAL AND METHODS

The Body Condition Index (BCI) of individuals was calculated using the formula proposed by Leloup 1976) were BCI=M/TM. M=actual body mass of the studied animal. TM, thetheoretical body mass of the studied animal is obtained from a reference table. The BCI is a measure of the deviation of the individual animals from this reference (Bonnet & Naulleau 1994).

72 females *Vipera aspis*, all of which died accidentally, were dissected to investigate relationship between BCI and main body reserves. All individuals were non-reproductive. Vitellogenic and pregnant females were excluded from this study to avoid sources of errors due to the transfer of body reserves to the growing follicles and possibly to the embryos during vitellogenesis and pregnancy. Total body length (to the nearest 0.5cm) and total body mass (to the nearest 0.1g) were recorded. The wet mass of the fat bodies, liver and carcass (muscles+skin+skeleton) were weighted to the nearest 0.1g. Means are expressed ±S.D. Comparisons between means were made using ANOVA.

RESULTS

The mean total body lengths in the 3 classes of females were not different ($F_{2,69}$=0.89, p>0.05), and were respectively 60.04±5.82cm in females with a BCI=0.70 (n=14, range 49.5-71.0cm), 58.91±4.62 cm in intermediate females (n=27, range 49.5-71.0cm), and 58.13±3.61cm, in thin females (n=31 range 49.5-64.5 cm).

Mean masses and ranges of the main maternal reserves were significantly different in the 3 groups of females (Table 1). In potentially reproductive females, maternal reserves are always abundant, intermediate females occupy an intermediate position, and in thin females body reserves are very reduced.

	Thin BCI <0.52 n = 31	Intermediate 0.52<BCI<0.7 n = 27	Reproductive BCI>0.7 n = 14
Fat Bodies (g)	1.76±1.48	7.79±4.51*	15.51±6.13*
range	0.10-4.70	1.10-18.50	8.80-31.90
conf. inter. 95%	1.23-2.31	6.02-9.59	11.98-19.03
Liver (g)	3.13±1.38	5.18±2.11 *	10.80±6.74 *
range	0.70-5.60	2.10-10.00	4.80-25.60
conf. inter. 95%	2.63-3.64	4.35-6.02	6.92-14.68
Carcass (g)	41.64±10.20	8.20±12.25*	77.49±23.85*
range	23.90-59.50	35.70-87.40	42.90-122.20
conf. inter. 95%	37.91-45.36	53.37-63.02	63.76-91.21

Table 1: Mean wet mass (± S.D.) of Body Reserves in 3 categories of female *Vipera aspis*. (Statistical difference with the preceding value * p < 0.01).

Relationship between maternal body reserves and BCI differed between the three groups (Table 2). In reproductive females BCI correlated only with the mass of the liver (p<0.01). In intermediate females we found a positive relationship between BCI and the mass of the fat bodies (p<0.05). In thin females, BCI correlated with the mass of the fat bodies (p<0.05) and strongly with the mass of the muscles (p<0.001).

Slope values obtained from the regressions between BCI and the mass of the liver in "fat" females (BCI=0.70, y=48.08x-29.28) and in intermediate females (y=15.17x-3.84) differed significantly ($F_{1,37}$=4.02, p=0.050).

Slope values of the regression between BCI and the mass of the fat bodies in intermediate females (y=41.41x-16.57) and in thin females (y=9.54x-2.09) differed significantly ($F_{1,54}$=5.06, p=0.027).

	BCI < 0.52 n = 31	0.52<BCI<0.7 n = 27	BCI>0.7 n = 14
Fat Bodies			
r	0.41	0.47	0.03
p	0.024	0.014	0.916
Slope	9.54	41.41*	-
Liver			
r	0.25	0.36	0.67
p	0.169	0.061	0.008
Slope	-	15.17	48.08*
Carcass			
r	0.62	0.18	0.47
p	< 0.001	0.378	0.093
Slope	101.15	-	-

Table 2: Correlations between Body Condition Index and body reserves in 3 categories of female *Vipera aspis*. (Statistical difference with the preceding value * p < 0.05).

DISCUSSION

In females *Vipera aspis* and *Colúber viridiflavus* BCI correlates strongly with main body reserves such as the mass of the fat bodies and the mass of the liver (Bonnet & Naulleau 1993). In *V. aspis*, as well as in other snakes, maternal body length shows great variations, and total body length correlated also with body reserves. However, BCI contributed more than BL to the variation of body reserves (Bonnet & Naulleau 1994). Thus, BCI is a good indicator of body reserves in living snakes. In wild population of female *V. aspis* we found two thresholds in BCI levels (a reproductive one,0.70, and a postparturition one,0.52) which determine three classes of individuals (potentially reproductive, intermediate and thin females). BCI correlates with body reserves within each of the three classes, but correlations are different among the groups.

In reproductive female *V. aspis*, the synthesis of the yolk precursor by the liver requires large body reserves (Saint Girons & Duguy 1992; Bonnet et al. 1994). The synthesis of vitellogenin necessitates large quantities of amino acids, stored prior to vitellogenesis mainly in the liver, and perhaps in the muscles (BONNET *et al.* 1994). The growing follicles need large amounts of lipids (SAINT GIRONS 1992; BONNET *et al.* 1994), and the fat bodies represent the major lipidic reserve (SAINT GIRONS & DUGUY 1992). However significant amounts of lipids may also be released by the liver during vitellogenesis (unpub.). The central position of the liver, both as the organ which synthetizes the precursor of the vitellus and as an important reserve of materials used during vitellogenesis, probably underlies the strong correlation between the BCI and the mass of the liver only found in potentially reproductive females.

After parturition, almost all females *V. aspis* are very emaciated, the mass of the liver was very low, fat bodies very reduced, and often only represented by the connective tissues. This may explain the weak correlation between the mass of the fat bodies and BCI in thin females. In the wild, numerous postparturient females have a low BCI (<0.52), but several non-reproductive females did too, even at the end of the feeding period (unpub.). When the BCI is less than 0.52 (range 0.29-0.51), it correlates with the mass of the carcass. Though the mass of the carcass was not adjusted to female body length, this correlation was strong ($p<0.001$). This suggests that such females started to metabolize their muscles to survive. Utilization of proteins to provide energy is known to occur in critical situation in many vertebrates, for example during long starvation periods, or when animals are stressed (GOODMAN *et al.* 1980; CHEREL *et al.* 1988), but no data are available on snakes.

Intermediate females (0.52¾BCI<0.70) were mainly represented by postparturient individuals during their first or second years after reproduction. Such females need to feed for one year or more to recover their body reserves in order to reproduce again (SAINT GIRONS 1957, SAINT GIRONS & DUGUY 1992). When BCI is between 0.52 and 0.69, it correlates with the mass of the fat bodies. This suggests that the energy provided by the preys is stored in the fat bodies, and/or invested in the growth of these females.

CONCLUSION

Reproductive female *V. aspis* have well developed fat bodies and liver at the beginning of the vitellogenesis. They invest almost all these body reserves in reproduction during vitellogenesis and pregnancy; after parturition they are very emaciated. They need to feed for one or more years to restore their body reserves in order to reproduce. In postparturient females, the energy provided by the food may be directed first to the fat bodies and/or to growth. When females have reached the reproductive threshold (,0.70), food energy may be then invested mainly in the liver. Using the BCI in wild population of *V. aspis*, we can make some predictions: almost all females with a BCI greater than 0.69 should reproduce, females with a BCI between 0.52 and 0.69 should increase their weight and/or their length, and females with a BCI of less than 0.52 should have a low survival in comparison to other categories.

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Structure of a *Natrix natrix* population from Northern Italy

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Abstract: The population ecology of Natrix natrix in a deciduous residual wood of Northwestern Italy has been studied in 1988-1989 (72 and 78 specimens respectively). Sexual dimorphism has been confirmed, nevertheless population structure has been slightly different from what has been reported for other localities of Italy and for some related species of North America (i.e. females rarely are longer than 90 cm). Habitat use was not dependent by a particolar dimension (mass, length) or sex; absence of large prey (Bufo bufo) seems to be the main factor dealing with the relative uncospicuous dimension of adult females.

Key words. Population ecology, dimorphism, habitat use, Natrix natrix, Northern Italy

INTRODUCTION

Few studies have been carried out on population ecology of the grass snake, Natrix natrix (DOLCE et al., 1982; DOLCE, 1983; MADSEN, 1984), and only a few other species belonging to the genus Natrix (GRUSCHWITZ, 1986; HAILEY & DAVIES, 1987) or related species (KING, 1986; TIEBOUT & CARY, 1987) have been studied. We analyzed a little population of the subspecies *helvetica* (Lacépède, 1789) and its relationships with ambience, with particular reference to population structure and habitat use.

STUDY AREA

This research was made in a deciduous residual wood inside the regional natural "Parco Lombardo della Valle del Ticino", along the Ticino river (Northwestern Lombardy, Northern Italy), on its right bank. The study area, called "Boschi del Vignolo" (45° 13' N, 3° 30' E), lies at about 72 m a.s.l. and is 45 ha. Climate is the typical sub-coastal temperate continental one, with two rainfall peaks (one in spring, one in autumn) and rainfall averaged 802 mm (at Pavia, about 15 Km E-SE).

The central area of the Vignolo is composed by Alnus glutinosa and a wet area with Salix caprea and Phragmites australis. Remaining parts are characterised by some mesophil woods, poplar groves, grass fields, canals and a few very altered zones.

MATERIALS AND METHODS

The study was carried out during 1988-1989, from March to October, for a total of 113 field-days. We used standardised routes throughout all available habitats, at different times of the day (ZUFFI, 1984; DONÀ et al., 1991), making also some trips during the night; infact it has been reported that some species can be active also after the sunshine (DOLCE, 1983; CHEYLAN, 1986).

Most snakes were captured under black rubber panels, a suitable part-time refuge present around a few abandoned garbage heaps. At each snake capture, sex, biometry and shaded temperatures of animal body and ambience were recorded (ZUFFI & GENTILLI, unpublished data); scale-clipping was used to mark snakes for future recognition.

RESULTS

Seventy two specimens (55.55% males; 34.72 % females; 9.73% juveniles) were captured in 1988 (49 field trips) and 78 specimens (42.3% males; 39.74%, females; 17.96% juveniles) were captured in 1989 (64 field trips). During first year male/female sex-ratio was 1.6, in the second year it was 1.064. Under binomial test, it has been shown that sex-ratio did not differ significantly from the expected 1:1; Wald-Wolfovitz test allow to demonstrate how that capture of both sexes was casual, and that male-female encounters were comparable. Used habitats amounted to about 4.5 ha, and the grass snake density (e.g. the overall number of captured snakes/the used area) was estimated as 16 and 17 specimens/ha per year. Biometrical features are shown in Tables 1, 2.

No statistical difference was found for weight and length between years for the same sex

(Mann-Whitney U test, p>0.05), whilst significant differences in weight were observed among sexes (1988, Z=2.32, p<0.05; 1989, Z=3.47, p<0.001; Mann-Whitney U test) and only in 1989 for length (Z=2.93, p<0.005).

	N	average	SE	min	max
males 88	38	56.18	3.82	8	103
females 88	26	86.60	10.62	10	190
males 89	33	51.76	3.54	13	106
females 89	30	108.13	11.97	10	285

Table 1: Weight of the grass snake population in 1988-1989.

	N	average	SE	min	max
males 88	37	59.86	1.57	26.5	78
females 88	26	63.08	3.43	30	90
males 89	33	57.82	1.57	34	70
females 89	30	57.50	3.28	30	99

Table 2: Length of the grass snake population in 1988-1989.

All captured grass snakes were then divided into 6 length classes, from 30 cm to 105, with a 15 cm range each class. On average females were longer than males, the 95% of the former beeing comprised between 31 and 90 cm, the 95% of the latter beeing comprised between 31 and 75 cm (Figures 1, 2). Length classes were not significantly different between years (Mann-Whitney U test, p<0.05). The "Boschi del Vignolo" was divided in 9 zones (i.e. northern grass fields; channels and adjacent wood, etc.) and in 3 subzones (i.e. 1: wood and grass fields; 2: wet areas; 3: anthropic areas), to verify if a relationship "snake dimension/habitat structure" did exist, but no difference was observed (Kruskall-Wallis test, p>0.05); neither sex preference for a given zone or habitat was found (^{2} test, p>0.05).

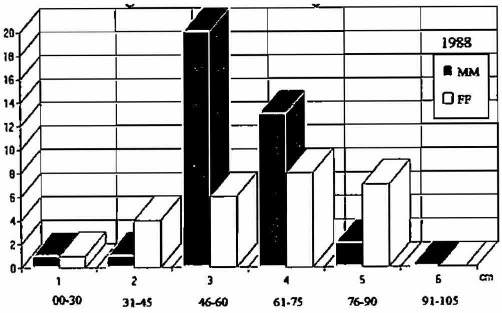

Figure 1: Distribution of length classes in 1988.

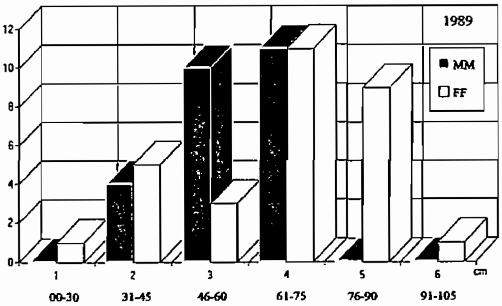

Figure 2: Distribution of length classes in 1989.

DISCUSSION

On average we found a medium sized population, well dimorphic in weight, with no one snake longer than 1 m and characterised by a relatively high density (cf. ZUFFI, 1984). Sex-ratio (male:female) does not differ significantly from 1:1, on the contrary it was frequently assessed for N.natrix as 3:1 (DOLCE et al., 1982; DOLCE, 1983) or for N.tessellata as 1:3 (GRUSCHWITZ, 1986). Also the frequency of observation of adults during the year appears to be constant, differently from what previously described for related genus (KING, 1986).

It has been reported that only adult females can reach an overall length ranging from 1.5 to almost 2 m and that this growth should be related to their trophic activity, varying from small sized preys (little amphibians, lizards) (DOLCE, 1983; FERRI, 1985) to medium or large sized preys (especially common toads, Bufo bufo, birds, small mammals) (DOLCE, 1983; LANZA, 1983). The prey availability does not follow what has generally been reported for this species (DOLCE et al., 1982; DOLCE, 1983; LANZA, 1983; ZUFFI, unpublished data) and it should be logical that older females do not reach the expected dimension inside the area. To be correct, there could be at least two main hypotheses. First, adult females after 3-4 years old, when are approximately at 80-90 cm long, could migrate to feed upon larger preys, but the observed sex-ratio did not seem indicate such a possibility. Second, adult females did not migrate outside the area, remaining on average in this habitat and, probably, not growing so much as though, this fact partly beeing explained accordingly to the observed 1:1 sex-ratio. Moreover the other two snake species present here, Coluber viridiflavus and Elaphe longissima, were markedly longer than the grass snake (DONÀ et al., 1991). In other parts of the

"Parco della Valle del Ticino" these three species were often found commonly to be longer than one meter (ZUFFI, 1988). Habitat use and preference was not shown to be different among sexes or dimensional classes, as already stated (MADSEN, 1984).

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On placing and thermobiology of polymorphic population of *Vipera dinniki* (Nikolsky, 1913) (Serpentes, Viperidae) at uppermost Msymta-river valley

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Abstract: Investigated area has about 5 hectares in subalpine belt (1750-1850 m) of uppermost Mzymta-river valley, West Caucasus. This area has two morains, rocky outcrops, high-grass and and mix elfin woodland-meadows. Both morains and rochs are the places of hibernation. There are distinguished 4 phenotypes of *Vipera dinniki*. Among thrm "tigrina"-morph prevails over "nebulosa" - and "bronze"-morphs. The most rare morph is "kaznakovi"-morph. Sexes ratio close to 1 (28 ♂♂ : 33 ♀♀). Adult vipers predominante in the age structure of population. Most not-numerous group is semiadults. Depend on slope exposition the begining and the end ofdaily activity have distinctions on the various places of this area but total length of surface activity was similar in each places and had 35% of daily cycle. It was picked out the distinctions in territorial placing between sexes and different age-grades. Thermobiological data of this area demonstrated the exceeding of the body temperature of active males than those of females. Body temperature of representatives of both sexes has stricty dependence on ground temperature than on air temperature. Results testify to existence of differences in thermobiology of various morphs.

Key words: Caucasus, Vipers, Morphs, Thermobiology.

INTRODUCTION

Snake's relations with environmental constrains (as poikilothems) are interesting withing temperature balance firstly. As rule data in literature have given an optimal interval of reptiles activity (PESTINSKY, 1939; BOGDANOV, 1950; 1962; 1965; PERSIANOVA, 1969; KHOZATZKY, ZAHAROV, 1970). *Vipera dinniki* isn't exception in this question (BOZHANSKY, 1986). At the same time thermal ecology of reptiles in various aspects was demonstrated in many articles (ORLOV, 1986; SEXTON *et*, 1992; PATTERSON, 1992; SEMENOV, BORKIN, 1992; PLUMMER, 1993).

Classical work was an article of SERGEYEV (1939) "The body temperature of reptiles in natural surroundings" in which Sergeyev described original method of investigation on temperature relations of reptiles. Based on this method *Echis multisquamatus* was investigated later (CHERLIN, 1977; CHERLIN, TZELLARIUS, 1981). Depend from elevation solar radiation increases up to the mountains. On the Caucasus the influence of solar radiation to hing-mountain reptile's ecology was noteed by STRELNIKOV (1944).

The thermobiology of polymorphic population of *Vipera dinniki* and caucasian vipers in general is unknown. We have tried to observe temperature relations of both sexes and different morphs of *V. dinniki* with ambient temperature during the most active seasonal period.

MATERIAL AND METHODS

Vipera dinniki was studied at the begining of river Mzymta, vicinity of Sochi, Western Caucasus, Russia (altitude 1750 - 1850 m). Investigated area had about 150 hectares and it placed from foothill of mt. Loyub and western shore of lace Kardyvach downwards to place Azmych (at the protected zone along the border of Caucasian Reserve). Two big Pleictocene moraines are on this east- and south-exposed area. Becide it rocky outcrops are present at place Azmych mt. Loyub. This valley cavered by subalpine vegetation (mix high-grass subalpine meadows and elfinwoodland with *Fagus orientalis*, *Betula litwinowii*, *Acer trautvetteri* and so on). Free-flowing parts of morains have some witnesses of Glacial period like lichen *Cladonia*. The same time there are xerophilouse shrubs (*Spiraea hypericifolia*, *Juniperus sabina*) on the rocks after Holocene. Both moraines and rocks are the places of viper's hibernation. Anomals were observed from 4 hibernation centres (5 hectares) of this area: "1st Morain", "2nd Morain", "Loyub", "Azmych".

There are distinguished 4 phenothypes of *V. dinniki*:

1. "kaznacovi"-morph (greyish-yellow or bright-yellow colour-ground with broad black zig-zug, usually combined with black pattern of head).
2. "tigrina"-morph (yellow or orange specimens with transverse diagonal blotchs, pronounced zig-zug is absent; head pattern is not total black

and it separates from dorsal blotchs).
3. "nebulosa"-morph is closed to "tigrina"-morph, but has some differences (greyish-brown colour-ground with indistinct undulate velvety dorsal pattern, usually brown head's pattern is unclear also can connect or separate with body pattern).
4. "bronze"-morph (goldern-grey or old-coopery colour-ground without any pattern or with rudimental zig-zug on the neck. Head pattern is similar with that of "tigrina"-morph).

It was observed the placing of 87 specimens (July-August) along this area. In the middle July cloacal temperature (Tb) of 61adult *V. dinniki* were recorded with a Schultheis fastregistering mercury theremometer (to the nearest 0.1°C).

Each snake was sexed and the following information was recorded: date of capture, time of capture, place of capture,weather, air temperature (Ta) 1.5 m above the ground, ground temperature (Tg) on the surface of land. After measuring snakes were released.

Becide it we recorded microclimate measures on the meteorological plot near lace Kardyvach (open sunny place): air temperature (Ta1) 0.2 m with a recording thermometer (AN-16 Model) in meteorological cabin., ground temperature with maximum and minimum thermometers, soil temperature (Ts) 0.1 m under the ground with a sling thermometer.

Morphs ratio among 61 termometrised vipers was 5 "tigrina": 1 "kaznakovi": 1.5 "nebulosa" : 1.3 "bronze". Vipers of these morphs were found at all 4 hibernation places but in various ratios. At the "1st Morain", there are 5 "bronze" : 2nd Morain" this ratio was 1:1:10:4, at the "Loyub" -2:5:10:1. At the "Asmych" "kaznakovi"-morph was absent and ratio of other 3 morphs was 1:1:2 correspondingly. According these data most common morph was "tigrina" and the most rare-one was "kaznakovi". The same time volume of "nebulosa"-morph and "bronze"-morph animals was approximatly equal.

Sexes ratio was about 1 (28♂♂:33♀♀). This ratio was constant among each, except "nebulosa"-morph (1♂♂:3.5♀♀). Sexes ratio was quite different at 4 hibernation places. "1st Morain" had 1♂♂:1.1♀♀; "2nd Morain" - 2.67 ♂♂:1♀♀; "Loyub" - 1♂♂:3♀♀ and "Azmych" - 1♂♂:3.75♀♀. But if we'll put into consideration high mobility of males (especially in breeding), we'll have the same ratio about 1 for total area of investigation.

Age-grades of this population had ratio 3.7 juveniles : 1 subadults : 15.7 adults. Among sexes this ratio was: ♂♂-2.3:1:6; ♀♀-4:1:29. Adult animals formed predominant age-grade and most rare group was subadults. This type of ratio describes this population as well as stisfactorily.

Molting is an important characteristic of population. It demonstrates the period of maximum trophic activity. Total number of molt vipers was 44.3%. Among females, there are 39.4% molt animals and among males - 50%. Specific ratio of molt specimens was noteed in different age-grades: 18.2% of juveniles, 100% of subadult and 46.8% of adults.

On the meteorological plot the amplitude of air temperature (Ta1) was from 3.5°C up to 31°C. Mean air temperature at 11oo (beginning of surfacely activity) was 14.0°C and mean air temperature at 14oo (snakes went away to shade and refuges) was 25.13°C (fig.1). Mean-maximum air temperature was 36.32°C. The least daily temperature amplitude was pointed out for soil temperature (Ts) 0.1 m under the ground (Fig.1): Range: 9.5 - 14.0°C and Mean 11.25±0.34.

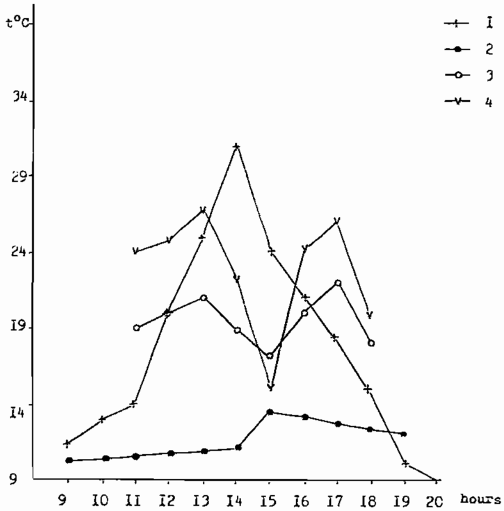

Figure 1: Microclimatical data of meteorological plot and places of capture of *Vipera dinniki* from the uppermost Mzymta-river valley. 1: Ta_1 (air temperature 0.2 m at the meteorologocal plot). 2:- Ts (soil temperature under 0.1 m at meteorological plot). 3: Ta (air temperature 1.5 m at places of viper's capture). 4: Tg (ground temperature at places of viper's capture).

Analogium data for Ts were given by CHERLIN & TZELLARIUS (1981) in Badhyz (Turkmenistan) and in Yew-box grove of

		Tb	Ta	Tg	Tb -Ta	Tb -Tg
♂♂	(range)	20.5-35.1	15.2-26.2	17.2-37.0	4.4-14.8	(-6)-9.8
	(x̄±S.D.)	28.47±0.58	19.93±0.52	24.88±0.91	8.54±0.51	3.58±0.74
♀♀	(range)	20.2-32.8	14.4-25.4	16.7-35.0	1.8-14.8	(-5.1)-12.8
	(x̄±S.D.)	26.74±0.59	18.78±0.44	22.93±0.71	7.97±0.46	3.93±0.62

Table 1: Comparison of body temperature and habital temperatures between sexes of *Vipera dinniki.*

morph		Tb	Ta	Tg	Tb -Ta	Tb -Tg
"nebulosa"	(range)	20.2-31.3	17.0-25.4	20.2-32.4	1.80-9.40	(-0.6)-3.6
	(x̄±S.D.)	26.49+1.37	19.9+0.86	24.56+1.36	6.58+0.84	1.9+0.58
"tigrina"	(range)	21.8-35.1	15.2-25.2	17.2-37.0	4.2-13.2	(-6.0)-9.80
	(x̄±S.D.)	27.91+0.59	19.81+0.55	24.24+0.82	8.10+0.43	3.67+0.84
"kaznakovi".	(range)	25.0-30.8	15.8-19.8	18.2-28.0	5.2-14.8	(-3.0)-10.2
	(x̄±S.D.)	27.50+0.85	17.60+0.61	22.70+1.62	9.90+1.40	4.80+1.96
"bronze"	(range)	21.2-33.6	16.8-23.8	19.4-35.4	4.4-12.0	(-1.8)-8.2
	(x̄±S.D.)	28.25+1.40	19.55+0.87	23.95+1.81	8.70+0.89	4.3+1.16

Table 2: Thermobiological data of 4 morps *Vipera dinniki* from the uppermost Mzymta-river valley.

Caucasian reserve (TUNIYEV & BEREGOVAYA, 1986).

Temperature data for each morph and sex at the places of capture are in the Tables 1 and 2.

DISCUSSION

During the period of observation (July-August) pregnant females were found at the 4 hibernation places as well as all molt specimens independ of sex. Dry females and males after molt were allocated throughout the stony subalpine meadows. All juveniles were caught at the most dens-grass part of meadows. We recorded bigest density of Orthoptera at the part of meadows. Representatives of genus Gomphocerus are main food objects for juvenile vipers in the hiph-western Caucasus. But it takes place in August-September when grasshoppers rich imago. Noted volume of molt juveniles (18.2%) showed low level of trophic activity in July. The same time subadults had maximum trophic activity (100%) because of difuse dissemination of not-numerous animals and absence of competitors for using equal size-limits of food.

Among adult vipers, there are bigest number of molt males (50%) than molt females (39.4%). Males are very lively and successful in getting prey in comparison with territory conservative females. From other hand, females keep energy ballance for development of embrions firstly. As rule, females don't come out completely on the surface of land. They lie under the ledges of stones and from time to time females put out abask different parts of body.

At middle-day time all population is in hiding under the hot stones and only single specimens go to the dense-grass parts or to the shady banks of streams. In July evening activity can absent if the first half of day was solar. Snakes are under the warm stones and later they go deepper to the burrows and cracks in the rocks and morain-bodis. At the cloudy-weather vipera can be find on the surface during all period of daily activity but males were observed rare than females. Similar notes we recorded at solar vindy weather, when females were found at the vindless warm places protected by stones and blocks. Only pregnant females and juveniles are on the surface during drizzle and formers have more long activity (even if Ta=13°C).

The temperature cut-off seasonal activity of *V.dinniki* was noteed as +5°C (from the end of April till middle of October) and the temperature cut-off of reproductive period was given as +10°C, according to BOZHANSKY (1986). But usually seasonal activity of *V.dinniki* is shorter (May-middle of September).

Depend on slopes exposition and sun-shining time the beginning and the end of daily activity have distinctions on the 4 hibernation places of investigated area (from 15 minutes between "1st" and "2nd Morains" up to 1.75 h between "Azmych" and "2nd Morain"). But total length of surface activity was similar in each places and had 8.5 h. or 35% of diurnal cycle. CHERLIN & TZELLARIUS (1981) pointed out strict time-limits of snake's activity. These authors have established dependence of daily activity's beginning from the minimum level of soil temperature in the burrows, when air temperature at the same burrows starts to exceed the soil temperature.

Data of temperature measurings (Tab. 1) show some interesting points. Firstly, maximum distance of ambient air temperature and body temperature on surface (Ta-Tb) was close to operative temperatures reported for beginning of the seasonal and diurnal activitis (+13°C). Secondly, body temperature of active males tended to de higher than those of females ($d=1.78$; $t=2.14$; $p<0.05$). Finaly, body temperatures of both sexes had strictly of air (Ta) at place of capture ($r=0.7$). Similar materials were reported for *Echis multisquamatus* (CHERLIN & TZELLARIUS, 1981) and it co-ordinates to those results of special investigations that body absorption of heat takes place mainly through substratum (DUVDEVANI & BORUT, 1974). STRELNIKOV (1944) suggested that it's not necassery high ambient air temperature in the mountains for favorable live of reptiles. Most important role has solar radiation which increases up to the mountains.

In the morning males get warm quick (Fig.2) and they rich maximum body temperature at middle-day time. Whereas females warm up more slowly and they rich of body temperature at 1 hour later, when males begin to reduce body temperature. Due to increasing of Ta and Tg (Fig.1) vipers of both sexes start their active thermoregulation and they choise shade places with cooler Ta and Tg. Distinkt-tracked inverse dependence has maximum at 15oo when due to maximum of ambient overheating snakes choise most cool parts of habitat and they maintain optimal body temperature. According to STRELNIKOV (1944) high-mountain caucasian reptiles die after 20 minuts of direct solar radiation and range of air temperature from 24 to 29°C. Their body overheating had 25°C.

In cloudy weather an ultraviolet radiation isn't so strong and animals can have more long surface activity. But in sunny weather *V. dinniki* reduce daily activity at middle-day time when ambient temperatures are high and they avoid basking.

At the second half of day due to reduction of solar radiation vipera correlate again active their body temperature. Mean body temperature of females riches maximal level again at 15-30 then it reduces to 26°C at 16oo and it stais constant till end of surface activity. Males don't have quite high mean temperature like in the morning but they keep up their body temperature higher than that of females by 3°C.

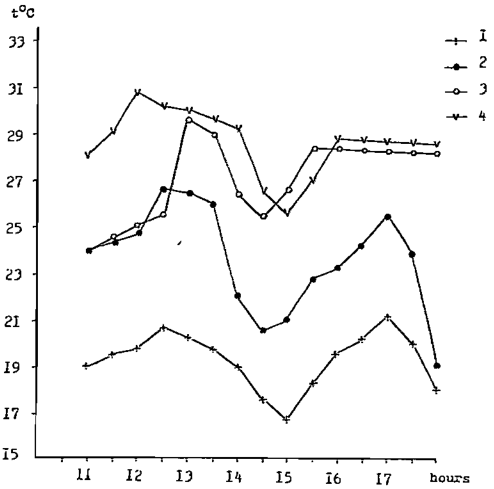

Figure 2: Daily pulsation of body temperature of males and females of *Vipera dinniki* and microclimatical data from the places of capture (uppermost Mzymta-river valley). 1: Ta (air temperature 1.5 m at the places of capture of vipera). 2: Tg (ground temperature at the places of capture of vipers). 3: Tb♀♀ (mean body temperature of females). 4: Tb♂♂ (mean body temperature of males).

Viper's behavioral thermoregulation correlates strictly with daily fluctuations in ambient temperatures of environment. Let's comparing data from investigated area and meteorological plot. In the morning air temperature (Fig.1) and snakes habitat's temperature are in direct correlation because air temperature raises quicke than soil temperature. Then soil temperature (Ts) go on higher when temperature pick of Ta1 has crossed and air got cool. At the capture places there are minimum means of Ta and Tg the same time because snakes go away of overheating. In the evening Tg1 get cool quicker than that of soil (Ts). Again snakes select behaviral much warm places. Thus habital data of temperature are in negative slope again with ambient temperature as whole. Total

	Tb		Ta		Tg		(Tb -Ta)		(Tb -Tg)	
compared-morphs	d	t	d	t	d	t	d	t	d	t
bronze-trigina	0.34	0.22	-0.26	0.20	-0.29	0.14	0.6	0.56	0.63	0.4
bronze-nebulosa	1.76	0.89	-0.35	0.28	-0.61	0.27	2.12	1.72	2.4	1.9
bronze-kaznakovi	0.75	0.42	1.95	1.71	1.25	0.49	-1.2	0.76	-0.5	0.23
tigrina-nebulosa	1.42	1.1	-0.09	0.08	-0.32	0.19	1.52	1.73	1.77	1.48
tigrina-kaznakovi	0.41	0.29	2.21	1.74	1.54	0.78	-1.8	1.59	-1.13	0.67
nebulosa-kaznakovi	1.01	0.55	2.3	1.97	1.86	0.87	-3.3	2.17*	-2.9	1.7

Table 3: Differences on thermobiological data betveen various morphs of *Vipera dinniki* from the uppermost Mzymta-river valley. d: distance between mean numbers ($\bar{x}_1$-$\bar{x}_2$); t: t-cristerion; *: $p<0.05$.

period of viper's active thermoregulation continues from 12-30 till 17-00.

It's well known that percentage of melanotic reptiles increases in the moderate latitudes as well as up to the mountains. It was reported in literature also (STRELNIKOV, 1944) about relationship between skin structure/colour and absorption of heat. We examined this question reference to 4 described morphs of *V. dinniki* (Tab. 2). Unfortunately volume of materials made it impossible to compare thermobiological data for each sex separately. That's why we have given joint comparison for both sexes of each morph (Tab. 3).

Even this way of examination has shown follow results which testify to existence of peculiarites of thermoregulation among representatives of various morphs:

1. Distance between body temperature and air temperature (Tb-Ta) of "nebulosa"-morph is significant lover that of "kaznakovi"-morph (d=-3.32; t=2.17; $p<0.05$).

2. Since "tigrina"- and "nebulosa"-morphs have rov of similar characteristics we compareed the unite sample of these morphs with "kaznakovi"-morph. Result demonstrates authenticity of examinated characteristic (Tb-Ta)(d=-2.4; t=2.05; $p<0.05$). Comparison of unite sample with "bronze"-morph didn't give significant distinction (d=o.96; t=1.01) in this mean.

These data can show high adaptation of Dinnik's vipers thermoregulation with daily and seasonal fluctuations of solar radiation in the high-mountain belts. Snakes tack between critical maximum and minimum of ambient temperature and they can realize behavioral normal stile of life in thermally favorable environment of the Western Caucasus. What is more, apparantely high polymorphism and thermal distincts in investigated population are results of microevolutional development of this comparatively young species since Pleistocene.

Among described morphs there is "kaznakovi"-morph which is most distinct in (Tb-Ta) mean. It may de indirect evidence as of increased heat-loosing as of possible thermal disadvantage of this morph. Anyhow, "kaznakovi"-morph takes minimal place in total volume of specimens. And this is fixed sign for Mzymta-river population. It's interesting to draw attention to specimens of *Vipera dinniki* from lake Kardyvach which were sampleed there by SOBOLEVSKY in 1928 (Collection of Zoological Museum of Moskow State University; No 2767). In that sample there are as all modern morphs as similar ratio of each morph. This fact corroborates long-period existance of these morphs and prosperity of population as whole.

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About the apparent inexistence of a spring mating in the catalan population of *Vipera latasti* (Reptilia: Viperidae), and note about the reproductive success

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Abstract: We are studying the annual cycle of an adult male and an adult couple of *Vipera latasti* in semi-natural conditions during three and one years respectively. The outdoor enclosure is located near the place they were captured, and both vipers feed, moult and hibernate normally in it. During these years, we haven not observed any sexual behaviour between the end of hibernation (middle of March) and the first moult (late May or June). It was a period of high passivity in which the male limited its activity to a thermoregulation, at no more than 50cm. from the winter refuge. The exploration activity of the male started with the first moult, and was directed exclusively to foraging till the end of August, when his feeding period finished. At this moment, the male activity increases in intensity, and this was related exclusively to sexual behaviour. This mating period had its peak in September, and finished at the beginning of November, with the winter latency. One year after of the mating period, the female gave birth to thirteen young in perfect condition. The apparent coincidence of this annual cycle with the monthly distribution of the field observations gathered in Catalonia, suggests that the inexistence of a spring mating is widely spread. If the information is confirmed, this would be a unique case not only among vipers but also among European snakes.

Key words: Viper, *Vipera latasti*, annual cycle, reproduction, mating.

INTRODUCTION

Sexual cycles of European vipers have been studied by several authors (SAINT-GIRONS 1973,1976,1982,1992, SAINT-GIRONS &DUGUY 1992, BONNET *et al.* 1992, BONNET & NAULLEAU 1993).

These studies differenciate two groups; one, *Vipera aspis*, *Vipera seoanei* and *Vipera latasti*, characterized by a spermiogenesis during the autumn and the beginning of spring and two mating periods: the principal one in spring, between the end of hibernation and the first moult, and another in the autumn, sometimes avoided by climatic conditions.

The second group, *Vipera berus*, *V. ammodytes* and *Vipera ursini*, is characterized by a spring spermiogenesis and only one mating period: in late spring, after the first moult (SAINT-GIRONS 1976,1992).

The studies about *Vipera latasti* in semi-natural conditions (big outdoor enclosures) have been made outside this distribution area and the references about *Vipera latasti* are usually incomplete in comparison with other European vipers (SAINT-GIRONS 1976, 1978, 1979, 1980 a,b, SAINT-GIRONS and NAULLEAU 1981).

The present study has been made within this distribution area (near the northern limit in the northeast distribution), also in an outdoor enclosure, and in this article, the first results that were obtained are shown.

MATERIAL AND METHODS

The observations have been carried out within an outdoor enclosure measuring 1.5 x 1.5 x 0.6 metres in 1991-2 and 2 x 2 x 0.8 metres in 1993. The enclosure is situated in the Garraf massif (the coastal mountain range of Barcelona) at an altitude of 400 metres and three kilometres from the place where the specimens were captured (410 metres in altitude). The climate of the location (Begues) is coastal Mediterranean with dry summer and an average rainfall of 704mm, distributed between autumn and spring. The average of the maximum air temperatures of the hottest month is 27.3°C, and the average of the minimum air temperatures of the coldest month is -0.5°C. The ground of the massif is calcareous with an advanced karstic process. The study area is situated in a zone where two communities of vegetation are in contact -typical coastal holm oak (Quercetum ilicis galloprovinciale) and macchia of kermes oak and Dwarf fan plant (Querco-lentiscetum) (BOLOS,1950). The observations of this study are based on three vipers belonging to the subspecies V.l.latasti, the information of which is shown in table 1.

During the years of the study, the vipers have fed, reproduced, moulted and hibernated normally inside the enclosure. From the 16th of September to the 30th of May, diurnal observations were carried out weekly (on two consecutive days) and almost daily during the rest of the year. However, during the spring season, a special effort was made to step up the frequency of the diurnal observations. Evening observations were carried out almost daily througout the year.

N°1- Male subadult-adult. In captivity from 24-9-90.
Total length 350mm. Weight 40 gr (Nov.1990)
Total length 585mm. Weight 112 gr (Oct. 1993)

N°2- Male adult. In captivity between 14 and 23-9-92.
Total length 632mm. Weight 158 gr (Sept. 1992)

N°3- Female adult. In captivity from 13-9-92.
Total length 590mm. Weight 202 gr (Sept. 1992)
Total length 610mm. Weight 279/158 gr (Sept. 1993, before/after the parturition).

Table1: Vipers studied in the enclosure

RESULTS

In Figure 1-A, the annual cycle of male n°1 during the years of the study is shown. This can be divided into three well-defined periods: The first is characterized by atypical passive behaviour, exclusively intended for thermoregulation, between the first spring emergence (middle of March) and the first moult (from the middle of May to the middle of June). During this period, the viper does not make any exploratory movements, limiting its activity - only on sunny weather- to a small area of 0,10-0,90m^2 close to the winter refuge. This behaviour did not vary after the emergence from the winter refuge of the female with which he mated the previous autumn.

The second period starts after the first moult and in this period, exploratory behaviour around all the surface of the enclosure (4m^2) begins, the motive being exclusively to forage. Exploration activity is only interrupted by the digestion of captured prey, or because of the proximity of moulting or unfavourable climatology. The presence of the female in the enclosure does not alter this behaviour. In this period, the dependency of activity to weather conditions is minimum.

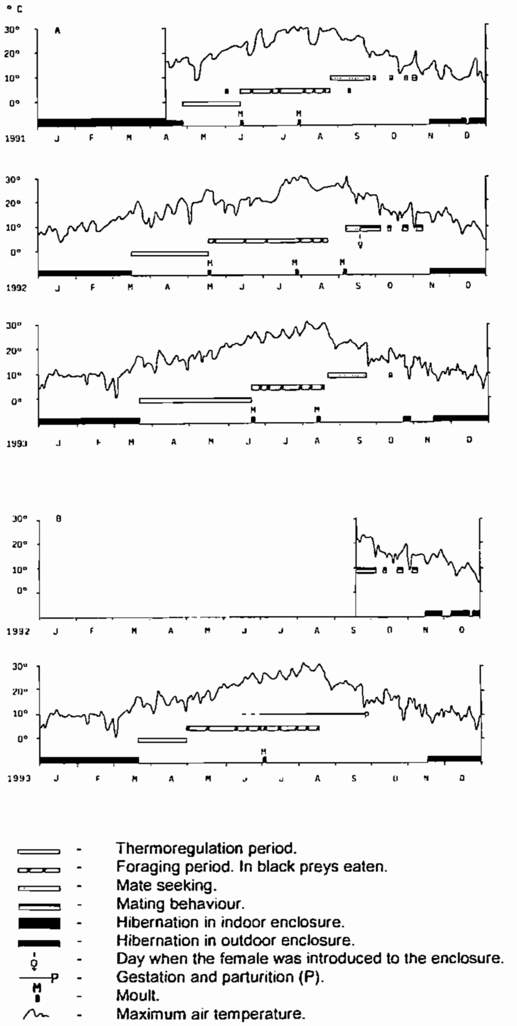

Figure 1: Annual cycle of male number 1 (A) and female (B) during the years of the study.

The third period starts at the end of August, when the male refuses the preys offered and increases his exploratory activity. This is only interrupted by unfavourable temperatures and can last almost all day if the heat is not excessive at midday (see figure 2, where the frequency of male's exploratory activity is shown). This period goes on until the middle of November, with the start of hibernation, although the least favourable climate of the last two months can inhibit exploratory activity. This exploratory behaviour, observed throughout the three autumns of the study, was only modified when the female was introduced into the enclosure on the 18th of September 1992 (see figures 1 and 2). The presence of this female (captured five days before, when she was found in the preliminary stages of mating with male n°2), caused male n°1 to stop his exploration activity, substituting it for the preliminary stages of mating. Mating was verified three days later, on the 21st of September. The preliminary stages lasting until the male began to hibernate. In the following autumn, as the female was not receptive (due to the gestation period and giving a birth), male n°1's exploratory movements, although not of a foraging nature, once again became his main activity.

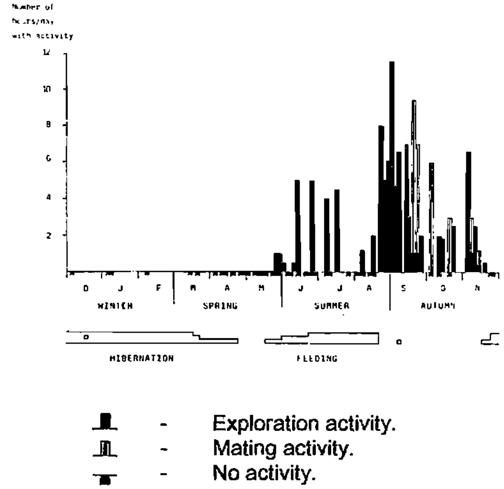

Figure 2: Exploration and mating activity of male number 1 in the enclosure (April 1991-October 1993) (N = 72).

In figure 1-B, the annual cycle of the female during the period of the study is shown. Introduced into the enclosure on the 18th of September 1992, she immediately accepted the preliminary stages of mating displayed by the two males. It is not possible to say whether she mated with male n°2 (with which she could have easily mated before her capture), even though both were found in the preliminary mating stages during their short coabitation in the enclosure.

Following hibernation -which finished just two hours after that of male n°1-, her activity was limited to thermoregulation until the 18th of April (when the first displacements were observed), with foraging activity commencing on the 1st of May. During the previous period, no indication of sexual behaviour was observed -she only was seen to be thermoregulating near the male-.

The feeding period ended with the capture of her last prey (on the 17th of August), which supplied her with nourishment until well into the gestation period This behaviour has already been described about V.aspis (BONNET & NAULLEAU, 1993). She gave birth on the 27th of September -one year after she mated, this means long-term sperm storage- to thirteen young in perfect condition, the characteristics of which a shown in table 2.

	Total length	Snout-vent length	Tail length	Weight
♂♂ (n=6)				
mean	190.5	161.1	29.5	6.45
rank	188-195	158-166	29-30	6.0-6.7
♀♀ (n=7)				
mean	185.5	159.7	25.8	6.57
rank	181-189	155-165	25-27	6.5-7.0
Total (n=13)				
mean	187.8	160.3	27.5	6.51
rank	181-195	155-166	25-30	6.0-7.0

Clutch mass = 84.7, Female length: 610mm
Female weight before/after parturition: 279/158

Table 2: Size and weight of the young and female. Length in mm, Weight in g.

Finally, male n°2 was only kept for ten days, during which time he was observed in the preliminary stages of mating with the female, in such a way as to clearly demonstrate antagonistic behaviour towards male n°1, which, because of his inferior size, avoided any encounters.

DISCUSSION

The results which were obtained are surprising because the non-existence of spring sexual activity between European vipers has never been described (SAINT-GIRONS 1976,1982,1992). On the other hand, a rise in exploratory activity is usually interpreted as a search for food, hibernaculum or, in the case of the males, as a search for a female to mate with it (NAULLEAU 1965,1966 GIBBONS & SEMLITSCH, 1987). In our study, it seems clear that autumnal activity corresponds to sexual activity and summer activity to foraging activity. Equally then, the non-existence of spring activity suggests the non-existence of sexual activity in the said period. Although this apparent anomaly does not seem to correspond to inadaptation to captivity, nor a lack of hibernation (this has been produced at a temperature between 2°C and 10°C, with a daily fluctuation of not more than 2°C and with a humidity near saturation), nor can it be discarded that it corresponds to an ethological anomaly of the male.

However, there is information which seems to indicate that the non-existence of spring mating is widespread within this species, at least in the greater part of Catalonia. Thus, Figure 3 shows the monthly variations of collected observations about *V.latasti* in Catalonia. In this figure, the scarcity of spring observations (19'6%), is surprising -there are even less than in the summer (28'5%), a period unfavourable for observations (SAINT-GIRONS 1975)-, when it is in spring (BERTRAND & COCHET 1992:117) or in spring and autumn (NAULLEAU 1965,1966, DUGUY 1972) that the other species of viper are more easily seen. On the other hand, this scarcity of spring observations completely coincides -as does the distinct peak in autumn (49'1%)- with figure 2, which shows the frequency of male nº1's exploratory activity in the enclosure.

It is interesting to point out that while the inhabitants of the Pyrenean area consider that the vipers (in this zone, *V.aspis*) are observed more frequently in spring, or in spring and autumn, the inhabitants of the Mediterranean area (area of *V.latasti*) think that it is more usual to see them only in autumn. Even some of the people who were interviewed (in Almatret, near the river Ebro at an altitude betwen 200 and 400 metres), have indicated that the elevation in autumnal observations correspond to their period of mating. In Andalucia (south of Spain), on the other hand, where *V.latasti* gaditana seems to mate in the spring, this period is popularily known as "la carrera" (the race), due to the frequency that their trails are found in the sand (CURT & GALAN 1982:159). In the mountains of Tortosa and Beseit in the south of Catalonia however (where V.latasti is found betwen 400 and 1500 metres), rangers have seen the same number of vipers in the spring and autumn, and our observations gathered also show the same. This fact, together with mating observations in April made by DELFIN GONZALEZ in the Vallés (pers.com.), suggest that in some places or years, spring mating occurs.

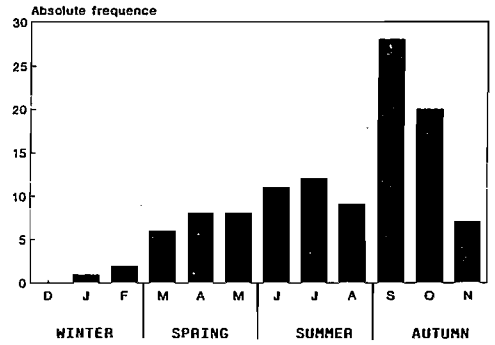

Figure 3: Monthly variation of *Vipera latasti* observations gathered in Catalonia (N=112).

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Structure of a wild population of *Vipera aspis* L., investigated using a Body Condition Index (BCI)

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Abstract: Seasonal variations in body condition of wild *Vipera aspis* were investigated using a Body Condition Index (BCI). The mean and the variance of BCI measured in the spring show differences among sexes. In female *Vipera aspis* the BCI measured during the spring ranges between 0.43 and 1.22, the mean was 0.71±0.15, CV=21% (n=221). 51.6% of females were potentially reproductive (BCI=0.70), 38.9% were intermediate non-reproductive (BCI between 0.52 and 0.69), and 9.5% were thin non-reproductive (BCI lower than 0.51). The male population was more homogeneous, BCI measured during the spring ranged between 0.40 and 0.75, mean BCI was 0.57±0.06, CV=10% (n=140). The proportion of males with large body reserves (BCI>0.60) was 34.3%, and the proportion of intermediate males (0.51<BCI<0.59) was 53.6%. Thin males, in a poor body condition (BCI<0.51), represented 12.1% of the total. The body condition of females varied more than males from year to year.

Key words: Body condition, Population, Reproduction, snakes, *Vipera*.

INTRODUCTION

Snakes devote considerable amounts of energy to reproduction. In female vipers this is mainly for vitellogenesis (SAINT-GIRONS, 1957; SAINT-GIRONS & DUGUY, 1992; BONNET *et al.*, 1992, 1994), in male for sexual behaviour, movements and intrasexual competition during the mating period (SHINE, 1978; MADSEN & SHINE, 1993). In numerous snakes and lizards, changes in body reserves and thus body condition occur during the annual cycle in relation to the reproductive events (MENDEZ DE LA CRUZ *et al.*, 1988; BROWN, 1991; ALDRIDGE, 1979; Diller and Wallace, 1984; SAINT-GIRONS & Duguy, 1992; Ramirez Pinilla, 1991). In temperate regions the sexual cycle is strongly influenced by the climatic conditions: in almost all species the end of hibernation is also the beginning of the reproductive season (SAINT-GIRONS, 1986).

Using a Body Condition Index BCI, body reserves, such as the mass of the fat bodies and the mass of the liver, can be estimated with a good precision in living snakes (Leloup, 1976; Bonnet & Naulleau, 1993, 1994). In *Vipera aspis* the induction of vitellogenesis requires large amounts of body reserves, females need to reach a reproductive threshold of BCI (¸0.70) at the beginning of mating season in order to reproduce (Bonnet *et al.*, 1994). After parturition females are very emaciated, the mean BCI is 0.52 (Bonnet *et al.*, 1994; SAINT-GIRONS, comm. pers.). In the wild, at the beginning of the reproductive season, it is possible to identify 3 classes of individuals: potentially reproductive females (high BCI=0.70), intermediate non-reproductive females (0.52¾BCI¾0.69), and thin females (BCI lower than the mean-postparturition one, BCI<0.52). Qualitative differences in body reserves have been found between the 3 classes. Vipers with a BCI greater than the reproductive threshold value have always very large body reserves and BCI correlates with the mass of the liver; thin vipers (BCI<0.51) have hardly any body reserves left and the mass of muscles declines due to starvation; intermediate non-reproductive females have moderate body reserves and BCI correlates with the mass of the fat bodies (Bonnet & Naulleau, 1993). In captive, but natural conditions, the BCI of reproductive females measured at the beginning of vitellogenesis correlates positively with litter size (Bonnet & Naulleau, 1994). In males, no reproductive threshold has been found (Unpub.). Spermatogenesis occurs in fat males as well as in thin males, and is apparently weakly influenced by the level of body reserves. However, a preliminary study carried out in captivity, suggests that males with a high BCI exhibit a most complete sexual behaviour and obtain more mating than thin males (VACHER, 1993). The aim of this study was to estimate the proportions of different classes determined by the BCI levels in a wild population of *Vipera aspis*.

MATERIAL AND METHODS

This study was carried out on a wild population of *Vipera aspis* during two years (1992 and 1993), situated in the Central Western of France near the Atlantic ocean at the northern limit of the *Vipera aspis* distribution (Les

Moutiers-en-Retz, 47°03'N-2°00'W). The study area, 30ha, is composed of fields and paths bordered by hedges. In some fields are marshes, and as they have not been used for farming for several years, brambles, brushwood and small trees are colonising the fields.

The snakes, adults males and females, were caught by hand during the spring period. Sexual maturity was determined using total body length (the minimal size for sexual maturity in *Vipera aspis* is 47.5cm in females, and 36.5cm in males; unpub. data). Each individual was sexed, total body length (to the nearest 0.5cm) and total body mass (to the nearest 1.0g) were recorded in the field. In 1992 the vipers were identified by clipping ventral scales (BLANCHARD & FINSTER, 1933). In 1993 they were marked by Identification tags (Sterile Transponder TX1400L, Rhône Mérieux, 69002 Lyon France, product of Destron/IDI Inc.). Each tag (11±1mm long) was implanted dorso-laterally beneath the skin about 5 cm from the cloak. As one tag was lost in such position and another one partly emerged from the skin, tags were later placed within the muscles. The tags were well tolerated during the whole study period (8 months). Vipers were identified very easily, and without error by electronic means (Mini-portable Reader HS5105L, Rhône Mérieux, 69002 Lyon France, product of Destron/IDI Inc.). The Body Condition Index (BCI) of individuals was calculated using the formula of Leloup (1976) were BCI=M/TM. M is the body mass of the studied animal; TM, the theoretical body mass of the studied animal is obtained from a reference table. The BCI is a measure of the deviation of the individual animals from this reference (Bonnet & Naulleau, 1994). Each viper was examined by palpation to detect the presence of prey in the digestive tract; when this examination was positive, the animal was excluded from analyses. Means are expressed ± S.D. Comparisons between percentages and means were made using X^2 and Student's t-test.

RESULTS

Captures

In 1992, 71 females and 17 males *Vipera aspis* were marked; in 1993, 150 females (49.3% recaptures), and 123 males (66.6% recaptures). All identifications were made in the spring. The population density was very high in the study area: for example 91 different adult females and 94 different adults males were caught in 10.7Ha between February 1992 and August 1993.

Population structure investigated by BCI measures

The mean BCI of females (0.71±0.15, CV=21.0%, n=221; 1992 and 1993) was greater than the mean BCI of males (0.57±0.06, CV=10.4%, n=140, 1992 and 1993), *F*=153.7, p<0.001 (Figure 1).

In females the BCI calculated during the spring ranged between 0.43 and 1.22, both in 1992 and in 1993 (Figure 1). The mean BCIs were 0.67±0.15 in 1992 (n=71) and 0.73±0.14 in 1993 (n=150), and were significantly different, *F*=7.71, p=0.005.

35.2% of the females were potentially reproductive (BCI greater or equal to 0.70) in 1992 (n=25), 59.3% in 1993 (n=89) and 51.6% in 1992 and 1993 (Figure 2). The proportion of potentially reproductive females was significantly different between 1992 and 1993 ($X^2=10.70$, p<0.001).

49.3% of the females were in an intermediate position (BCI between 0.52 and 0.69, non-reproductive females) in 1992 (n=35), 34% in 1993 (n=51), and 38.9% in 1992 and 1993 (Figure 2). The proportions were different between 1992 and 1993 ($X^2=3.96$, p<0.05).

15.5% of the females were in a poor body condition and were non-reproductive (BCI lower than 0.52) in 1992 (n=11), 6.7% in 1993 (n=10) and 9.5% in 1992 and 1993 (figure 2). The difference between 1992 and 1993 was close to significance ($X^2=3.08$, p=0.08).

The composition of the male population was less heterogeneous than females (CV=10.4 %). The BCI ranged between 0.40 and 0.73 in the spring (Figure 1). The mean was 0.55±0.06 in 1992 (n=17), and 0.57±0.06 in 1993 (n=123). There were no difference between 1992 and 1993, *F*=1.66, p=0.19.

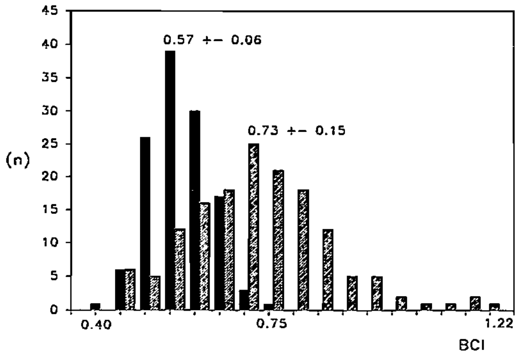

Figure 1: The range of the BCI in a wild population of *Vipera aspis* (1993). Comparison between males and females. Hatched bars: females (n=150), solid bars: males (n=123). Mean BCIs in each sex are indicated ± S.D.

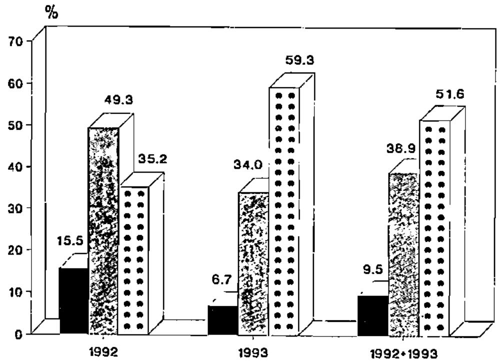

Figure 2: The proportion of adult females in a wild population in different BCI classes. Comparison between 1992 and 1993. Solid bars: thin non-reproductive females (BCI¾0.51), hatched bars: intermediate non-reproductive females (0.52¾BCI¾0.69). Dotted bars: potentially reproductive females (BCI=0.70).

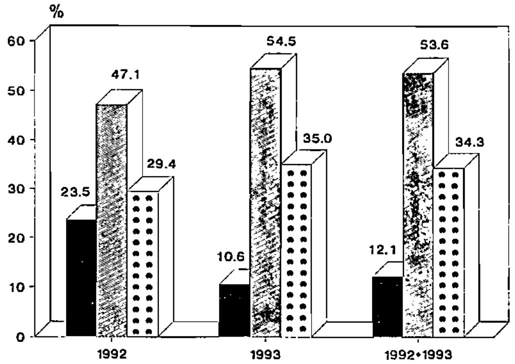

Figure 3: The proportion of adult males in a wild population in different BCI classes. Comparison between 1992 and 1993. Solid bars: thin males (BCI¾0.50), hatched bars: intermediate males (0.51¾BCI¾0.59). Dotted bars: males with a BCI=0.60.

29.4% of the males had large body reserves (BCI greater or equal to 0.60) in 1992 (n=5), 35% in 1993 (n=43) and 34.3% in 1992 and 1993 (Figure 3). The proportions were not significantly different between 1992 and 1993 (X^2=0.48, p=0.49).

47.1% of the males had a BCI between 0.51 and 0.59 in 1992 (n=8), 54.5% in 1993 (n=67) and 53.6% in 1992 and 1993 (Figure 3). The proportions were not different between 1992 and 1993 (X^2=0.82, p=0.37). This group represents about half the population of males.

23.5% of the males were in a poor body condition (BCI lower than 0.51) in 1992 (n=4), 10.6% in 1993 (n=13) and 12.1% in 1992 and 1993 (Figure 3). The proportion differed between 1992 and 1993 (X^2=5.01, p=0.025).

DISCUSSION

During the spring females are on average in better body condition than males. This difference is probably due to their different reproductive cycles. Females need large body reserves to reproduce while males not, and females feed during the mating season while males starve.

Both the range and the variability of BCI values are greater in females than in males. Before reproduction females are able to store very large body reserves (BCI can exceed 1.00), for use during vitellogenesis and pregnancy, about 6 months. After parturition almost all females are emaciated. Thus in the wild, there is a wide range of females, from potentially reproductive to postparturiant ones. In males the influence of body reserves on reproduction is less important as they do not need to store very large reserves and the energetic investment is limited to a shorter period, the mating season (about 2 months); as a result the male population is more homogeneous.

The structure of the female population shows differences between the two years of the study, particularly in the class of potential reproductives (35.2% in 1992 and 59.3% in 1993). Such fluctuations are probably influenced by several factors, among which food availability, climatic conditions, and the proportion of reproductive females the previous year may be important. Gestation length (NAULLEAU, 1986), digestion rate (NAULLEAU, 1983), and thus the possibility in recovering body reserves between parturition and hibernation in a given year are dependent on weather conditions. When 1992 and 1993 are pooled, the proportion of potentially reproductive females is near from 50%, this suggests that the duration of the female reproductive cycle is about two years in our study area, which is close to estimates using different methods (DUGUY, 1963; SAINT-GIRONS & DUGUY, 1992).

All males of the population are potentially reproductive at least as far as spermatogenesis is concerned, since cloacal massage always showed the presence of spermatozoa. However, as males starve during the whole mating period, body reserves may play a role in reproductive performance, males in good body condition may have higher reproductive success (VACHER, 1993). Thin vipers seem to have difficulty in recovering their body reserves (capture/recapture unpublished data), the thinnest may have low survival rates.

CONCLUSION

Our results show that in the wild population of *Vipera aspis* studied, adult females and males comprise vary classes with regard to body condition. The proportion of different classes of vipers, identified using the BCI values, vary among years, but the structure of the population of females varying more than males. The influence of such variations on the reproductive strategy of vipers is not yet understood. Further field studies are necessary to quantify precisely the relationships between body condition and reproductive success, in males and in females.

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The reptiles in the diet of the Otter (*Lutra lutra* L., Carnivora, Mammalia) in Europe

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Abstract: We work on 36 different studies about otter's food in Europe. Reptiles are taken south of 43° N. It can be explained by the distribution of the dominant components of this reptilean parts of diet: water snakes (*Natrix maura* and *N. tesellata*). Both species are the 95-6 % of the eaten reptiles (snakes as a whole the 97.8 %). We collected a total of 738 spraints of otter, containing 2140 prey-items. We found that reptiles are taken mainly during the summer. In the river Noguera Ribagorçana there are important interannual variations (% of occurrence: 3-45 %). Ecological and conservation implications are discuted.

Key words: Otter, *Lutra*, Reptiles, *Natrix*, Europe.

INTRODUCTION

The diet ot the otter (*Lutra lutra*), a semiaquatic Mustelid, has been extensively studied (see MASON & MACDONALD, 1986, and Table 1). The main components of this diet are fish, crustaceans and amphibians, and in smaller proportions mammals, birds and insects. It was not untill the work of CALLEJO *et al.* (1979) that the habitual consumption of reptiles by otter was reported. After this, only some aspects of the trophic ecology of other herpetos have been considered (WEBER, 1990; TEIXEIRA, 1989).

METHODS

Reptile consumption

A literature search on otter diet in Europe (36 studies, from 16 different countries) found results expressed as relative frequency RF (number of reptile prey-items x 100 / total number of prey-items) and/or occurrence frequency OF (number of spraints containing reptile prey-items x 100 / total number of spraints). In two cases results were presented by the authors as occurrence but not relative frequency (ADRIAN & MORENO, 1986; ADRIAN & DELIBES, 1987); the RF has been calculated from those cases in Table 1 in which both indices were present ($RF = 0.84\ OF - 0.084$; $n = 9$; $r = 0.97$; $p < 0.0001$).

In studies where individual reptile species were identified (CALLEJO *et al.*, 1979; CALLEJO, 1984; LOPEZ-NIEVES & HERNANDO, 1984; RUIZ-OLMO, 1985; ADRIAN & MORENO, 1986; PRIGNIONI *et al.*, 1986; ADRIAN & DELIBES, 1987; ARCA & PRIGNIONI, 1987; DELAKI *et al.*, 1988; RUIZ-OLMO *et al.*, 1989; GOMES *et al.*, 1989; SKAREN, 1993) the number of captures for each reptile species was summarized.

Temporal variations in reptile consumption

A total of 804 spraints (cotaining 2419 prey-items) were collected from three rivers in the river Ebro basin (N.E. of the Iberian Peninsula): Algars (152 spraints; 292 prey-items), Matarranya (143 spraints; 359 prey-items), Noguera Ribagorçana (348 spraints; 1181 prey-itmes) and Montsant (201 spraints; 387 prey-items). First three during the period summer 1984 to spring 1985, and the last one between 1986 and 1987.

In addition, 195 spraints were collected from the river Noguera Ribagorçana during the summers of 1985, 1988, 1991, 1992 and 1993 (representing 308 additional prey-items). Summer diet was prefered for the study of interannual dietary fluctuations as maximun diversity is attained during this period and reptiles are more frequent (RUIZ-OLMO *et al.*, 1989).

Spraint analysis followed conventional methodology (see RUIZ-OLMO *et al.*, 1989).

RESULTS

Reptiles in the diet of the otter

Reptiles are almost entirely absent, or purely incidental, in the diet of *L. lutra* in the Centre and North of the Continent, but become increasingly significant nearer the Mediterranean area, where they can form up to 17.8 % of the diet (Figure 1). This is reflected as a important increase in reptiles in otter diet south of 43° N.

Species taken

A total of 363 reptiles from 10 species were recorded (Table 2). The main prey-items were

			REPTILES IN DIET	
LOCALITY	SAMPLE SIZE	REFERENCE	FR	%OCCURRENCE
Central Finland	2255	Skaren (1993)	0.04	-
Estonia	4307	Laanetu (1989)	0	0
Belarus	2173	Sidorovich (1992)	0	0
S. Sweden	14615	Erlinge (1967)	0	0
S. Sweden	350	Erlinge (1969)	0	0
Denmark	61	Erlinge & Jensen (1981)	0	0
Mayo (Eire)	706	Gormally & Fairley (1982)	0	0
Midlands (Eire)	4448	Kyne *et al* (1989)	0	0
Devon (GB)	2222	Wise *et al* (1981)	0	0
Devon (GB)	960	Chanin (1981)	0	0
Somerset Levels	858	Webb (1975)	0	0
Dyfed (GB)	258	Henshilwood (1981)	0	0
Norfolk (GB)	2260	WEIR *et al* (1973 & 1977)	0	0
Ceslovenko		Kozena *et al* (1992)	1.2	2.3
Ceslovenko		Toman (1992)	0	0
Britain (France)	251	Libois *et al* (1987)	0	0
Creuse (France)	491	Bouchardy (1986)	0	0
E. France	141	Lode (1989)	0	0
Hungary	811	Kemenes (1989)	0.1	-
Drina (Bosnia)	836	Taylor *et al* (1988)	0.1	-
Central Italy	280	Arca & Prignioni (1987)	13.6	25.7
Albania		Prignioni *et al* (1986)	6.9	12.1
Galicia (Spain)	260	Callejo *et al* (1979)	16.3	-
Galicia (Spain)	632	Callejo (1988)	0.79	1.21
N Spain	549	Callejo & Delibes (1983)	0.9	1.4
NE Spain	628	Ruiz-Olmo *et al* (1989)	1.4	3.0
NE Spain	1181	Ruiz-Olmo *et al* (1989)	1.0	3.2
Mondego	63	Simoes-G.& F.ALM. (1983)	17.8	21.4
Vouga (Portugal)	130	Gomes *et al* (1989)	0	0
Paiva (Portugal)	265	Gomes *et al* (1989)	1.3	1.3
Greece		Gaethlich (1988)	8.33	-
Greece	80	MacDonald & Mason (1982)	0	0
SE Spain	2841	L-Nieves & Hernando (1984)	6.77	-
S. Portugal		Teixeira (1989)	0.9	-
SW Spain	334	Adrian & Delibes (1987)	2.6	7.1
SW Spain	264	Adrian & Delibes (1987)	0.3	0.8
SW Spain	145	Adrian & Moreno (1986)	4.6	9.7

Table 1.- Presence of Reptiles in the diet of eurasian otters in freshwater habitats of Europe.

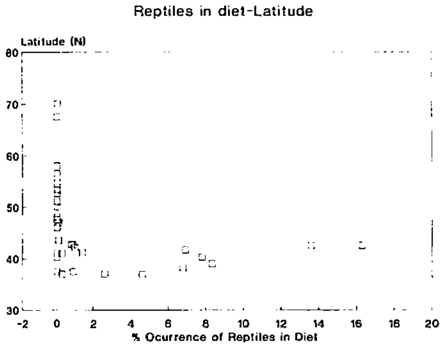

Figure 1: Relation between latitude and presence of reptiles in the diet of otter in Europe

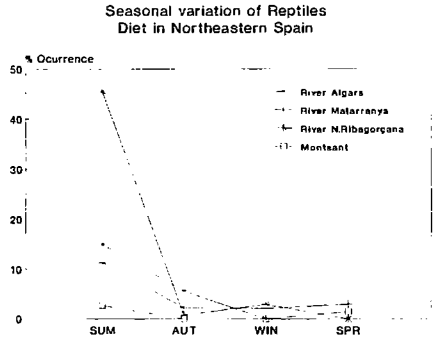

Figure 2: Seasonal variation of the occurrence of reptiles in the diet of otter in four rivers of the Iberian NE

snakes (97.8%), in particular the genera *Natrix* (95.6 % of the reptiles) and, where identified, the species *N. maura* and *N. tesellata* (Viperine and Dice Snakes respectivelly).

Terrestrial species formed only a very small proportion of the diet (4.8 %).

Seasonal fluctuations

Reptiles are taken prinarily during the summer (Figure 2) and values are low or null for the remainder of the year. Differences are significant in the rivers Noguera Ribargorçana ($X^2 = 166.61$; dF = 3; $p < 0.0001$), Algars ($X^2 = 9.78$; dF = 3; $p = 0.021$) and Matarranya ($X^2 = 12.38$; dF = 3; $p = 0.006$), but not significant in the river Montsant ($X^2 = 1.76$; dF = 3; $p = 0.623$).

Likewise, considerable interannual fluctuations can exist, as Noguera Ribagorçana river (Figure 3).

DISCUSSION

Reptiles as a component in the diet of the otter had not been considered in depth to date. The most striking feather observed is the increase from north to south which reflects the sharp increase in abundance and diversity of semiaquatic reptiles, occurring south of 43° N; even though many reptile species are present north of this latitude.

This pattern can be readily explained by the distribution of the dominant components of this reptilian part of the diet, *N. maura* and to a lesser extent, *N. tesellata*, which occur in the south of Europe under the 45-48 ° N (ARNOLD & BURTON, 1978). Thus otter reptile consumption depends almost entirely on the availability of these semiaquatic snakes. It is interesting to note that Pond Terrapins are hardly taken despite being abundant in may parts of Mediterranean Europe; the hard shell seems to account for this.

The effect of temperature on reptile consumption has not been studied. CALLEJO (1988) however, demonstrated the importance of temperature (particularly minimum air temperature and water temperature) on the consumption of mammals, amphibians and fish. Temperature could similarily be expected to be a determinig factor for ectothermic especies such

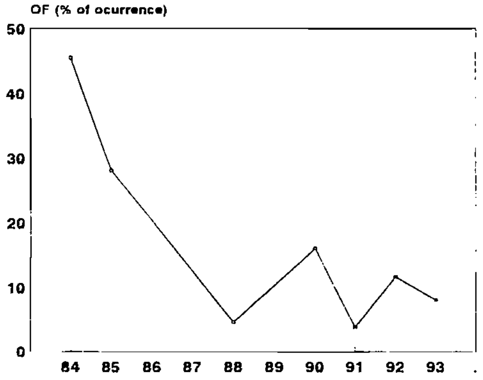

Figure 3: Annual variation of occurrence of reptiles in the august diet of the otter in the Noguera Ribagorçana river

	IBERIAN PENINSULA	ITALY	ALBANIA	OTHER	TOTAL	%
Ophidia	-	-	-	-	355	(97.8)
Natrix maura	280	0	0	0	280	77.1
Natrix tesellata	0	0	4	0	4	1.1
Natrix sp	22	38	0	0	60	15.1
Malpolon monspesulanus	1	0	0	0	1	0.3
Vipera sp	4	0	0	0	4	1.1
Colubridae indet.	3	0	0	3	6	1.7
Sauria						
Psammodromus algirus	-	-	-	-	6	(1.7)
Lacerta schreberi	2	0	0	0	2	0.6
Lacerta sp	1	0	0	0	1	0.3
Anguis fragilis	0	0	0	1	1	0.3
	2	0	0	0	2	0.6
Quelonia						
Mauremys sp	-	-	-	-	2	(0.6)
	2	-	-	-	2	0.6
n	317	38	4	4	363	-
Number studies with data	8	1	1	2	12	-

Table 2.- Reptiles found in the diet of the otter in Europe considering only studies giving information at species level (see references in Table 1).

as reptiles, as indicated by the higger reptile intake in summer (this paper; LOPEZ-NIEVES & HERNANDO, 1984; ARCA & PRIGNIONI, 1986). High reptile consumption in summer has been reported in *Lutra canadensis* in Notrh America (CHABRECK *et al.*, 1982; TUMLISON *et al.*, 1986) and *Lutra perspicillata* in the India (TILER *et al.*, 1986).

Overall, it would seem that otters take those reptile species most abundant (specially during summer), most easily caught and living in the inmediate aquatic vicinity.

Interannual fluctuations of *N. maura* in the diet of otter may additionally give an insight into population variations of this reptile. This could be important as changes in *N. maura* abundance during fish and crayfish shortages (being an alternative food source) may affect otter abundance and conservation. There is, however, evidence for the existance of a contrary situation (high abundance of otter could affect water snakes abundance and conservation, during crayfish and fish shortages).

Fish populations were very abundant in this area from 1984 to 1990, while from 1991 to 1993 a shortage of fish was recorded (RUIZ-OLMO, 1993 and 1995). In some years, the low *N. maura* consumption observed would reflect the great abundance of fishes; however the peak of fish abundance in 1984 is coinciding with peak in water snake (fishes are also one of the main preys of this snake). The effect of the fish shortages has been noted in the same area for the water vole (*Arvicola sapidus*), where the proportion in the otter diet rose from OF = 1.9 % in 1984 (RUIZ-OLMO *et al.*, 1989) with hight availability of fish, to 14.0 % in 1992 during the fish shortage (our unpublished data). For this reason, low occurrences in diet during fish shortages, indicates water snakes shortages.

It is evident, therefore, that the shortages in fish abundance could have negative implications for water snake populations and conservation: directly for shortage in food and indirectly for increase in depredation (otters and, likewise, for other predators of semiaquatic and aquatic reptiles such as herons, ospreys, etc.).

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Sialic acid in the skin of some Amphibia

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Abstract: Homogenates of ventral and dorsal skin of the anurans *Bufo marinus*, *Rana cyanophlyctis*, *Xenopus laevis* and *Pipa carvalhoi* and two urodelan species (*Ambystoma mexicanum* , *Taricha torosa*) were assayed for sialic acids, using the thiobarbituric acid technique of WARREN (1959). The findings are discussed with regard to possible sites of sialic acid occurence within the skin. Skin from the dorsal surface appears to contain higher concentrations of sialic acid than that from the ventral surface in most species. At the present time a correlation between the concentration the of sialic acid and habitat selection is not obvious.

Key words: Sialic acids, skin, proteoglycans, glycoconjugates

INTRODUCTION

Sialic acids are present in many tissues of vertebrates. They are mostly bound to macromolecules, usually lipid or protein, and perform a great variety of functions (e.g. GOTTSCHALK, 1966; SCHAUER, 1978).

In amphibians sialic acid was recently found to be responsible for the binding of exogenous protein and water retention by the egg jelly (BERNER & INGERMANN, 1990). Lectin histochemical staining namely with WGA (wheat germ agglutinin) revealed the presence of acidic glycoconjugates including sialic acid in the membranes of the epidermis of larval A. tigrinum (ZACCONE *et al.*, 1987), possibly in the tadpoles of *Rana perezi* (VILLALBA & NAVAS 1989), in the adult epidermis and dermal glands (DANGUY & GENTEN, 1989) and possibly in the dermal connective tissue of *Bufo bufo* and *Xenopus laevis* (GENTEN & DANGUY, 1990).

When studying the glycosaminoglycans in the skin of *Xenopus laevis* and *Bufo marinus* (ROSIEPEN & GREVEN, in prep.), we determined the amount of sialic acid (N-acetyl-neuraminic acid) as a possible source of the polyanionic nature of the amphibian skin. The results prompted us to perform a comparative study of sialic acids on the ventral and dorsal skin of other Amphibia with different ecological requirements.

MATERIALS AND METHODS

One individual of each of the anurans *Bufo marinus*, *Rana cyanophlyctis*, *Xenopus laevis*, *Pipa carvalhoi* and the urodelans *Ambystoma mexicanum* and *Taricha torosa* was studied. Animals were sacrificed, tissue samples were taken immediately from dorsal and ventral skin and dried in acetone. Dried tissue from the tail of was a gift of Prof. Dr. G. Clemen, University of Münster.

Pieces of the tissue were treated with 0.1 N H_2SO_4 for 1 h at 80° C in order to liberate sialic acids. 0.2 ml of the hydrolyzed samples and purified N-acetyl-neuraminic acid (Merck) as control were taken for assay using the Warren technique (WARREN, 1959). Photometrical determinations were made with a Beckmann DU-64 photometer.

RESULTS AND DISCUSSION

Values for sialic acids, namely N-acetyl-neuraminic acid (NANA) expressed as µmol per 100g dry weight of skin are presented in Table 1. The amount ranges from 200 (ventral skin of *T. torosa*) to 1044 µmol/ 100 g dry weight (tail of *A. mexicanum*). In all specimens (with the exception of *A. mexicanum* where only the tail was investigated) concentrations of sialic acid appear to be higher in the dorsal skin. The exact site for the bulk of the sialic acid concentration is not clear.

Histochemical studies of the skin of several Amphibia employing lectin binding patterns gave evidence of sialic acid containing glycoconjugates in the epidermis, dermal glands and possibily in dermal connective tissue (see literature cited above). Interpretation was based on the affinity of WGA, a lectin sensitive to N-acetylated glucosamine and sialic acid residues. In previous studies, binding of WGA to human connective tissue was interpreted as binding to glucose (SÖDERSTROM, 1987).

In fish extracellular matrix WGA-binding to dermal components was attributed to the presence of hyaluronate containing N-acetyl-D-glucosamine and keratan sulfate which contains sialic acid (DANGUY & GENTEN, 1990; see also SPICER *et al.*, 1992) Both are proteoglycans (polyanionic macromolecules with a protein core to which glycosaminoglycans are attached) of the vertebrate connective tissue matrices. Binding of biotinylated WGA to the epidermal basement lamella (for terminology see FOX, 1986) and to the stratum spongiosum (the upper dermis containing a loose network of collagen fibers) of *Xenopus laevis* and *Bufo bufo* was not commented on by GENTEN & DANGUY (1990). To our knowledge keratan sulfate has not been (unequivocally) demonstrated in the amphibian skin to date (e.g. LIPSON & SILBERT, 1968).

Species	Sialic acid (µmol/100g skin dry weight) dorsal	 ventral
Anura		
Pipa carvalhoi	597	447
Xenopus laevis	495	392
Rana cyanophlyctis	746	623
Bufo marinus	348	340
Urodela		
Taricha torosa	243	200
Ambystoma mexicanum	1044*	

Table 1. Sialic acid content in the dorsal and ventral skin of six amphibian species (* only the tail was investigated).

Preliminary tests using FITC-conjugated LPA (*Limulus polyphemus* agglutinin) - a lectin more specific for sialic acid than WGA, which did not stain collagen strands in human tissues (SÖDERSTROM, 1987) - reveal significant binding in the stratum spongiosum and the epidermal basement lamella comparable to the WGA-staining described by GENTEN & DANGUY (1990). Staining of the epidermis was weak and dermal glands show some autofluorescence. Thus specifity of the staining and efficiency of the methods must be improved in further studies. Wherever the main bulk of sialic acid may be localized - in glycoconjugates of the connective tissue, in epidermal membranes or in the glands - they are involved in the water economy and the hydratation state of the skin (for similar functions of the acellular matrix of cartilage see CARNEY & MUIR, 1988).

Although there are species-specific differences in the content of sialic acid, a correlation of concentrations and ecological requirements of the species examined is not obvious at the present time. In Amphibia relationship between dermal proteoglycans i.e. their acidic glycosaminoglycans and habitat selection has been discussed (e.g. JÜRSS & SCHLISIO, 1973; NAKASHIMA & KAMASHIMA, 1990; DANGUY & GENTEN, 1990). Preliminary results suggest variations in the concentrations of sialic acid of the anuran skin in response to the reproductive cycle and thermal stress (unpublished). These relationships, however, await further study.

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Immunofluorescent localisation of thyroxine in tissues of developing *Xenopus laevis*

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Abstract: In *Xenopus laevis* thyroxine (T4) was detected in erythrocytes (smears) and in fixed and sectioned tissue (thyreoidea, integument, gills, muscles, cartilage) from stage 50 - staging (NF) according to the table of NIEUWKOOP & FABER - to adults. Triiodothyronine (T3) was clearly seen only in the thyreoidea.

Key words: Thyroid hormones, immunofluorescence

INTRODUCTION

The thyroid hormones (T3, T4) play a decisive role in metamorphosis. Their different amounts allow amphibians to remain in the larval form and become neotenic or to metamorphose and (usually but not necessarily) become terrestrial. Circulating hormone (T4) increases from low levels in early larval stages to maximum levels during metamorphic climax. Thyroxine (T4) was detected and localized by indirect immunofluorescent staining in the blood plasma and the erythrocytes of individual *Rana catesbeiana* throughout the larval period, i.e. from early limb bud stages to the end of metamorphosis (PIOTROWSKI & KALTENBACH, 1985) and of adult amphibians (STIEFF & KALTENBACH, 1986). The method was found to be more sensitive than earlier radioimmunoassays on pooled samples of larval serum which revealed a surge of T4 in the blood plasma, mainly during climax stages (eg. LELOUP & BUSCAGLIA, 1977).

To broaden the knowledge of distribution and localization of thyroxine not only in the circulating system but also in other tissues of developing Anura, we applied this technique to blood smears and to histological sections of some selected stages of the clawed frog *Xenopus laevis*.

MATERIAL AND METHODS

For the investigation the following developmental stages (according to NIEUWKOOP and FABER, 1975) were available: NF 46/47, 50, 55, 62, and juveniles a few days after metamorphosis.

Blood was obtained by inserting a capillary in the truncus arteriosus of anesthetized specimens (not NF 46/47, 50).

Smears were air - dried, fixed in 95% ethanol and stored in a refrigerator before use. Larvae were fixed in Bouin, embedded in Paraplast and sectioned in 7 m sections. Smears and deparaffined sections were subjected to an indirect immunofluorescence assay for thyroxine: Sheep anti-T4 and anti-T3 serum (Henning GmbH, Berlin) and fluorescent isothiocyanate-labeled anti-sheep-IgG-serum (Sigma) were diluted 1:80 (anti-T4 and anti-T3) and 1:10 (anti-IgG-FITC) with PBS (0.02 mol/l, pH 7.3). The first and second incubation were carried out 30 minutes with a 50 l drop of anti-T4 or anti-T3 resp. of anti IgG FITC. To check specifity incubations were made in the presence of the first or the second antibody or without antibodies (to check autofluorescence) (for further details see PIOTROWSKI & KALTENBACH, 1985).

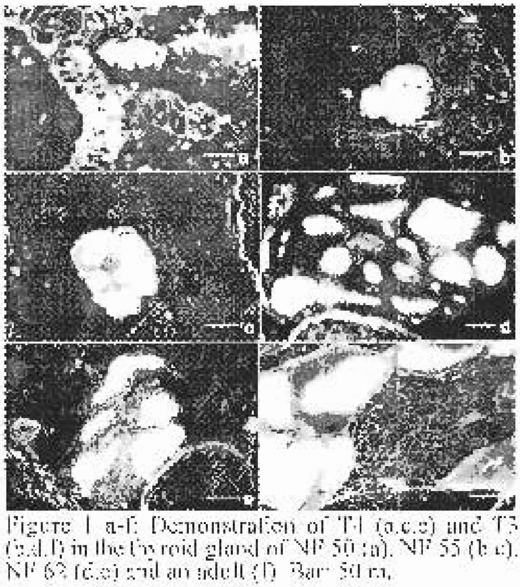

Figure 1 a-f: Demonstration of T4 (a,c,e) and T3 (b,d,f) in the thyroid gland of NF 50 (a), NF 55 (b,c), NF 62 (d,e) and an adult (f). Bar: 50 m.

Slides were viewed with an Olympus BH2-RFL epifluorescence microscope equipped with the appropriate filter combination.

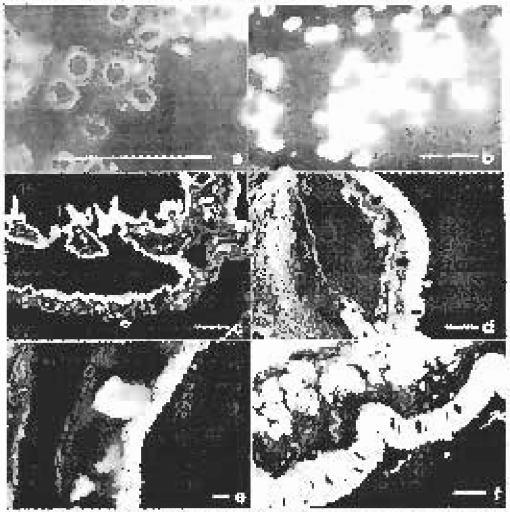

Figure 2 a-f: Demonstration of T4 in erythrocytes of NF 55 (a) and NF 62 (b), in the gill epithelium of NF 55 (c), in the epidermis of NF 62 (d) and a juvenile a short time after metamorphosis (e), and in the adult oral epithelium (f). Bar: 50 m.

RESULTS AND DISCUSSION

In erythrocytes and different tissue of NF 46/47 neither T3 nor T4 was demonstrable. In larval tissues of NF 50 and following stages the hormones were localized only in the thyroid gland (Fig. 1 b,d,f). Localisation of T4 in different tissues is summarized in Table 1 and illustrated in the Figures 1 a, c,e-f, 2 a-f). Confidence of the method was supported by the controls (absence of autofluorescence in the tissues pictured and the absence of fluorescence when the primary or secondary antibody was omitted).

Findings on erythrocytes and blood plasma as well as the sensitivity of the technique agree very well with the results obtained by PIOTROWSKI & KALTENBACH (1985) and STIEH & KALTENBACH (1986). T4 was demonstrable with some variation in intensity at different stages and in different fields of the same smear from early through late stages of development. Main sites of specific fluorescence were plasma, mainly around the blood cells, cytoplasma of eryhrocytes and nuclei, particulary at nuclear perimeters. Whether erythrocytes are actually targets for T4 appears, however, unclear.

In addition T4 was demonstrable in some tissues throughout larval development, indicating its significance also in prometamorphic stages (see GALTON & COHEN, 1980) and illustrating the wide spectrum oftarget cells. T4 possibly may bind to cytoplasmatic proteins (e.g. GALTON, 1980).

Although it is not easy to define a general basic function of thyroid hormones to any basal metabolic process in adults, demonstration of T4 in erythrocytes and several tissues of juveniles and adults stress the significance of this hormone also after metamorphosis.

Absence in NF 46/47 and presence of specific fluorescence from NF 50 upwards as well as differences in the intensity of fluorescence indicate different hormone levels, i.e. the general increase in thyroid hormone concentration and perhaps gradually appearing of hormone sensitivity of tissue during the course of larval development. For *X. laevis* very low concentrations of T4 were found in the plasma of tadpoles (pooled from 4 to 10 individuals) as early as stage 54 (LELOUP & BUSCAGLIA, 1977).

Stage (NF)	46/47	50	55	62	juvenile (a few days after metamorphosis)
Thyroid gland					
follicular cells	-	-	+	+	++
colloid	-	−++	+++	+++	+++
oral epithelium	-	++	+++	++	++
epidermis	-	−++	+++	+++	+++
gill epithelium	-	−++	+++		
cartilage	-	−+	++	++	++
connective tissue	-	−+	++	++	++
muscles	-	++	+++	+++	+++

Table 1: Immunofluorescence of T4 in tissues of developing *X. laevis*. Stages (NF) according to NIEUWKOOP and FABER (1975) (-): no fluorescence. (−+): weak fluorescence, (++): middle fluorescence, (+++): strong fluorescence.

The method, although only semiquantitative at best, allows visualization of hormone in individuals of rather early stages and results are largely consistent with findings obtained by other quantitative, but obviously less sensitive methods (e.g. chromatography, radioimmunoassay).

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First observations on the immunological materno-foetal relationships in *Typhlonectes compressicaudus*, a viviparous Gymnophionan Amphibia

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Abstract: Explants from gills or total *Typhlonectes compressicaudus* larvae were cultivated in vitro under several conditions. We were able to show that pregnant female spleen cells possessed a cytotoxic effect against its own larvae. We could also shown that only maternal serum prevented this toxicity.

Key words: Amphibia, Gymnophiona, pregnancy, immunology.

INTRODUCTION

Immunological materno-foetal relationships are poorly known in viviparous and ovoviviparous Amphibian species. Only *Salamandra salamandra* has been the object of this kind of study (BADET *et al*, 1975, 1977). In Gymnophiona Amphibians, 50% of species are viviparous (or ovoviviparous) (WAKE, 1977). Few of them have been studied (*Dermophis mexicanus*, WAKE, 1980; *Typhlonectes compressicaudus*, EXBRAYAT, 1986). The immunological relationships have never been investigated.

In *Typhlonectes compressicaudus*, the embryonic development lasts about 6 months (EXBRAYAT, 1986). At the end of their development, the intra-uterine larvae possess a pair of vesiculous gills that are narrowly applied against the uterine wall, realizing a placental-like structure (DELSOL *et al*, 1986).

Some aspects of development and materno-fetal relationships have been studied in this species (EXBRAYAT, 1986 ; SAMMOURI *et al*, 1990 ; EXBRAYAT & HRAOUI-BLOQUET, 1991 ; HRAOUI-BLOQUET & EXBRAYAT, 1992). In this work we have tried to understand the immunological relationships between intra-uterine larvae and pregnant female.

MATERIAL AND METHODS

Two pregnant females with 12 stage 32 intra-uterine larvae and one non-pregnant female have been used. The embryonic stages have been determined according to the table of SAMMOURI *et al* (1990).

Larvae were extruded from the uterus. They were dissected in small gills explants (1mm^3) and small fragments of total embryos with integument (1mm^3). Some of them were cultivated in a M199 medium (Institut Pasteur productions, Paris, France), to which had been added 10% fetal calf serum. Maternal spleen cells were dissociated and cultivated in presence of gills or larval explants in the same medium. Other samples were cultivated in presence of maternal serum.

Females blood added with heparin was centrifugated at 4°C. Sera was them used in certain culture experimentations.

Several types of culture were realized. They are summarized as follows:

1) Culture medium + larval tissue; 2 samples with gills and 2 with larval fragments.
2) Culture medium + larval tissue + maternal serum; 6 samples with gills and 6 with larval fragments.
3) Culture medium + larval tissue + maternal spleen cells; 12 samples with gills and 12 with larval fragments.
4) Culture medium + larval tissue + maternal spleen cells + maternal serum; 12 samples with gills and 12 with larval fragments.
5) Culture medium + larval tissue + other pregnant female serum; 2 samples with gills and 2 with larval fragments.
6) Culture medium + larval tissue + other pregnant female serum + maternal spleen cells ; 4 samples with gills and 4 with larval fragments.
7) Culture medium + larval tissue + non pregnant female serum; 2 samples with gills and 2 with larval fragments.
8) Culture medium + larval tissue + non pregnant female serum + maternal spleen cells; 4 samples with gills and 4 with larval fragments.

The experimentation was carried out during 41 days (experiments 1 to 4) or 105 days (experiments 5 to 8).

RESULTS

Evolution of cell cultures is similar for gill explants and total larvae fragments.

1 - Samples with or without maternal serum. After two days, we observed the multiplication of cells coming from the explants (Fig.1.1). This multiplication was uninterrupted during the duration of the experimentation (41 days) (Fig. 1.2).

2 - Samples with maternal spleen cells. After two days, many spleen cells were observed on the larval epithelium (gills or integuments) (Fig. 1.3). Next, these cells invaded the explants and finally, they prevented the multiplication of explant cells (Fig.1.4).

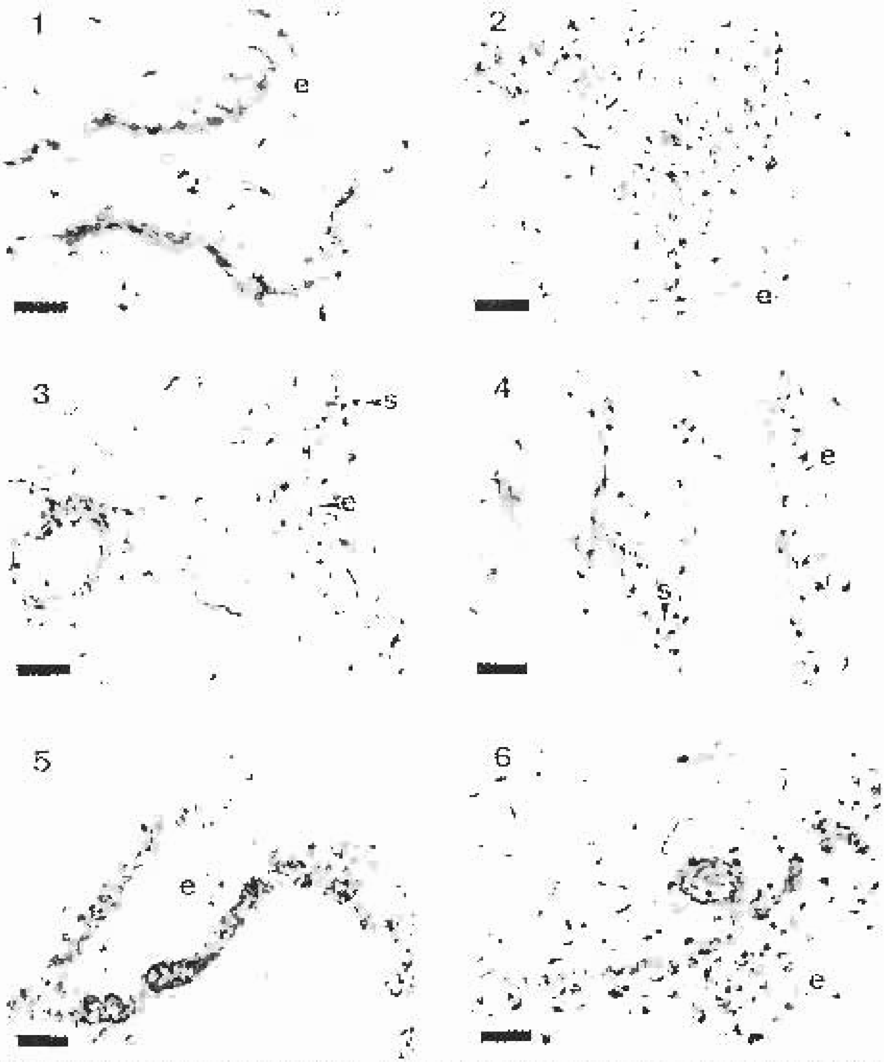

Figure 1: Gill explant with culture medium. 1.1- 2 days after the beginning of the experimentation. Scale mark 5 µm. 1.2- 41 days after. Scale mark 10 µm. **1.3**- and mother spleen cells. 2 days after. Scale mark 10 µm. **1.4**- and mother spleen cells. 41 days after. Scale mark 10 µm. **1.5**- mother spleen cells and mother serum. 2 days after. Scale mark 10 µm. **1.6**- mother spleen cells and mother serum. 41 days after. Scale mark 10 µm. e: explant cells; s: mother splenocytes

3 - Samples with maternal spleen cells and maternal serum. After two days, some spleen cells were observed on the epithelial surface of the gill explants or total larval tissues (Fig. 1.5). Then the explant cells multiplicated. After 41 days, spleen cells were rare or even absent (Fig. 1.6).

4 - Samples with maternal spleen cells and other pregnant female serum or non pregnant female serum. After two days, maternal spleen cells proliferate. A lot of degradated explant cells were observed on the bottom of culture vessel. After 105 days, the explants were dead and the structures unrecognizable with a microscope. The spleen cells were numerous on the surface of dead cells.

CONCLUSIONS

In this study, we could show that maternal spleen cells of *Typhlonectes compressicaudus* have a cytotoxic effect against the explant of their own intra-uterine larvae. This effect was suppressed by the presence of maternal serum and not by the serum of another pregnant or non pregnant females. We suppose that, at the end of pregnancy, when larval gills are applied against the uterine wall, the maternal serum contains a factor that prevents the cytotoxicity and protects the development of intra-uterine larvae. Our results concerning *Typhlonectes compressicaudus* and those obtained in *Salamandra salamandra* (BADET *et al*, 1975, 1977) are comparable. It seems to exist similar mechanisms of larvae rejection and facilitation in Amphibians.

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In vitro androgen secretion by testicular seminiferous tubules and interstitial compartment in the lizard *Podarcis s. sicula* Raf.

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This paper is dedicated to Prof. Gianfranco Ghiara in recognition of his fundamental contribution on the biology of reproduction studies.

Abstract: In the male lizard *Podarcis s. sicula* Raf., displaying annual spermatogenetic cycles, the testis releases androgens *in vitro*. This hormone release varies during the sexual cycle, showing a maximum level in early spring, when gonadal recrudescence is evident. The two testicular compartments (seminiferous tubules and interstitial compartment), isolated by enzymatic digestion, were also competent to release androgens. This hormonal production appeared enhanced when pituitary aqueous extracts or LH were added to the incubation media. The seminiferous tubules and the interstitial compartment showed different patterns of hormone release in relation to the spermatogenetic cycle. During gonadal recrudescence, androgens appeared to be mainly released from the interstitial compartment (Leydig cells) rather than from seminiferous tubules; their steroidogenic rate was consistent with the high plasma-androgen levels observed in this period and with secondary sexual characters development. During gonadal regression (late summer), instead, the maximal steroidogenic activity occurred in the seminiferous tubules (Sertoli cells ?). In this phase, the circulating androgen level was very low, whereas hormone content was quite abundant in the gonad. Our results show that in *Podarcis s. sicula* the two isolated testicular compartments have steroidogenic potentiality. However, further studies are necessary to better define the contribution of the different types of testicular somatic cells to steroidogenesis.

Key words: Spermatogenesis, Leydig cells, Sertoli cells, Androgens, Lizard.

INTRODUCTION

Spermatogenesis is known to be regulated by pituitary hormones (FSH, LH) and androgens (STEINBERGER, 1971; STEINBERGER & STEINBERGER, 1975; LOSTROH 1975; PARVINEN, 1982). In the lizard *Podarcis s. sicula* Raf., as in other vertebrates (LOFTS & BERN, 1972; CHRISTENSEN, 1975; KIME, 1987), the testis appears to be the main source of circulating steroids since the level of these hormones decreases after castration (ANDÒ *et al.*, 1992).

The males of this lizard, like most reptiles inhabiting the temperate zone, display a reproductive cycle with seasonal gonadal recrudescence and regression (ANGELINI & GHIARA, 1984; ANGELINI & BOTTE, 1992). Specific changes in plasma sex-hormone levels occur during the different phases of the gonadal cycle (ANDÒ *et al.*, 1990). Plasma androgens increase during gonadal recrudescence (early spring) and are low during the regression (late summer), when in the interstitial compartment Leydig cells are morphologically reduced (VARANO *et al.*, 1973), and only spermatogonia and large Sertoli cells are present in seminiferous tubules. The same steroids are found in the testis, but, during the sexual cycle, their level does not always follow the same trend as in the plasma: the level of intragonadal androgens is higher in late summer (ANDÒ *et al.*, 1992), when the gonad is regressed. The androgens are continuously released also *in vitro*, indicating that the isolated testes have significant steroidogenic potentialities (CIARCIA *et al.*, 1990).

In non-mammalian vertebrates, sex-hormone synthesis has been chiefly assigned to Leydig cells (LOFTS & BERN, 1972). However, the appearance and disappearance of Δ5-3βHSD, lipid, and agranular reticulum has been reported to occur also in Sertoli cells of numerous fishes, amphibians, and birds. This is indicative of some steroid secretory activity in this cell type (CALLARD *et al.*, 1978).

In order to investigate the role of the somatic testicular component in the steroidogenesis of the lizard *Podarcis s. sicula,* we isolated seminiferous tubules and interstitial compartment to study their androgen production *in vitro*.

MATERIALS AND METHODS

Animals. Adult males of *Podarcis s. sicula* Raf. were captured in the neighbourhood of Naples (Arzano) during gonadal recrudescence (early spring) and regression (late summer).

Their weight ranged from 12 to 14 g and their snout-vent length was about 7-8 cm. The animals were maintained in terraria for two weeks under natural photoperiodic and thermal regime, in order to repair the effects of acute stress on gonadal hormone secretion (GREENBERG & WINGFIELD, 1987; CREE *et al.*, 1990; MANZO *et al.*, 1994). After anesthesia by immersion in an ice bath, cardiac blood was immediately collected by puncture into heparinized capillary tubes, and the separated plasma was stored at -20 °C until analysis. Successively, the animals were killed by decapitation. The testes were excised and some of them were immediately frozen in liquid nitrogen until assayed for androgens; the remaining testes were utilized for *in vitro* experiments.

Hormone. Luteinizing hormone (LH, Sigma, Lot 70H02641) from equine pituitary was used. The dose-response relationships of LH for androgens were investigated on superfused testes (n=18) during gonadal recrudescence and regression, after washing for 2 hours in medium alone to establish a baseline output. The curve was constructed using the androgen release in response to six different doses of LH (1, 10, 25, 50, 100, 200 ng/ml). Each experiment was carried out in triplicate. Maximal response by testis was obtained with 50 ng LH/ml both during recrudescence and regression (Fig. 1). Therefore the dose of 50 ng/ml was chosen for each experiment in which LH was employed.

In vitro superfusion. Superfusion was carried out by the method previously reported (CIARCIA, 1993). The testes (n = 9) and pituitary glands were rapidly excised and placed in incubation medium (Dulbecco's Modified Eagle medium, DME, - Gibco), which contains Penicillin and Streptomycin (100 µg/ml) and Amphotericin B (10 µg/ml). For the superfusion, DME was pushed upwards by peristaltic pumps (Gilson, France) at a flow rate of 30 µl/min. The fractions were collected every 30 minutes for 3 hours, after having established a baseline output for 2 hours in DME alone, and stored at -20 °C until use. In each experiment, three incubations were run in parallel with: the medium alone; the medium containing LH (50 ng/ml); the autologous pituitary kept in incubation chambers arranged in cascade in order to deliver the pituitary effluents to the testis. Each experiment was carried out in triplicate. The testes were removed from the superfusion system at the end of the incubations and stored at -20 °C until assayed for androgens.

Preparation of pituitary extracts. The aqueous extract of pituitaries was obtained homogenizing six glands with a glass homogenizer in 500 µl DME. The homogenate was centrifuged at 2000 rpm for 10 minutes at 4 °C, and the supernatant was used immediately.

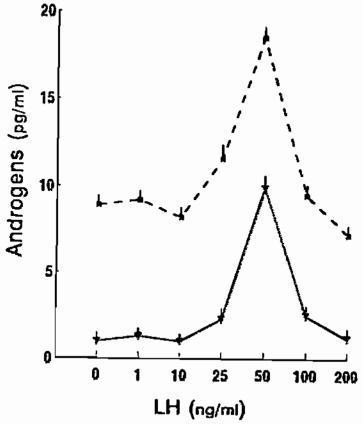

Figure 1: LH dose-response curve for androgens production, over basal secretion, by *Podarcis* testes during gonadal recrudescence (dashed line) and regression (solid line). Values represent the means ± SEM of the maximal responses, for each dose, reached 180 minutes after the addition of LH.

Static incubation. Six testes were utilized in order to obtain the seminiferous tubules and the interstitial compartment by enzymatic digestion. The tunica was removed, and the testes were incubated in DME containing 0.25 mg/ml collagenase (Sigma, type XI) and 0.02 mg/ml of DNase (Sigma, DN-25) at room temperature with constant stirring for 15 minutes. After incubation, the cell suspension was allowed to sediment for 3 minutes; then the supernatant, containing the interstitial components, and the sediment, consisting of seminiferous tubules, were separated. Intact seminiferous tubules and interstitial compartments were washed three times with ten volumes of medium; then they were transferred into test tubes, and added with medium to a final volume of 5 ml. The tubes were positioned vertically inside a recirculating water bath at 28 °C for 3 hours in the presence of oxygen and with constant shaking; under these conditions optimal responsiveness had been observed in previous experiments (CIARCIA, 1993). Aliquots (800 µl/fraction) of medium were collected every 30 minutes and immediately stored at -20 °C until analysis. In each experiment, three incubations were run in parallel with: medium alone; medium containing LH (50 ng/ml); medium containing aqueous extract of pituitaries. Experiments were carried out in triplicate.

Determination of androgens. Androgen content was measured in the testis, plasma and medium by the radioimmunoassay analysis (RIA) previously validated (CIARCIA *et al.*, 1985; ANDÒ *et. al.*, 1992). Since the antiserum used (G.F. Bolelli, Bologna) was cospecific for testosterone and 5 -dihydrotestosterone, the results are expressed as androgens. The sensitivity was 3 pg/tubes. Intrassay and interassay coefficients of variation were 6 and 12%, respectively.

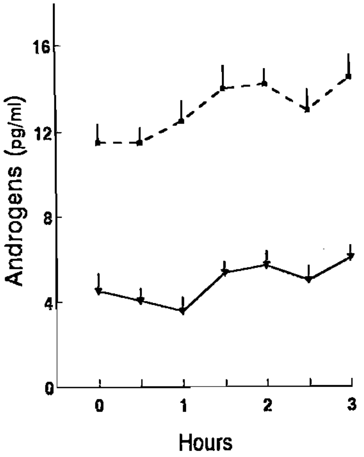

Figure 2: Variations in androgen release from superfused *Podarcis* testes. Data show means ± SEM from three separated experiments. Gonadal recrudescence: dashed line; gonadal regression: solid line. Time 0 represents the start of the superfusion.

Statistical Analysis. Data were analyzed by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test.

RESULTS

The testis of the lizard *Podarcis s. sicula,* kept in an *in vitro* superfusion system. synthesized androgens. Hormone release showed a fluctuating pattern, varying during the reproductive cycle. It generally increased during gonadal recrudescence, when all germ cell stages were present (Fig. 2). Addition of a homologous pituitary or LH to the superfusion system increased hormone output significantly only when early spring gonads were utilized (data not shown).

Hormone variations were also found within the testis, and the maximum level was reached during gonadal regression, when plasma levels are low (Fig. 3).

The seminiferous tubules and the interstitial compartment of the lizard testis, isolated by enzymatic digestion, were able to release androgens *in vitro* throughout the reproductive cycle. During gonadal recrudescence, androgen output was more abundant in the interstitial compartment (0.6 pg/testis/3h) than in seminiferous tubules (0.09 pg/testis/3h). On the contrary, during gonadal regression, the maximum output was obtained from seminiferous tubules (1.22 pg/testis/3h) (Fig. 4a). When homologous pituitary aqueous extracts (Fig. 4b) or LH (Fig. 4c) were added to the incubation media, the same results were obtained, although hormone release was enhanced.

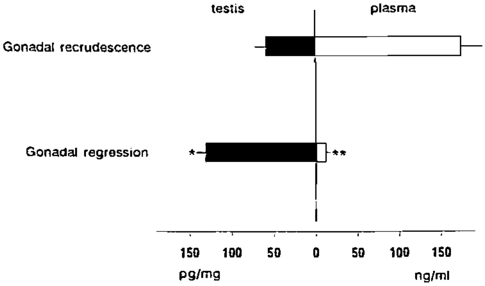

Figure 3: Intratesticular and plasma variations of androgen content in the lizard *Podarcis s. sicula* during gonadal recrudescence and regression. Data show means ± SEM from five determinations. Levels of significance versus the mean preceding values. *$P < 0.05$, **$P < 0.01$. Note the scale difference between testis and plasma.

DISCUSSION

Our results confirm that the testis of the lizard *Podarcis s. sicula* releases androgens *in vitro.* However, the pituitary or the gonadotrophin (LH) is involved in the regulation of this gonadal activity. Androgen release varies during the sexual cycle, showing a maximum level in early spring (gonadal recrudescence), when *in vitro* the testis also displays the highest responsivenes

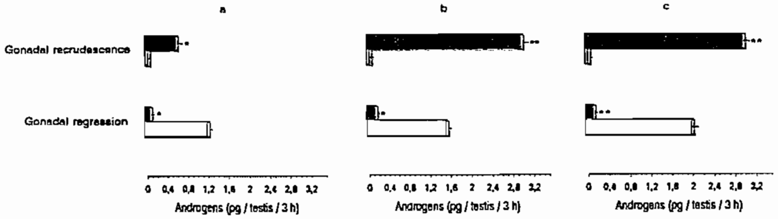

Figure 4: Androgen release from seminiferous tubules and interstitial compartment of *Podarcis* testis during gonadal recrudescence and regression. Seminiferous tubules and interstitial compartment were incubated with **a:** DME; **b:** homologous pituitary aqueous extract; **c:** LH (50 ng/ml). White bars: seminiferous tubules; black bars: interstitial compartment. Values represent means ± SEM of triplicate cultures. Levels of significance of interstitial compartment versus seminiferous tubules. $^*P < 0.05$, $^{**}P < 0.01$.

to gonadotrophin treatments.

We demonstrated that the two isolated testicular compartments are also competent to release androgens. The seminiferous tubules and the interstitial compartment show different patterns of hormone release in relation to the reproductive cycle. During gonadal recrudescence, androgens appear to be mainly released from the interstitial compartment (Leydig cells); their steroidogenic rate is consistent with the high plasma androgen levels observed in this period and with secondary sexual characters development. During gonadal regression (late summer), instead, the maximal steroidogenic activity occurs in the seminiferous tubules (Sertoli cells?). It is to be pointed out that, in this phase, the circulating androgen level is very low, whereas hormone content is quite abundant in the gonad. The synthesized hormone might be entrapped in the gonad, as has been observed in other reptiles (CALLARD *et al.*, 1976; COURTY & DUFAURE, 1979). Since in the lizard *Podarcis s. sicula,* during gonadal regression, Leydig cells are reduced (VARANO *et al.*, 1973) and seminiferous tubules contain only spermatogonia and Sertoli cells, the latter might be the main source of androgens in this period.

Androgen production by Sertoli cells has also been proposed for numerous reptilian species (MAHMOUD *et al.*, 1985; CALLARD *et al.*, 1976). It might occur during the phases of the sexual cycle characterized by complete regression of the gonad (only spermatogonia and Sertoli cells). These androgens might be supplied directly to germinal cells without being released in the blood (CALLARD *et al.*, 1989).

Androgen synthesis by Sertoli cells is, however, still controversial. In the dogfish shark *(Squalus acanthias),* it has been reported that Sertoli cells are the primary steroidogenic element of the testis (PUDNEY & CALLARD, 1984). In mammals, *in vitro* studies have shown significant androgen biosynthesis by seminiferous tubules (CHRISTENSEN & MASON, 1965), though a contamination by Leydig cells might also be possible. However, most investigations on mammals are concerned with species, like the rat, that after sexual maturity, show active spermatogenesis, with Leydig cells continuously secreting androgens. Therefore, in these species, a full complement of steroidogenic enzymes in Sertoli cells would seem to be redundant (CALLARD *et al.*, 1989). Instead, in other mammalian species with seasonal spermatogenic recrudescence, as the squirrel *Citellus lateralis,* the development of the seminiferous tubules at every reproductive cycle is associated with increases in steroidogenic enzyme activities and massive development of smooth membranes in Sertoli cells (PUDNEY, 1986).

It has been reported that, in mammals, Sertoli cells contain activity of each of the enzymes responsible for androgen synthesis, with the exception of 3β-HSDH (DORRINGTON & FRITZ, 1975; WIEBE *et al.*, 1988). However, this enzyme has been found in Sertoli cells of numerous non-mammalian vertebrates (CALLARD *et al.*, 1978), and its appearance, together with typical steroidogenic organelles (CHRISTENSEN & FAWCETT, 1961), supports a role of these cells in androgen production.

Peritubular cells, too, are known to have steroidogenic potentiality, since, in mammals, during pubertal maturation, newly differentiated Leydig cells may arise from peritubular cells that leave the lamina propria to gain access to interstitial tissue (KERR & SHARPE, 1985). This would also happen in adults following interstitial cell destruction by experimental treatment

(O'SHAUGHNESSY & MURPHY, 1991).

An origin of Leydig cells from peritubular cells has been proposed in the lizard *Lacerta muralis* (VEGNI-TALLURI & BIGLIARDI, 1983) and the skink *Chalcides chalcides* (LIMATOLA *et al.*, 1993). Therefore, in *Podarcis s. sicula,* androgen production by the regressed seminiferous tubules might also be related to peritubular cells, that during enzymatic digestion remain attached to the tubular walls, rather than to Sertoli cells. Further studies will be necessary to obtain large quantities of pure somatic cells (Leydig, Sertoli and peritubular cells) for better defining the role of these cells in lizard steroidogenesis.

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Preliminary data on the helminth communities of the Loggerhead Turtle, *Caretta caretta* (Linnaeus, 1758), in the Western Mediterranean

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Abstract: We examined the helminth fauna of the digestive tract of 29 Loggerhead Turtles (*Caretta caretta*) off Western Mediterranean. Nine helminth species were detected. Trematoda: *Calycodes anthos*, *Enodiotrema* sp., Hemiuroidea sp., *Orchidasma amphiorchis*, *Pachypsolus irroratus*, *Pleurogonius* sp., *Rhytidodes gelatinosus*. Nematoda: *Kathlania leptura*, Nematoda sp. The finding of Hemiuroidea sp. is a new parasite record for this host. The trematode *Enodiotrema* sp. showed the highest prevalence and abundance. Values of diversity, total abundance and species richness revealed depauperate helminth communities, being well within the range of those of ectothermic vertebrates.

Key Words: Parasites, Digenea, Nematoda, Helminth communities, Chelonia, Sea turtles, Mediterranean Sea.

INTRODUCTION

Marine turtles harbour a great variety of metazoan parasites belonging to the trematoda, cestoda, nematoda and hirudinea groups (LAUCKNER, 1985). Of these, digenetic trematodes are remarkable due to their species richness. Over 50 fluke species have been detected only in the green turtle (*Chelonia mydas*) and at least 35 in *Caretta caretta* (KINNE, 1985). These parasite faunas are completely different from those of terrestrial tortoises, which are dominated by nematodes (PETTER, 1966). However, marine turtles share some trematode and nematode families with freshwater species, although not any genus.

Most work on the parasite fauna of these reptiles is restricted to the analysis of a few hosts, being focused basically in systematics (LOOSS, 1901; LUHMAN, 1935; MARTIN & BAMBERGEN, 1952), corology (EUZET *et al.*, 1972; ALMOR *et al.*, 1989), pathological effects (WOLKE *et al.*, 1982; GLAZEBROOK & CAMPBELL, 1990a and b) and, scacerly, on life cycles (BERRY & CANNON, 1981). In this context, it is important to remark that the taxonomy of most sea turtle parasites is needed of revision (BLAIR & LIMPS, 1982).

Interest on the ecology of parasite communities has increased over the last years, reflecting a shift in emphasis away from descriptive studies towards more quantitative approaches to identify processes responsible for creating community patterns. To date, few attempts have been made to study the parasite communities of reptiles (AHO, 1990) and none concerning marine turtles. Moreover, the evolution of the host/parasite system makes of great interest the study of the origin and development of these parasite communities.

This work presents some preliminary data on the parasite fauna of the digestive tract of *Caretta caretta* in the western Mediterranean waters, focusing in the characterization of its helminth communities.

MATERIALS AND METHODS

Twenty-nine specimens of *Caretta caretta* were studied. The hosts came from two different sources:

Group A: 2 animals stranded on the coast of the Comunidad Valenciana, collected with the cooperation of the Conselleria de Medi Ambient of the Generalitat Valenciana in 1991 and 1992. Group B: 27 turtles seized in Barcelona in 1991. These animals had been frozen to be used illegally for commercial purposes. The samples were made available to us by the Generalitat de Catalunya and Universitat Autónoma de Barcelona (UAB). Data of the localities and dates of capture of these animals are unavailable, although they probably came from the Western Mediterranean. This hypothesis is supported by two lines of evidence. Firstly, the swordfish longline fisheries in the Spanish Mediterranean produce a number of by-catches of *C. caretta*, estimated in more than 20,000 individuals a year (AGUILAR *et al.*, 1993). Secondly, the sizes of individuals are well within the range of Western Mediterranean loggerhead turtles, which is markedly different from other Atlantic or Mediterranean populations (AGUILAR *et al.*, 1993). Therefore, in this work we will assume that all turtles were caught approximately at the same geographic area and time.

Viscerae of animals of group A were frozen at -20 °C. Organs of group B were fixed and preserved in 10% formalin. Stomach and intestine were examined separately. The latter was divided into 10 equal sections. In 20 turtles, the first three sections were also divided into three equal subsections in order to obtain more detailed information about the linear distribution of helminths. The contents of each section or subsection were flushed through sieves 0.40 mm mesh. All parasites were identified and counted.

RESULTS

Overall, nine helminth species were detected, seven digenean trematodes and two nematodes (see Tables 1 and 2). All turtles were infected with one or more helminth species. Prevalence, range of intensity and mean abundance of the parasites found in turtles of group B are shown in Table 2.

	ind. 1	ind. 2	site
Trematoda:			
Calycodes anthos		*	small intest.
Enodiotrema sp.	*	*	small intest.
Hemiuroidea sp.		*	stomach
Orchidasma amphiorchis	*		stomach
Pleurogonius sp.	*		large intest.
Rhytidodes gelatinosus	*		stomach/small int.
Nematoda:			
Kathlania leptura	*		large intest.

Table 1: Helminth species found in the gut of two stranded loggerhead sea turtles (*Caretta caretta*) on Valencian coasts (spanish Mediterranean) (ind. 1= from Javea (Alicante); ind. 2= from Sta. Pola (Alicante)).

Considering the assumption stated previously, the component community of intestinal helminths of the turtles of group B is composed by (i.-) a single core species (*sensu* BUSH & HOLMES 1986), *Enodiotrema* sp., showing the highest prevalence and abundance, (ii.-) two secondary species, *Calycodes anthos* and Hemiuroidea sp., with intermediate values and (iii.-) three satellite species, with very low values of prevalence of abundance: *Pachypsolus irroratus*, *Rhytidodes gelatinosus* and Nematoda sp. The general parameters at infracommunity level (worms of all species found within a single host) are shown in Table 3.

Concerning linear distribution patterns, we found that most species occurred in the stomach and first sections of the small intestine. *Enodiotrema* sp. showed an unimodal distribution with a peak in the first intestinal section (duodenum). The distribution of *Calycodes anthos* showed a peak in the second intestinal section but overlapped widely with that of the latter species (Figs. 1-2). Two species occurred in the large intestine: the nematode *Kathlania leptura*, a single specimen, and *Pleurogonius* sp., 3 specimens in a single turtle (Table 2). Four larvae of Nematoda sp. were found attached to the stomachal mucosa in one turtle and in poor conditions, being so, difficult to identify.

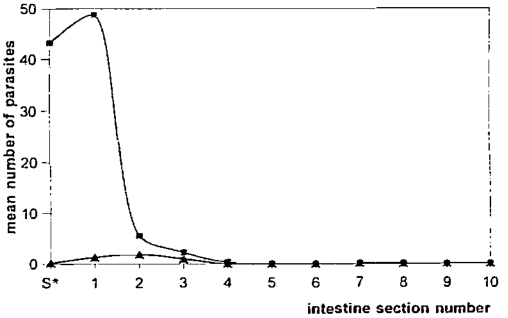

Figure1: Distribution of *Enodiotrema* sp and *C. anthos* along the stomach (S*) and intestine sections of *Caretta caretta*. ■ *Enodiotrema* sp. ▲ *Calycodes anthos*

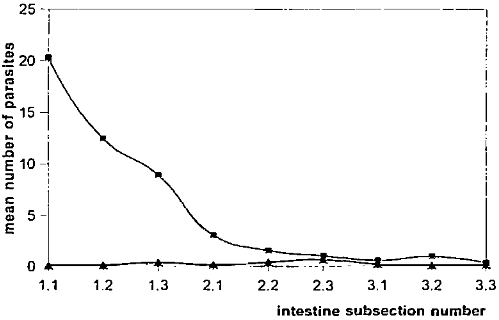

Figure 2: Distribution of *Enodiotrema* sp. and *C. anthos* along the three first intestine subsections of *Caretta caretta*. ■ *Enodiotrema* sp. ▲ *Calycodes anthos*

DISCUSSION

All helminth species, except Hemiuroidea sp. have been previously reported in *C. caretta* (Dodd, 1988). Hemiuridoids are commonly

	prevalence	range	abundance X ± SD	site
Trematoda:				
Calycodes anthos	13(48.1%)	0- 28	4.2 ± 8.1	stom./small int.
Enodiotrema sp.	25(92.6%)	0-377	100.1 ± 114.8	stom./small int.
Hemiuroidea sp.	10(37.0%)	0- 4	0.6 ± 0.9	stom./intestine.
Pachypsolus irroratus	2(7.4%)	0- 8	0.3 ± 1.5	stom./duodenum
Rhytidodes gelatinosus	1(3.7%)	0- 1	0.0 ± 0.2	small intestine
Nematoda:				
Nematoda sp.	1(3.7%)	0- 4	0.2 ± 0.9	stomach

Table 2: Helmith species found in 27 *Caretta caretta* specimens caught in western Mediterranean with the parasite prevalence, abundance and site (X = Mean; SD = Standard Deviation).

known as parasites of fishes (GIBSON & BRAY, 1979). However, BLAIR (1984) described a hemiurid, *Elytrophallus carettae*, parasitizing loggerhead sea turtles in Australian waters. Specimens detected in the present study occurred in low numbers and were immature. So, in spite of its considerable prevalence (37%), we consider that *C. caretta* is not what has been called its main or required host (HOLMES *et al.*, 1977).

	X ± SD	Range
Species richness:	1.88 ± 0.86	1-4
Helminth abund.:	105.41 ± 115.56	1-379
Diversity*:	0.17 ± 0.205	0-1.04
Eveness:	0.43 ± 0.269	0.03-1

*(Brillouin's index)

Table 3: General parameters of the infracommunities of *Caretta caretta* of group B (X= Mean; SD= Standard Deviation).

The nematode found attached to the stomachal mucosa would belong, according to the literature, to the species *Sulcascaris sulcata* (Nematoda: Ascaridoidea). It appears, however, with very low prevalence to respect to previous surveys (SEY, 1977; LICHTENFELS *et al.*, 1978). *Pleurogonius* sp. and *Kathlania leptura* showed also low prevalences and intensities. The occurrence of these three species seemed to be determined by stochastic factors.

The high number of species found in turtles of group A, should indicate at first sight a richer parasite community, but the low size of the sample (n=2) and the distance in time of the two strandings (almost a year) makes inappropriate any comparison between them or with group B. Further studies based on larger sample sizes could provide a better understanding of the compounding of the helminth communities of *C. caretta* in this area. Concerning turtles of group B, values of diversity, abundance and species richness point out to depauperate helminth communities, being well within the range of those of ectothermic vertebrates. However, the community richness is over the average among reptilia and even testudines (although data is only available from fresh water turtles and terrestrial tortoises)(KENNEDY *et al.*, 1986; AHO, 1990). At component community level, there is no clear bimodality in the distribution of helminth prevalence (see Fig.3), differing slightly respect to the core-satellite systems (BUSH & HOLMES, 1986); so we suggest that the processes involved in the shaping of these communities would be different.

Considering that distribution of parasites along the intestine have not changed due to postmortem movements, the high degree of overlapping along the first four intestine sections of two species of the same guild (ESCH *et al.* 1990)(*Enodiotrema* sp. and *Calycodes anthos*), appearing at similar sites and co-occurring frequently, might provide some possibilities for inter-specific interactions (HOLMES & PRICE, 1986). This possibility will be examined in future work.

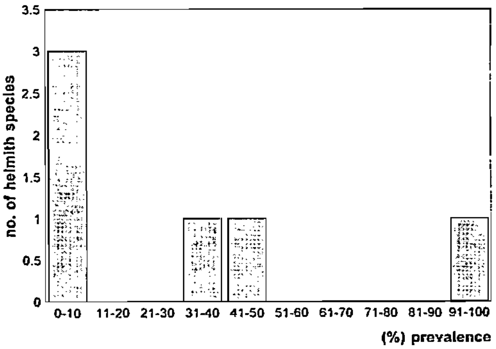

Figure 3: Prevalence patterns of the intestinal communities of *Caretta caretta*.

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An approach to the knowledge of the helminth infracommunities of Mediterranean insular lizards (*Podarcis* spp.)

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Abstract: Seven lizard species of the genus *Podarcis* living in several islands of the Mediterranean Basin, have been helminthologically searched. Nineteen helminth species have been found: 2 trematodes, 4 cestodes, 12 nematodes, and 1 acanthocephalan. The structure of the helminth infracommunities of the hosts has been analysed. There are a few component species mainly the Pharyngodonidae nematodes and the trematode *Paradistomum mutabile*. Also the larval forms of cestodes, nematodes, and acanthocephalans are qualitatively important in the helminth faunas of Mediterranean lizards. Their presence indicate that these saurians are intermediate hosts when they are taken as prey by small carnivorous or birds of prey. The diversity parameters such as species richness, abundance, and Brillouin's index, indicate that the helminth infracommunities of these lizards are depauperate and isolationist as it occurs in most saurians.

Key words: helminth infracommunities; diversity patterns; trematodes; cestodes; nematodes; acanthocephalans; *Podarcis* lizards; islands; Mediterranean Basin.

INTRODUCTION

The ecological studies of the parasite communities of reptiles are at the present time, scarce and unsystematic. AHO (1990) gives general information on the structure of the helminth communities of several reptiles and amphibians. Recently, DOBSON & PACALA (1992) and DOBSON *et al* (1992) analysed the helminth communities of ten *Anolis* species from seven islands of the Caribbean. In the Palearctic region, this kind of studies has been made only on lizards and gekkoes of the Iberian Peninsula and nearby islands (ROCA, 1993; LAFUENTE & ROCA, 1993; ROCA & HORNERO, 1994).

In the islands and islets of the Mediterranean Basin there exists a great number of lizard species of the genus *Podarcis* Wagler, 1830 probably originated in themselves (ALCOVER, 1988). All these species, together with other continental forms, came from one or very few stocks which colonized the Mediterranean area during the Mesinian period; fossils of the genus *Podarcis* are known from Majorca, Minorca, Eivissa, Formentera, Sardinia, Sicily, Malta, and Crete, so at least one of the species of the genus *Podarcis* was part of the first wave of mesinian colonizings (ALCOVER, 1988). From these *Podarcis* spp., several species occur in restricted areas, as *Podarcis pityusensis* (Boscá, 1883) and *Podarcis lilfordi* (Günther, 1874) being endemic of some islands and islets of the Balearic Islands (Western Mediterranean), or *Podarcis milensis* (Bedriaga, 1882) being endemic of a small number of islands of the Cyclad Archipelago (Eastern Mediterranean). Other species have a great distribution area living in different islands and also in continental lands of the Mediterranean Basin, as it occurs with *Podarcis muralis* (Laurenti, 1768).

In these Mediterranean islands and islets the parasitological studies of the lizards are especially interesting because: (i) the isolation of the host populations; (ii) the peculiar food habits of some of these populations; (iii) the high population densities found in some of the islands (HORNERO, 1991; ROCA, in prep.).

A total of 12 Mediterranean *Podarcis* spp. inhabit islands; some of them are only insular while others have insular and continental populations (ARNOLD & BURTON, 1978; CASTILLA *et al.*, 1991): thus, *Podarcis hispanica* (Steindachner, 1870) living in some small islets of the Iberian Mediterranean coast; *Podarcis pityusensis* and *Podarcis lilfordi* in islands and islets of the Balearic Archipelago; *Podarcis sicula* (Rafinesque, 1810) in some islands of the Mediterranean Basin; *Podarcis muralis* also in some islands of the central area of the Mediterranean sea; *Podarcis tiliguerta* (Gmelin, 1789) in Corsica and Sardinia; *Podarcis wagleriana* (Gistel, 1835) in Sicily; *Podarcis filfolensis* (Bedriaga, 1876) in Malta; *Podarcis melisellensis* (Braun, 1877) in some islands of the Adriatic sea; *Podarcis taurica* (Pallas, 1814) in some Ionic islands; *Podarcis erhardii* (Bedriaga, 1876) in some Cyclad islands and in Lesvos island; and *Podarcis milensis* in Milos and nearby islets. Seven of these insular populations have been investigated helminthologically (*P. pityusensis, P. lilfordi, P. tiliguerta, P. muralis, P. sicula, P. erhardii,* and

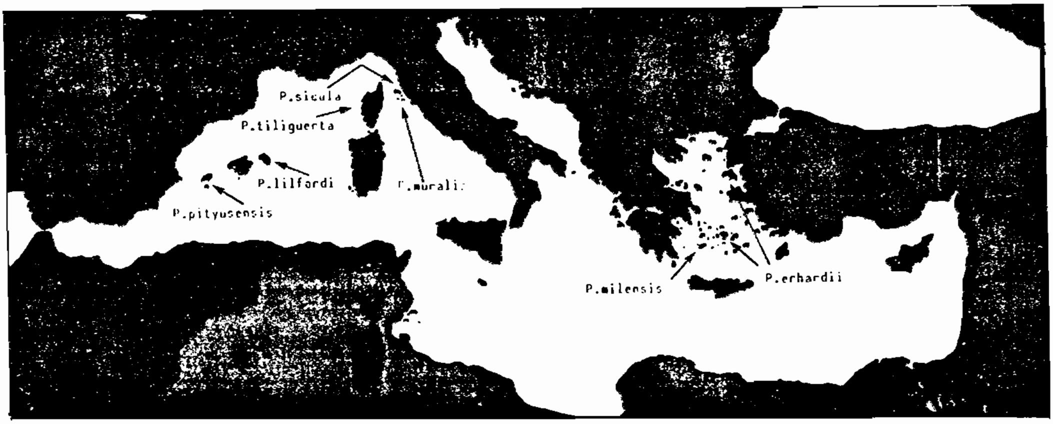

Figure 1: Sampled lizards from different islands of the Mediterranean Basin.

P. milensis) (Fig. 1) while for the other five species no data are known of their insular populations (*P. hispanica, P. wagleriana; P. filfolensis, P. melisellensis*, and *P. taurica*).

The present work includes some published data from *Podarcis pityusensis* and *Podarcis lilfordi* (ROCA, 1993; ROCA & HORNERO, 1994) and offers unpublished data on the remainder hosts.

MATERIAL AND METHODS

Some of the hosts were collected by hand. Also specimens from museums and private collections were studied after the following distribution. *Podarcis pityusensis*: -110 specimens caught by the author in the years 1987, 1988, and 1989; - 242 specimens from the private collection of Dra. Antonia Mª Cirer; -212 specimens from the private collection of the author. *Podarcis lilfordi*: - 20 specimens caught by the author in 1989; 408 specimens from the collection of Museo Nacional de Ciencias Naturales de Madrid (Spain); -113 specimens from the private collection of the author. *Podarcis tiliguerta*: -17 specimens handed over by Dr. Pierre Bartoli from the Université d'Aix-Marseille III. *Podarcis muralis*: -73 specimens from the collection of the Museo Zoologico "La Specola" di Firenze. *Podarcis sicula*: -77 specimens from the same collection. *Podarcis erhardii*: - 8 specimens handed over by Dr. Efstratios Valakos from the University of Athens; -18 specimens caught by the author in 1990. *Podarcis milensis*: -21 specimens caught by the author in 1990. All the captures were made with the appropriate permits. The provenance of the hosts is reflected in figure 1. For more detailed information see HORNERO (1991) AND ROCA & HORNERO (1990).

Helminths were collected, fixed and mounted according to routine techniques (ROCA, 1985; HORNERO, 1991). The terms referring to infestation parameters (prevalence, intensity and abundance) follow the definitions of MARGOLIS *et al.* (1982). Ecological terms referring to the parasites such as infrapopulation, infracommunity, component community, etc. are in accordance with ESCH *et al.* (1990). We chose Brillouin's index of diversity because it is especially recomended for fully censed communities (MAGURRAN, 1988) to produce results comparable with other parasitological studies (e.g. BALBUENA & RAGA, 1993; ROCA & HORNERO, 1994).

RESULTS

Systematics

TREMATODA
Family Dicrocoeliidae (Looss, 1899)
  Genus *Paradistomum* Kossack, 1910
  Paradistomum mutabile (Molin, 1859)
Family Brachylaimidae Joyeux et Foley, 1930
  Genus *Brachylaima* Dujardin, 1843
  Brachylaima sp. (*metacercariae*)
CESTODA
Family Linstowiidae Mola, 1929
  Genus *Oochoristica* Lühe, 1898
  Oochoristica gallica Dollfus, 1954
Family Nematotaeniidae Lühe, 1910
  Genus *Nematotaenia* Lühe, 1899

Nematotaenia tarentolae López-Neyra, 1944
Family Dypilidiidae Mola, 1929
Genus *Diplopylidium* (Beddard, 1913)
Diplopylidium acanthotetra (Parona, 1886) (*larvae*)
Family Mesocestoididae Perrier, 1897
Genus *Mesocestoides* Vaillant, 1863
Mesocestoides sp. (*larvae*)
NEMATODA
Family Pharyngodonidae Travassos, 1919
Genus *Skrjabinodon* Inglis, 1968
Skrjabinodon medinae (García-Calvente, 1948)
Genus *Spauligodon* Skrjabin *et al.*, 1960
Spauligodon cabrerae Castaño *et al.*, 1988
Spauligodon sp. aff. *saxicolae* Sharpilo, 1961
Spauligodon paratectipenis (Chabaud et Golvan, 1957)
Genus *Parapharyngodon* Chatterjii, 1933
Parapharyngodon bulbosus (Linstow, 1899)
Parapharyngodon micipsae (Seurat, 1917)
Parapharyngodon sp.
Family Seuratidae (Hall, 1916)
Genus *Skrjabinelazia* Sypliaxov, 1930
Skrjabinelazia hoffmanni Li, 1934
Family Strongyloididae Chitwood et McIntosh, 1934
Genus *Strongyloides* Grassi, 1879
Strongyloides ophiusensis Roca et Hornero, 1992
Family Physalopteridae Diesing, 1861
Genus *Abbreviata* Travassos, 1920
Abbreviata abbreviata (Rudolphi, 1819)
Family Acuariidae Seurat, 1913
Genus *Acuaria* Bremser, 1811
Acuaria sp. (*larvae*)
Spirurida gen. sp. (*larvae*)
ACANTHOCEPHALA
Family Gigantorhynchiidae Hamann, 1892
Genus *Centrorhynchus* Lühe, 1911
Centrorhynchus sp. (*larvae*)

All these species are well known (ROCA, 1985; HORNERO, 1991) so they are not morphologically or taxonomically studied in this paper.

Structure of the helminth infracommunities

Podarcis pityusensis (Table 1)

In this host all the helminth groups usually found in lizards, are performed. So we found two trematodes (one as larval form), four cestodes (two as larval form), seven nematodes (two as larval form), and one acanthocephalan as larval form. Only *P. mutabile, S. medinae, S. cabrerae, P. bulbosus*, and *P. micipsae* are component species (species found at least in 10% of the hosts, *sensu* BUSH *et al.*, 1990) so they constitute the component community of which four species are Pharyngodonidae nematodes.

Podarcis lilfordi (Table 2)

Its helminth fauna is similar to that of *Podarcis pityusensis* but with some differences. In this host no larval forms of cestodes have been found. The intestinal nematodes are different in both hosts. We found *Strongyloides ophiusensis* in *Podarcis pityusensis* and *Skrabinelazia hoffmanni* and *A. abbreviata* in *Podarcis lilfordi*. Only four species constitute the component community being *S. cabrerae* the species with higher values of infestation parameters.

Podarcis tiliguerta (Table 3)

Only one cestode and two nematodes have been found. The nematodes are the component community. We notice the absence of *S. cabrerae* but the presence of its possible counterpart *Spauligodon* sp. *aff. saxicolae.*

Podarcis muralis (Table 4)

The helminth infracommunity of this lizard is similar to those of the Balearic lizards but with the absences of intestinal nematodes, acanthocephalans and larval forms of *Mesocesotides* sp. The component community is formed by *P. mutabile, S. medinae, S. cabrerae*, and *P. bulbosus* being *S. medinae* the species with higher prevalence, and *S. cabrerae* the one with higher values of intensity and abundance.

Podarcis sicula (Table 5)

Only one trematode, one cestode, and four nematodes (one as larval form) have been found in the Italian Wall lizard. *P. mutabile, S. medinae*, and *S. cabrerae* are component species. Neither larval forms of cestodes, nor intestinal nematodes have been found.

Podarcis milensis (Table 6)

As occurs in *Podarcis tiliguerta*, in *Podarcis milensis* the nematode *S. cabrerae* is replaced by its counterpart *S. paratectipenis*, being the species with higher values of infestation parameters. We also notice the high presence of larval forms of Spirurida gen. sp. (*larvae*) reaching the component species level. *P. mutabile* is not a component species in this lizard.

Podarcis erhardii (Table 7)

All the species of the helminth infracommunity are component species having high prevalences. As it occurs in *Podarcis milensis*, the nematode *S. paratectipenis* is the species with higher values of prevalence, intensity and abundance.

Diversity of the helminth infracommunities of the hosts

Table 8 shows diversity parameters for the helminth infracommunities of all hosts. Values of species richness, helminth richness, and Brillouin's index are low, showing the low diversity of these parasite communities.

Excepting *P. mutabile*, the monoxenous helminths show the highest prevalences and intensities of infection so they make a greater contribution to the infracommunities structure. Thus the Pharyngodonidae *S. medinae, S. cabrerae, P. bulbosus*, and *P. micipsae* in *Podarcis pityusensis*; the same species except for *P. bulbosus* in *Podarcis lilfordi*; *S. medinae*, and *S. saxicolae* in *Podarcis tiliguerta*; *S. medinae*, and *S. cabrerae* in *Podarcis sicula*; *S. medinae*, and *S. paratectipenis* in *Podarcis milensis* and in *Podarcis erhardii*. Apart from *P. mutabile*, only the heteroxenous nematodes *S. hoffmanni* in *Podarcis erhardii* and Spirurida gen. sp. (*larvae*) in *Podarcis milensis* show high prevalences but not high abundances.

The following helminth species have been found as larval forms: *Brachylaima* sp. (*metacercariae*), *Mesocestoides* sp. (*larvae*), *Diplopylidium acanthotetra* (*larvae*), *Acuaria* sp. (*larvae*), Spirurida gen. sp. (*larvae*). Except for *Brachylaima* sp. which is an accidental species (ROCA & HORNERO, 1991), all occurs in the body cavity. Their prevalences are not high (except for Spirurida gen. sp. in *Podarcis milensis*) but they are an important part of the parasite fauna of almost all hosts.

		Prevalenc		Int. infest.		Abundance	
Helminth species	Site	n/Sampl.	%	Range	x	N	x
P. mutabile	gall-bladder	129/564	22.9	1-56	8.1	1040	1.8
Brachylaima sp.	intestine	1/564	0.2	-	-	1	-
O. gallica	intestine	25/564	4.4	1-264	30.4	759	1.3
N. tarentolae	intestine	19/564	3.4	1-10	2.6	50	0.1
D. acanthotetra	body cavity	16/56	2.8	1-35	10.1	161	0.3
Mesocestoides sp.	body cavity	9/564	1.6	1-56	12.4	112	0.2
S. medinae	cloaca	67/564	11.9	1-20	4.8	323	0.6
S. cabrerae	cloaca	193/564	34.2	1-222	20.9	4037	7.2
P. bulbosus	cloaca	104/56	18.4	1-134	17.6	182	3.2
P. micipsae	cloaca	123/564	21.8	1-15	4.6	536	1
S. ophiusensis	intestine	7/564	1.2	1-37	9.7	68	0.1
Acuaria sp.	body cavity	5/564	0.9	1-8	3.8	19	0.03
Spirurida gen. sp.	body cavity	6/564	1.1	1-6	2.8	17	0.03
Centrorhynchus sp.	body cavity	4/564	0.7	1-4	2.5	10	0.01

Table 1.- Infestation parameters of the helminths parasitizing *P. pityusensis*

		Prevalence		Int. infest		Abundance	
Helminth species	Site	n/Sampl.	%	Range	x	N	x
P. mutabile	gall-bladder	80/541	14.8	1-26	6.2	498	0.9
Brachylaima sp.	intestine	3/541	0.6	1-2	1.3	4	0.008
O. gallica	intestine	3/541	0.6	1-5	2.3	7	0.01
N. tarentolae	intestine	1/541	0.2	-	-	2	0.003
S. medinae	cloaca	101/541	18.7	1-17	4.2	429	0.8
S. cabrerae	cloaca	203/541	37.5	1-187	12.6	2565	4.7
P. bulbosus	cloaca	25/541	4.6	1-22	8.6	215	0.4
P. micipsae	cloaca	80/541	14.8	1-40	4.8	380	0.7
S. hoffmanni	intestine	4/541	0.7	1-5	2.0	8	0.01
Abbreviata sp.	intestine	1/541	0.2	-	-	1	-
Acuaria sp.	body cavity	10/541	1.8	1-20	4.2	42	0.08
Spirurida gen. sp.	body cavity	9/541	1.7	1-6	3.0	27	0.05
Centrorhynchus sp.	body cavity	5/541	0.9	1-5	2.0	10	0.02

Table 2.- Infestation parameters of the helminths parasitizing *P. lilfordi*.

		Prevalence		Int. infest.		Abundance	
Helminth species	Site	n/Sampl.	%	Range	x	N	x
Mesocestoides sp.	body cavity	1/17	5.9	-	-	102	-
S. medinae	cloaca	5/17	29.4	1-3	2.2	11	0.6
S. saxicolae	cloaca	4/17	23.5	1-6	3.8	15	0.9

Table 3.- Infestation parameters of the helminths parasitizing *P. tiliguerta*.

		Prevalence		Int. infest.		Abundance	
Helminth species	Site	n/Sampl.	%	Range	x	N	x
P. mutabile	gall-bladder	11/73	15.1	1-35	13.2	145	1.9
O. gallica	intestine	6/73	8.2	1-8	4.0	24	0.3
N. tarentolae	intestine	1/73	1.4	-	-	1	-
D. acanthotetra	body cavity	1/73	1.4	-	-	5	0.07
S. medinae	cloaca	25/73	34.2	1-44	5.6	141	1.9
S. cabrerae	cloaca	14/73	19.2	2-57	13.1	184	2.5
P. bulbosus	cloaca	9/73	12.3	4-33	13.3	120	1.6
P. micipsae	cloaca	5/73	6.8	1-10	5.4	27	0.4
Acuaria sp.	body cavity	1/73	1.4	-	-	1	-
Spirurida gen. sp.	body cavity	2/73	2.7	2-4	3.0	6	0.08

Table 4.- Infestation parameters of the helminths parasitizing *P. muralis*.

		Prevalence		Int. infest.		Abundance	
Helminth species	Site	n/Sampl.	%	Range	x	N	x
P. mutabile	gall-bladder	25/77	32.5	1-23	7.4	185	2.4
O. gallica	intestine	1/77	1.3	-	-	1	-
S. medinae	cloaca	16/77	20.8	1-18	4.5	72	0.9
S. cabrerae	cloaca	26/77	33.8	1-34	8.6	223	2.9
Acuaria sp.	body cavity	2/77	2.6	1-4	2.5	5	0.06
Spirurida gen. sp.	body cavity	4/77	5.2	3-41	12.5	50	0.6

Table 5.- Infestation parameters of the helminths parasitizing *P. sicula*.

		Prevalence		Int. infest.		Abundance	
Helminth species	Site	n/Sampl.	%	Range	x	N	x
P. mutabile	gall-bladder	2/21	9.5	3-12	7.5	15	0.7
S. medinae	cloaca	5/21	23.8	1-27	6.6	33	1.6
S. paratectipenis	cloaca	8/21	38.1	3-35	15.3	122	5.8
Parapharyngodon sp.	cloaca	1/21	4.8	-	-	1	0.05
A. abbreviata	cloaca	2/21	9.5	1-4	2.5	5	0.2
Spirurida gen. sp.	body cavity	3/21	14.3	1-2	1.3	4	0.2
Centrorhynchus sp.	body cavity	1/21	4.8	-	-	1	0.05

Table 6.- Infestation parameters of the helminths parasitizing *P. milensis*.

		Prevalence		Int. infest.		Abundance	
Helminth species	Site	n/Sampl.	%	Range	x	N	x
P. mutabile	gall-bladder	4/26	15.4	1-34	17.8	71	2.7
S. medinae	cloaca	8/26	30.8	1-12	5.9	47	1.8
S. paratectipenis	cloaca	12/26	46.2	2-103	32.9	395	15.2
S. hoffmanni	cloaca	6/26	23.1	1-5	2.2	13	0.6

Table 7.- Infestation parameters of the helminths parasitizing *P. erhardii*.

Host	Sample size	Number of species/host	Number of helminths/host	Brillouin's Index	P
P. pityusensis	564	x = 1.35±1.02 range: 0-5	x = 16.44±31.22 range: 0-420	x = 0.242±0.292 range: 0-1.211	0.60
P. lilfordi	541	x = 0.97±0.78 range: 0-4	x = 7.74±14.43 range: 0-187	x = 0.094±0.198 range: 0-0.906	0.79
P. tiliguerta	17	x = 0.59±0.71 range: 0-2	x = 7.53±24.96 range: 0-104	x = 0.034±0.121 range: 0-0.499	0.88
P. muralis	73	x = 1.03±0.93 range: 0-3	x = 8.96±14.24 range: 0-61	x = 0.147±0.249 range: 0-0.935	0.69
P. sicula	77	x = 0.96±0.94 range: 0-3	x = 6.96±10.39 range: 0-54	x = 0.141±0.248 range: 0-0.830	0.74
P. milensis	21	x = 1.05±0.92 range: 0-3	x = 8.62±12.13 range: 0-35	x = 0.097±0.216 range: 0-0.767	0.76
P. erhardii	26	x = 1.12±0.95 range: 0-3	x = 20.23±34.57 range: 0-135	x = 0.108±0.201 range: 0-0.574	0.73

Table 8: Overall diversity parameters of the helminth infracommunities from the searched hosts. (P): Proportion of sample with 0 or 1 helminth species.

DISCUSSION

In spite of the differences in the parasite species number among hosts, we can see quite homogeneous helminth fauna in Mediterranean lizards, several species parasiting almost all hosts, as *P. mutabile, O. gallica, S. medinae*, and *S. cabrerae*. This fact migth be in accordance with the hipothesis of ALCOVER (1988) regarding the common origin of these lizards.

The presence of *S. paratectipenis* in the Eastern lizards (*Podarcis milensis* and *Podarcis erhardii*) enlarges the list of the parasitized hosts and also its distribution area, which was limited to the Western part of the Mediterranean Basin (GARCÍA-CALVENTE, 1948; ROCA *et al.*, 1985), corroborating its palearctic origin and its limit to the circummediterranean area (ROCA, 1993).

The low diversity shown by the helminth infracommunities of all hosts (Table 8) suggests that they are depauperate communities. This agrees with the typical pattern of helminth infection of many reptiles (ROCA & HORNERO, 1994), poverty being related with several features of hosts such as ectothermy, simplicity of the alimentary canal, low vagility, simple diet, and generalist feeding (KENNEDY *et al.*, 1986; ROCA & HORNERO, 1994). So, helminth infracommunities of these lizards are isolationist (HOLMES & PRICE, 1986; STOCK & HOLMES, 1988; ROCA & HORNERO, 1994).

The finding of larval forms of cestodes, nematodes, and acanthocephalans in Mediterranean lizards, involves that they are intermediate or paratenic hosts of these helminths. *D. acanthotetra* (*larvae*) has been found in many places of the Mediterranean Basin (JOYEUX & BAER, 1936; LÓPEZ-NEYRA, 1947). The experiences made by PARROT & JOYEUX (1920) and the observations of LÓPEZ-NEYRA & MUÑOZ-MEDINA (1919) and ROCA & HORNERO (1991) point out cats and genets as possible definitive hosts for this cestode because they sometimes are predators of lizards. For *Mesocestoides* sp. (*larvae*), ROCA & HORNERO (1991) suggest that some bird of prey could be the definitive host, although the small carnivorous may also be definitive hosts of some species of this genus. Probably the acanthocephalan *Centrorhynchus* sp. (*larvae*) has also as definitive host a bird of prey (YAMAGUTI, 1963; HORNERO & ROCA, 1992). For the nematodes *Acuaria* sp. (*larvae*) and Spirurida gen. sp. (*larvae*), lizards could be second paratenic hosts, being the first intermediate host an invertebrate (POINAR, 1983) and the definitive host perhaps an herpetophagous bird (ROCA & HORNERO, 1991).

One of the effects of the insularity on the helminth fauna of small mammals has been noted by MAS-COMA & FELIU (1984) and MAS-COMA *et al.* (1987) as a higher prevalence of infection of the hosts with respect to hosts living in continental lands. In lizards this effect should not be generalized. The known data on lizards from the Iberian Peninsula are: p = 66.1% for *Podarcis hispanica*; p = 45.7% for *Podarcis muralis*; and p = 66.1% for *Podarcis bocagei* (HORNERO, 1991). Figure 2 shows the global prevalences of insular Mediterranean lizards. There is a group of four species, *Podarcis pityusensis, Podarcis lilfordi, Podarcis milensis*, and *Podarcis erhardii* whose prevalences (higher than 70%) are always superior to the prevalences of continental lizards. Two species, *Podarcis muralis*, and *Podarcis sicula* have higher prevalences than continental *Podarcis muralis* and *Podarcis bocagei* but lower than continental *Podarcis hispanica*. *Podarcis tiliguerta* has similar prevalences to continental lizards.

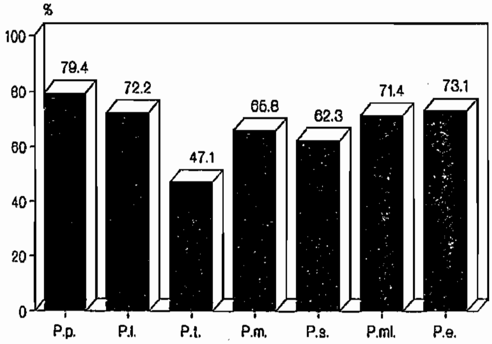

Figure 2: Histogram showing global infection prevalences of Mediterranean lizards. P.p.: *Podarcis pityusensis*; P.l.: *P. lilfordi*; P.t.: *P. tiliguerta*; P.m.: *P. muralis*; P.s.: *P. sicula*; P.mi.: *P. milensis*; P.e.: *P. erhardii*.

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Studies on endo- and ectoparasites of Canarian Lizards

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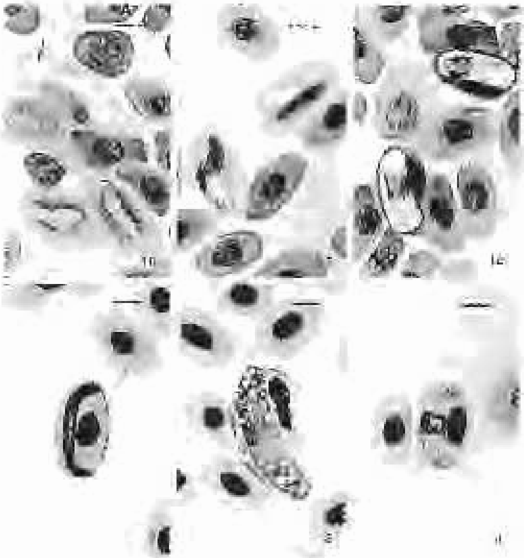
Abstract: The lizards of the genus *Gallotia*, which are endemic to the Canary Islands, are studied for their endo- and ectoparasites. The island-dwelling omnivorous lizards harbor protozoan parasites of the genus *Sarcocystis* which reveal an unusual cannibalistic mode of transmission. Haemogregarine blood parasites of as yet undetermined taxonomic status have recently been found in three *Gallotia*- species. An undescribed blood-sucking mite of the genus *Ophionyssus*, which was found on *G. galloti* from Tenerife, is suspected to be involved in the life cycle of the protozoan blood parasites. Further investigations on the presented parasites concerning their taxonomy, morphology, biology, and ecology are necessary.

Key words: *Gallotia*, Haemogregarinidae, Lacertidae, *Ophionyssus*, *Sarcocystis*.

INTRODUCTION

Island populations offer many advantages for investigating natural parasite-host relationships as for example the limited study area, the paucity of an island fauna and the relatively high population densities of many species. In this respect the Canary Islands offer almost ideal conditions with their endemic reptiles, especially the lizards of the genus *Gallotia*, whose ancestors colonized the Islands probably from the northwest of Africa developing over millions of years to these forms living today.

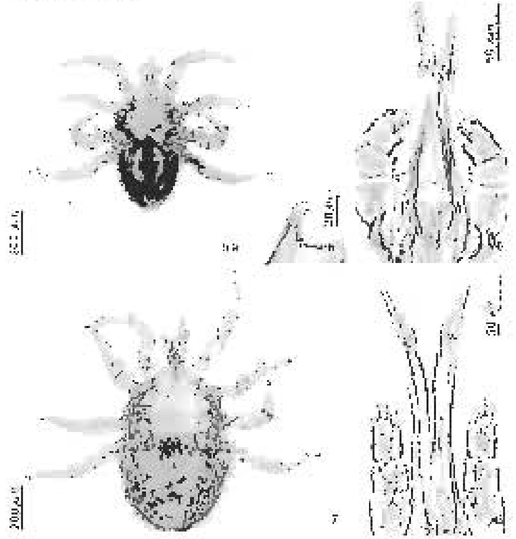
First investigations on the parasites of *Gallotia*-species lead to the discovery of an unusual life cycle of protozoa of the genus *Sarcocystis* parasitizing *G. galloti* and *G. stehlini*, which is based on a cannibalistic mode of transmission (MATUSCHKA & BANNERT 1987, 1989). Only recently, *Sarcocystis simonyi* was described from the endangered Giant lizard of El Hierro, *G. simonyi*, (BANNERT 1992). This *Sarcocystis* species exhibited a dihomoxenous life cycle with *G. simonyi* being both intermediate and definitive host. Thus far, dihomoxenous Sarcosporidia have been found only in lizards from the Macaronesian Archipelago. In 1992 we discovered for the first time haemogregarine-like parasites in blood smear preparations of three different *Gallotia*-species. The identity of these parasites remains to be elucidated. An undescribed mite of the genus *Ophionyssus*, which we found on *G. galloti* from Tenerife, is suspected to be involved as vector in the life cycle of these protozoa transmitting the blood parasites to the lizards. Further investigations concerning the taxonomy, morphology, biology, and ecology of these endo- and ectoparasites are needed to elucidate epizootiological phenomena.



Figures 1-4: Different morphological forms of haemogregarines in erythrocytes of *Gallotia* spp. Bars equal 5 µm. **1a** Newt-larvae-like form (arrow) and two intranuclear forms found in G. g. galloti from Tenerife. **1b** Spindle-like form found in *G. g. galloti* from Tenerife. **1c** Cigar-shaped form from *G. g. galloti* from Tenerife. **2** Sickle-like form found in *G. stehlini* from Gran Canaria. **3** Crescent form found in *G. g. caesaris* from Hierro. **4** Banana-shaped form found in *G. atlantica* from Lanzarote

MATERIALS AND METHODS

Blood samples were taken from Canarian lizards of the genus *Gallotia* from the Islands of Tenerife, El Hierro, La Gomera, Gran Canaria, and Lanzarote. Blood smear preparations were air dried, methanol fixed, and stained with Giemsa's solution according to standard methods. Mites were collected from *G. galloti* from Tenerife and fixed in polyvinyllactophenol or in glycerine alcohol. Light micrographs were taken with a Zeiss Axiophot photomicroscope using Agfapan 25 film. Measurements of fixed parasites were made with a calibrated ocular micrometer.



Figures 5-8 Mites of the genus *Ophionyssus* from *Gallotia galloti*. 5a Male. Dorsal view. The dorsal plate of the male, which covers almost the whole abdomen, possesses only a few bristles. 5b Hypertrophic bristle of femur III. 6 Chelicera of a male. The chelicera are in general of the Ornithonyssinae-type, however, the basal part is broader. 7 Female. Ventral view. 8 The chelicera of the female differ from those of the male by being longer and not as broad.

RESULTS AND DISCUSSION

In the blood smear preparations of *G. atlantica*, *G. galloti galloti*, *G. g. caesaris*, *G. g. gomerae* and *G. stehlini* we found haemogregarines which differ morphologically from each other (Figs. 1-4). Only in *G. g. galloti* from Tenerife we found several morphological forms of haemogregarines in single host lizards (Figs. 1a-c). Measurements of parasites are given in Table 1. The parasite stages persist in the blood cells of the lizards, which are kept in terraria without ectoparasites, for many months.

The apicomplexan family Haemogregarinidae

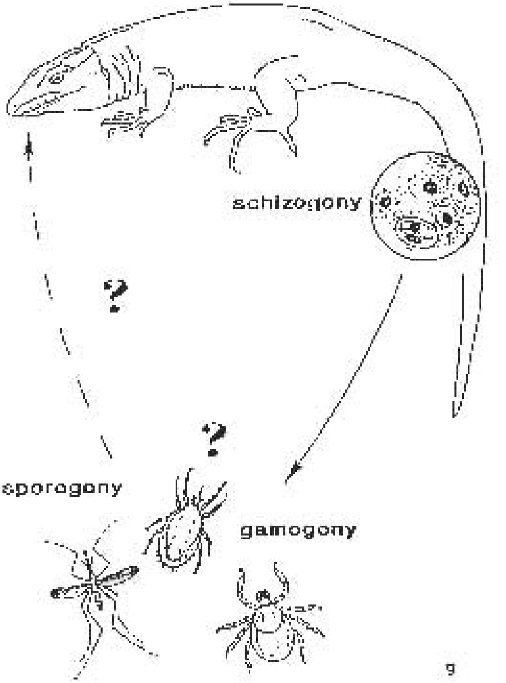


Figure 9: Haemogregarines of diverse genera are known from all groups of vertebrates. These parasites are heteroxenous cycling between a vertebrate host and a blood-sucking arthropod. An asexual proliferation (schizogony) takes place in the blood cells and inner organs of the intermediate host (vertebrate). Sexual development (gamogony) and a further asexual reproduction (sporogony) occur in the final host (arthropod vector). The life cycles of the discovered haemogregarines of the *Gallotia*-species are unknown. It remains to be elucidated whether mites or other arthropods like ticks or mosquitoes may function as vectors. The route of transmission is also unclear. If mites are involved as a vector, it could be possible that infected mites are eaten directly by the lizards or that they are swallowed accidentally due to the lizards habit of eating each other's autotomized tails. The parasites might also be transmitted by the mites during the process of blood-sucking.

contains four genera, *Haemogregarina*, *Karyolysis*, *Hepatozoon*, and *Cyrilia*. The life cycles and vectors of the great majority of haemogregarine species are unknown, so in many cases their assignment to one or another of these genera seems doubtful (LEVINE 1982). The taxonomic classification of the haemogregarines found in the *Gallotia*-species is uncertain until their life cycles become known and stages of gamogony and sporogony will be found in the vector. The scarce information in the literature concerning haemogregarines of lacertid lizards points at the mites as potential vectors (REICHENOW 1913, SVAHN 1974).

The mites which we found on *G. galloti* from Tenerife (Figs. 5-8) possess all morphological features typical for the genus *Ophionyssus*, but they differ from the species *O. lacertinus*, *O. natricis*, and *O. saurarum*, which are known from lacertid lizards (see: EVANS & TILL 1966; MICHERDZINSKI 1980). Particularly, the large conspicuous hook-shaped hypertrophic bristle on femur III of the males (Fig. 5b) differs considerably. The description of these mites as a new species will be published separately.

			Sizes		
				Host cell	
Host species	Locality	Shape parasite	Parasite	Parasitized	unparasitized
G.g. galloti	Tenerife	Intranuclear form	8.3x2.5 (6.2-10.0x2.3-3.1) n=20	14.6x8.1 (12.5-14.8x7.8-8.6) n=20	13.9x7.6 (13.3-15.6x7-9.4) n=20
G.g. galloti	Tenerife	Spindle-like	10.4x1.9 (9.4-12x1.6-2.3) n=20	15.2x9.3 (14-16.4x8.6-10.1) n=20	
G.g. galloti	Tenerife	Newt-larvae-like	15.0x2.6 (14.8-17.5x1.6-2.7) n=20	18.8x7.5 (17.2-19.5x7-10.8) n=20	
G.g. galloti	Tenerife	cigar-shaped	12.8x4.4 (11.7-15.4x3.1-4.7) n=15	20.4x9.2 (18.9-28x6.2-10.8) n=15	
G.g. gomerae	Gomera	Crescent form	12.5x2.3 (7.8-17.9x1.6-3.9) n=20	12.9x7.4 (12.5-13.3x7-7.8) n=20	13.7x7.4 (11.7-15.6x6.2-8.6) n=20
G.g. caesaris	Hierro	Crescent form	12.8x4.5 (8.1-25x2.7-9) n=16	19.4x9.6 (17.1-22.5x8.1-12.6) n=10	13.9x8.3 (9-17.1x7.2-9) n=10
G. atlantica	Lanzarote	Banana-shaped	14.5x4.7 (11.7-18x3.6-5.6) n=16	16.8x10.6 (13.8-18.9x7.2-13.5) n=10	14.1x9.2 (10.8-15.3x8.1-10.8) n=10
G. stehlini	Gran Canaria	Sickle-shaped	19x2.1 (13.5-24x1.8-2.7) n=15	18.6x6.3 (16.2-27x3.6-9) n=10	14.9x8 (13.5-17x7.2-9) n=10

Table 1: Sizes of hemogregarines and host blood cells from Canarian lizards of the genus *Gallotia*. (All measurements are given in µm.)

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Distribution and status of the Reptiles in Switzerland, a preliminary report

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Abstract: Fifteen reptile species are considered native to Switzerland. Of the six species occurring in most parts of the country *(Anguis fragilis, Lacerta agilis, Lacerta vivipara, Podarcis muralis, Natrix natrix, Coronella austriaca)*, particularly the two snakes are continuously rarefying in the heavily exploited midlands, *Coronella* now being regionally extinct. The venomous snakes *vipera aspis and berus* are predominantly inhabiting the Jura and Alp massifs, being essentially allopatric. *Aspis* has become very rare in the northeastern Jura. Another five species (*Lacerta viridis, Coluber viridiflavus, Elaphe longissima, Natrix maura, Natrix tessellata)* are confined to the southern parts of Switzerland. *Natrix maura* and *N. tessellata* have lost much of their natural habitat in the last decades and today are the most endangered of all Swiss reptiles. Whether *Emys orbicularis* and *Podarcis sicula* are autochthonous species remains uncertain. *Emys* has been introduced severas times for at least two-hundred years but today doesnlt reproduce in most parts of the country. *Podarcis sicula* has repeatedly been brought in by goods transports from Italy. The presence of the only potentially autochthonous populations in the southernmost tip of the country hasnlt been confirmed since 1987. Today the only known population lives along a railway embankment in Basel.

Key words: reptiles, Switzerland, distribution, status

INTRODUCTION

The first distribution maps covering all Swiss reptiles have been published by KRAMER and STEMMLER (1986). These were based chiefly on the authors' personal field data which appear extrapolated as shaded areas on a relief map. Meanwhile, recent surveys provided us with additional information, there we consider a new presentation of distribution maps justified. They are derived from the reptile data of the KARCH (Swiss Amphibian and Reptile Conservation Programme), which consist of personal observations, including Kramer's and Stemmler's data, and official survey projects. Although still incomplete, they are adequate to reflect the **general distribution** of all species. However, information on a species' local status is irregular and sparse for areas without any recent survey and would be misleading if included in the maps. Therefore we restrict it to brief comments in the text.

A dot on the map reflects a 5x5 km surface on which the species has been observed at least one time. Except for *Emys orbicularis*, observations date back not more than 70 years. Stars refer to introduced populations.

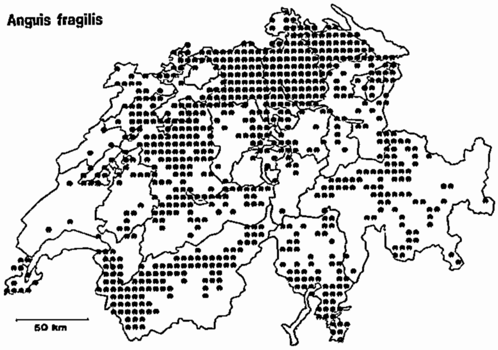
COMMENT ON THE MAPS

Switzerland covers 41'293 km^2 and consists essentially of two west-east orientated mountain chains, the *Jura* (highest peak 1607m) in the north and the *Alps* (highest peak 4807m) in the south, separated by a lowland corridor about 50 km broad, generally referred to as the *Swiss Midlands* (ranging from about 360 to 900 m).

Reptiles occur in all parts of the country up to about 2700m. Of the fifteen species considered native, five have a very limited distribution (*Lacerta viridis, Elaphe longissima, Coluber viridiflavus, Natrix maura, Natrix tessellata*), two are absent from the Midlands (*Vipera aspis, Vipera berus*) and the autochthonous status of recent populations of*Emys orbicularis* and *Podarcis sicula* is doubtful. Eight of the species reach the border of their general distribution, which is mainly due to the effectiveness of mountain chains as dispersal barriers.

Anguis fragilis

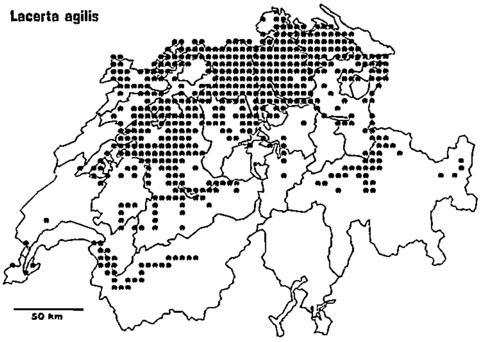
The slow worm is still a widespread animal in all parts of Switzerland up to about 2100m.

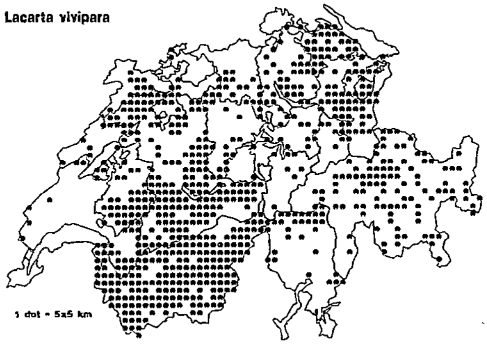


Rather ubiquitous it is found in a broad variety of habitats, ranging from marshlands and gardens to dry meadows and alpine screes. Although not on the red list, some threats accountable for a local decline are obvious.

Lacerta agilis **and** ***Lacerta vivipara***

Lacerta agilis is the lizard of the lowlands, confined to the Midlands and the lower parts of the Jura and major Alpine valleys and completely absent from the canton of Ticino.



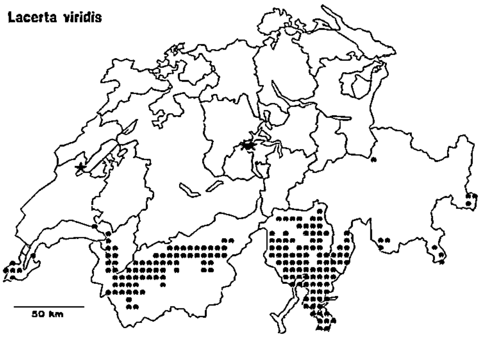


Although fairly common in the most of its Swiss range, population densities are generally shrinking. A typical inhabitant of embankments, wastelands and extensive meadows, this species is particularly suffering from habitat loss caused by ongoing over-exploitation and intensivation in agriculture.

In contrast to *L.agilis*, *L.vivipara* is the lizard of the mountains and hilly regions, with its largest populations on alpine meadows and pastures between 1200 and 2500m. The species is equally found in most parts of the Midlands, where it is generally confined to marsh- and woodlands. Unlike *agilis*, the common lizard tends to avoid human settlements and is hardly encountered in gardens or parks.

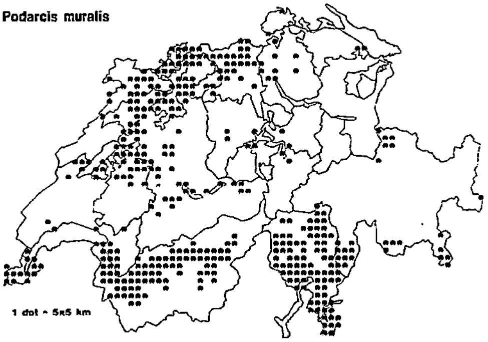
Lacerta viridis

The largest of the Swiss lizards is confined to the southern parts of the country. In the Valais and Ticino populations seem to be fairly stable, whereas along Lake Geneva the species is almost completely extinct due to severe habitat loss in the wine-growing zone. *L.viridis* occurs up to about 1400m, with some populations found at exceptional altitudes of over 2000m.



Podarcis muralis

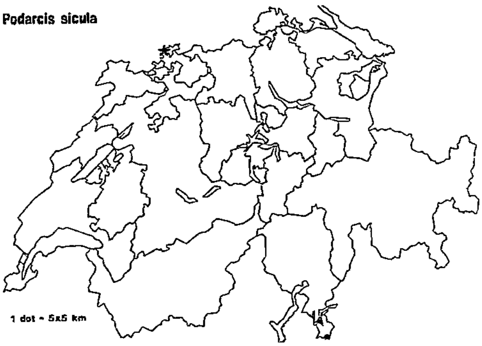
While its main range lies in the Jura and southern parts of Switzerland, recent surveys have uncovered many wall lizard populations in the Midlands and the Alps, where this thermophilous species was found in one case at 2200m (canton of Valais, Pillet pers.comm.). In northern Switzerland and at altitudes above 1000m the Wall lizard is strongly dependent on well-exposed rock cliffs and screes which provide a xerothermal microclimate. Its ability to reach high population densities in remote areas as well as in anthropic habitats together



with a considerable potential for dispersal render this species less vulnerable than the other Swiss lizards.

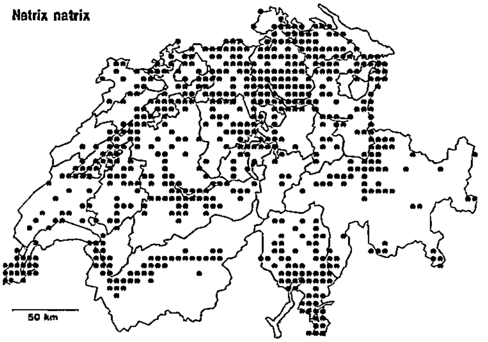
Podarcis sicula

If ever native to Switzerland, this species was confined to the southernmost tip of the canton of Ticino, where no reliable observations are available since 1987. Today, the only extant population lives at the opposite boundary of the country, along a railway embankment in Basel.



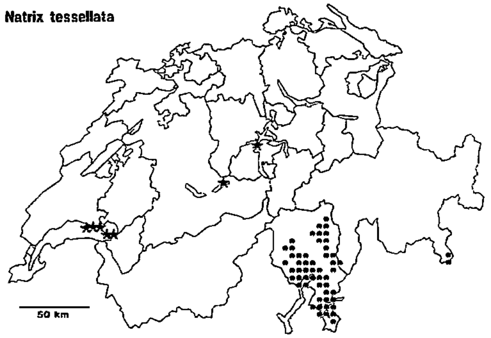
Natrix natrix

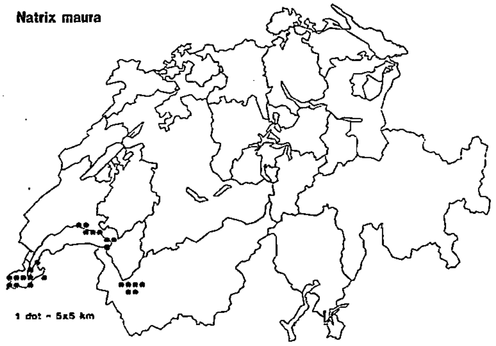
Grass snakes are found in a wide range of habitats, sometimes far away from open water bodies. In most parts of the country the main factor limiting their distribution is probably a lack of extensive undisturbed habitats, in some areas prey-density or suitable spots for oviposition. Single individuals have been observed up to 1900m in the Alps, but populations seem to be confined to altitudes of less than 1500m. The nominate form is found in the northeasternmost parts of Switzerland, the rest of the country is inhabited by the subspecies *N.n.helvetica*.



Natrix tessellata **and** ***Natrix maura***

Apart from *Podarcis sicula*, these two snakes have the smallest range of all reptiles in Switzerland. *N.maura* is confined to the bottom (below 500m) of the Rhone Valley, from the Geneva Basin along Lake Geneva up to the lower Valais. The eastern populations are severely endangered by habitat loss and may in one case also compete with introduced *N.tessellata* .

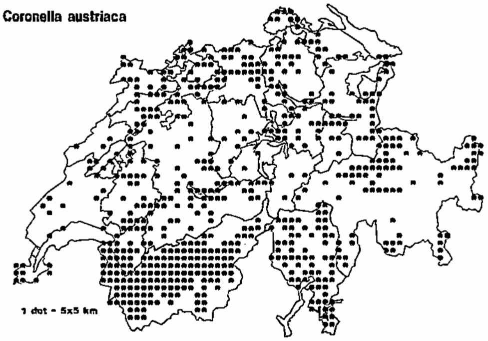


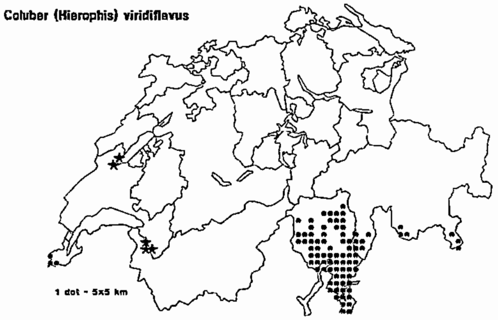


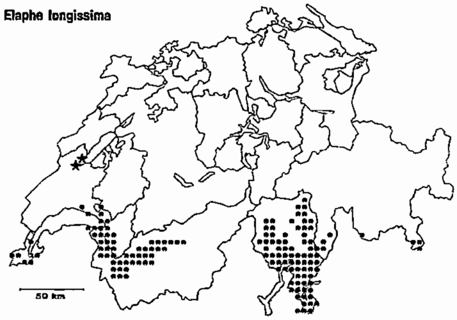
The dice snake, native to river- and lakesides of the canton of Ticino and the Posciavo up to about 900 m, has illegally been introduced to several lakesides north of the Alps, where populations are surprisingly successful. In contrast the species has rarefied markedly in large parts its original range and is now classified as endangered on the red list. Today *N.tessellata* as well as *N.maura* are in urgent need of species-specific conservation measurements in Switzerland.

Coronella austriaca

The smooth snake is found in all parts of the country up to about 2200m, but populations are probably confined to altitudes under 1700m. In the Midlands due to habitat destruction the species disappeared heavily within the last 30 years and is now locally extinct, the few remaining populations in most cases being widely separated. In the two mountain regions (Jura and Alps) several larger habitats with probably good populations remain.





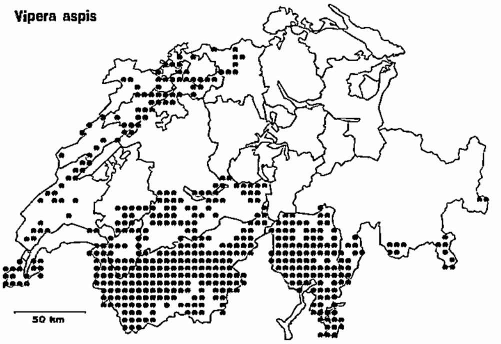


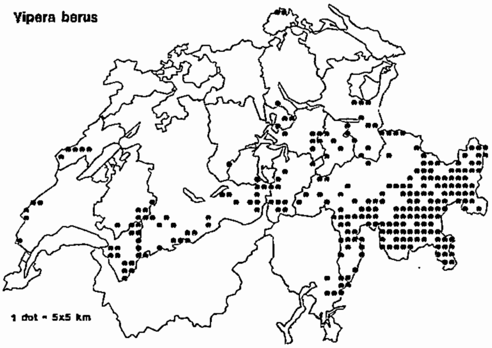
Coluber (Hierophis) viridiflavus and *Elaphe longissima*

The two largest Swiss snakes are still fairly common in most parts of their ranges, which are very similar and restricted to the southern parts of the country. While *C.viridiflavus* is mainly concentrated on the canton of Ticino, *E.longissima* is also found in larger populations in the lower Valais, where it is the most abundant snake in some areas (Pillet, pers.comm.). Both species often coexist and occur in a broad variety of habitats. They also thrive well in the vicinity of man.

Vipera aspis and *Vipera berus*

These are the two reptiles with the best known range in Switzerland. Several comments and maps on their distribution have been published (e.g. Müller 1884, Kathariner 1913, Baumann 1924, Hänseler 1965). They are essentially allopatric: *V.spis* is the typical viper of the western and southern parts of the country, *V.berus* the venomous snake of eastern Switzerland, with its largest populations in the southern Grisons. In the regions where both species occur, the asp viper generally inhabits lower altitudes up to the timber line, whereas the adder replaces it in higher altitudes up to about

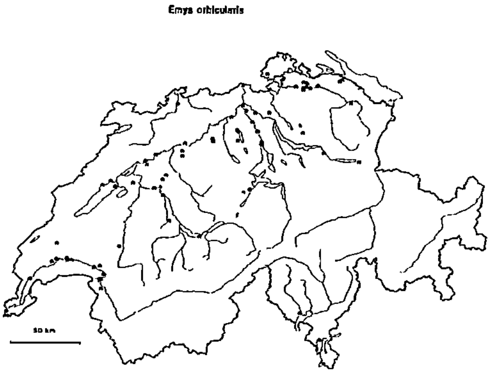




2700 m. So far we know of some six sites where both species coexist at least temporarely. Today *V.spis* is severly endangered in the northern parts of the Jura. Reaching the climatic limit of its overall distribution, here the species reacted particularly sensitive to the habitat alterations of the past decades. The northernmost populations (canton of Schaffhouse) of *V.berus* are considered extinct, whereas an assessment of its status in the western and cetral parts of the country proves difficult due to a lack of more recent surveys.

Emys orbicularis

Whether the pond tortoise ever was native to Switzerland remains in the dark. Populations inhabiting the lowland wetlands have been mentioned by several authors (Kramer and Stemmler 1986:797), but some doubted their origin, assuming that the species has regularly been introduced as a Lent food in the Middle Ages. In more recent times it most probably rarefied constantly and finally disappeared completely due to habitat loss and possibly long-term climatic changes.



Several reintroductions have been tried, but in most parts of the country the reproductive success of the populations seems to be too sparse for their survival. The map reflects all presently known observation localities.

RED LIST OF THE REPTILES OF SWITZERLAND

This list replaces the one of Hotz and Broggi (1982) and is an excerpt from the "Rote Listen der gefährdeten Tierarten der Schweiz", compiled by Peter Duelli and published in the BUWAL Red List series. The Reptile list has been revised by Kurt Grossenbacher and Ulrich Hofer. The only two species not considered endangered in Switzerland are *Anguis fragilis* and *Lacerta vivipara*. **Legend:** - absent; 0 extinct; 1 prone to extinction; 2 endangered; 3 vulnerable; 4 potentially vulnerable (small populations at edge of range); n presently not vulnerable; North: river systems of Rhine and Doubs; South: river systems of Rhone, Ticino, Adda, Etsch, Inn; CH whole country.

Species	North	South	CH
Emys orbicularis	0	0	0
Lacerta agilis	3	3	3
Lacerta viridis	0	3	3
Podarcis muralis	3	n	3
Podarcis sicula	-	4	4
Natrix natrix	2	3	3
Natrix maura	-	1	1
Natrix tessellata	-	2	2
Coronella austriaca	2	3	3
Coluber viridiflavus	-	3	3
Elaphe longissima	-	3	3
Vipera aspis	3	3	3
Vipera berus	3	2	3

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Amphibian diversity in Portuguese Natural Parks: a study project

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Abstract: In spite of their relative abundance and diversity, until now Portuguese natural populations of Amphibians have not been the subject of regular and extensive studies, aiming at monitoring their status and at detecting eventual situations of decline. The authors are undertaking a study project in two Portuguese natural parks, where Amphibian diversity is being approached at genetic, specific and community levels. The study zones are the Natural Parks of Serra da Estrela (central plateau) and of Serra de S. Mamede, where the following goals are adopted: assessment of presence/absence of species per biotope, inventory of breeding sites, quantification of populations, evaluation of conservational threats; analysis of genetic diversity, differentiation and structure of populations of some selected species of different ecology and distribution patterns; integration of biological data collected with biophysical characteristics of the regions studied through a Image Processing and Geographical Information System. This study is the first project aiming at broad knowledge of Amphibian diversity in Portugal and at providing basis for their conservational management in protected areas. Details on the sampling plan, field methods, genetic analysis, and geographic information processing are given, along with results achieved so far.

Key words: Amphibians, diversity, conservation.

INTRODUCTION

Amphibians in Portugal

Portuguese amphibiofauna includes 17 species, of which 11 Anurans and 6 Urodeles, with 6 Iberian endemisms (CRESPO AND OLIVEIRA, 1989). Their abundance and diversity is considerable in Western European terms, fact which, apart from evolutionary history, is probably connected to the relatively reduced industrial impact in much of the territory. However, growing developmental pressures of several kinds (industry, roads, forestry, urbanism) are likely to affect Amphibian communities in many regions of Portugal, potentiating populational fragmentation or declining phenomena. In this context, protected areas in general, and particularly natural parks and reserves, may have an important role in assuring habitat preservation, recolonizing potential, or in Amphibian diversity conservation in general.

Although the biology and ecology of Portuguese Amphibians has been studied from several points of view, no regular extensive studies seem to exist on Amphibian diversity and abundance in selected regions, with monitoring of species distribution per habitat, reproduction sites and seasons, populational densities, etc.. Studies of this kind are important, as they provide fundamental data for conservation, or for detection of eventual situations of menace or decline.

Aims

The aim of our project is to provide such an extensive study for two selected protected areas, approaching Amphibian fauna at genetic, specific and community levels, through:

1. Assessment of presence/absence of species per biotope, inventory of breeding sites, quantification of populations, evaluation of conservational threats.
2. Analysis of genetic diversity, differentiation and structure of populations of some selected species of different ecology and distribution patterns.
3. Integration of biological data collected with biophysical characteristics of the regions studied through a Image Processing and Geographical Information System.

Study Areas

Natural Park of Serra de S. Mamede

This Natural Park is situated in Central East Portugal, near the border with Spanish Extremadura (Figure 1). It has a total of 31750 ha, including the mountain system of S. Mamede (1025 m) and surroundings. Its altitute determines a climate of Atlantic characteristics, especially in northern slopes, contrasting with the mediterranean plateaux and rolling hills that surround it. Thus, many plants have in S. Mamede their meridional range limit, or occurr as isolates (MALATO-BELIZ, 1986). This is also reflected in the herpetofauna, which is enriched by the presence of species that are usually found more septentrionally, and that seem to constitute populational isolates - cases of *Rana iberica, Alytes obstetricans* or *Lacerta schreiberi*. S. Mamede's region is one of the most diverse in Amphibians, as 15 of the 17 Portuguese species are present (Table 1). This was one of the reasons to select it as study area.

Natural Park of Serra da Estrela

This Natural Park includes the highest mountain system of continental Portugal (1998 m), situated in Central North Portugal (Figure 1), and extending through 100000 ha. The area of the Park situated above 1400 m high (the Central Plateau) is characterized by sub-alpine biotopes of reduced human impact, for which a biogenetic reserve has been proposed. Serra da Estrela has an interesting and abundant herpetofauna (MALKMUS, 1985), with an Amphibian community composed of 11 species (Table 2). The extension of the Park and its relatively central position, make it an important area for Amphibian conservation. This and the differences in geo-climatology in relation to Serra de S. Mamede (altitude, temperature, pluviometry, etc.), favoured its selection as study area, restricted to the Central Plateau.

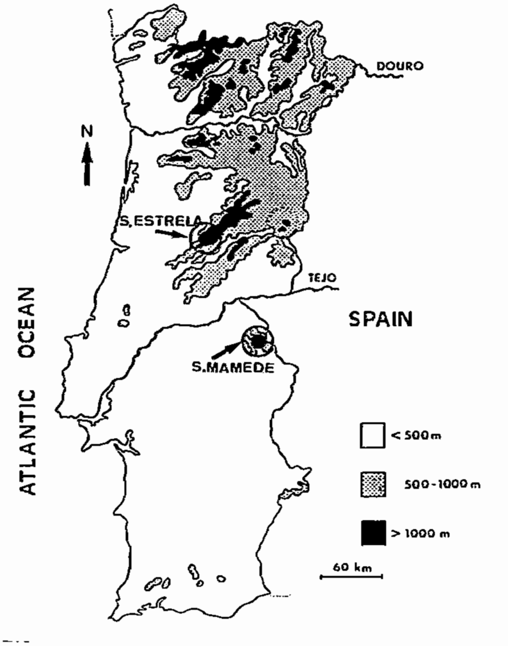

Figure 1: Localization of the study areas in Portugal

METHODS

Sampling plan

For definition and choice of sampling stations, the UTM (1 km x 1 km) grid of each zone was characterized for parameters likely to be relevant for Amphibiofauna distribution: altitude, water availabitily, pluviometry, temperature. UTM squares were chosen in paired replicates covering the classes defined for these parameters, and covering the main water lines and sectors of each area. The sampling strategy defined resulted in the selection of 48 stations (UTM squares) for Serra de S. Mamede and 30 for Serra da Estrela, to be sampled monthly during one year.

Discoglossus pictus
Alytes cisternasii
Alytes obstetricans
Pelobates cultripes
Pelodytes punctatus
Bufo calamita
Bufo bufo
Hyla arborea
Hyla meridionalis
Rana iberica
Rana perezi
Pleurodeles waltl
Salamandra salamandra
Triturus boscai
Triturus marmoratus

Table 1: Amphibians of Serra de S. Mamede

Field methods

Methods for field work followed the reccomendations of HEYER *et al.* (1993), adapted to our field situations as follows. For each station, a circuit has been established including transects along one main water line and a course on land. In each water line 3 x 100 m were marked for visual encounter survey, and 4 x 10 m were marked for quantitative sampling.

The course on land include the main biotopes of the UTM square for visual encounter survey, and they include sampling in any relevant water point (pits, fountains, pools). Pools are sampled visually along 2 x 100 m of margin (or all the margin if of smaller perimeter), and quantitatively for 10 m. Pits are sampled visually and through the use of traps. Diurnal sampling is complemented by nocturnal controls in each station, for visual survey of at least one of the 10 m segments marked in the water line, and for acoustic survey of anuran activity.

Alytes obstetricans
Pelobates cultripes
Bufo calamita
Bufo bufo
Hyla arborea
Rana iberica
Rana perezi
Chioglossa lusitanica
Salamandra salamandra
Triturus boscai
Triturus marmoratus

Table 2: Amphibians of Serra da Estrela

The observer and hour of survey for each station are alternated each month, to avoid biases. Data are collected in especially designed sheets, and they include information on the individuals observed (species, number, developmental stage), and on characteristics of its habitat (vegetation cover, stream width and depth, weather, time of the day, associated species, etc.).

Genetic analisys

Genetic diversity and differentiation will be approached through standard allozyme electrophoresis, following methods of PASTEUR *et al.* (1987), comparing samples of populations of selected species from the study areas and from other regions. Six species of anurans were chosen, belonging to three genera, and having different ecologies and distribution patterns: *Alytes obstetricans, A. cisternasii, Rana iberica, R. perezi, Hyla arborea,* and *H. meridionalis.* Sampling will cover 3 to 4 zones additional to the study areas, and will be based on 20 to 30 protein loci, screened through standard starch gel and cellulose acetate electrophoresis. Genetic diversity will be evaluated through parameters such as mean heterozygosity and polymorphism, and genetic differentiation through genetic distance indexes. Replicate sampling for some species will provide information on eventual intra-area genetic heterogeneity.

Geographic information

A Image Processing and Geographic Information System (TNT-MIPS) will be used for data processing, and for integration of information on biophysical characteristics of the study areas with information on Amphibian fauna. The cartography of the study zones will be digitalized, along with data on hidrography, climate, vegetation, and other information available. These will be integrated and correlated with the biological data collected, for evaluation of the factors underlying Amphibian distribution, diversity and abundance.

RESULTS

Our project on Amphibian diversity has started in April 1993, and is planned to be concluded by December 1994. Results achieved so far have revealed the following aspects:

- The methodology adopted seems to cover the essential of specific diversity of each study area, as in few months of field work almost all species known to occurr in the study areas have been detected.
- Local distribution for some species has revealed to be broader than supposed from previous field missions; for example, both *R. iberica* and *A. obstetricans* seem to be widespread in Serra de S. Mamede, and not restricted to few small areas.
- A sucession of species in activity throughout the year becomes evident from numbers obtained in surveys, and from acoustic controls; these, and also egg clutches, larvae, and individuals in reproductive period, are providing precise information on breeding seasons.
- The importance of different habitats for life and reproduction of each species is also becoming evident from the methodology and sampling strategy adopted.
- Allozyme pilot-studies suggest different patterns of genetic structure of populations according to the species, with some revealing scarce differentiation between all populations sampled (cases of *A. cisternasii* and *H. arborea),* and others revealing a high degree of differentiation and populational fragmentation (such as *R. iberica* and *A. obstetricans).*
- Situations of populational isolation in Serra de S. Mamede, inferred from known distribution of *R. iberica* and *A. obstetricans,* seem to be confirmed in different degrees by field and genetic data.

The authors hope that the study project presented herein will give basis for standardized monitoring techniques of Amphibian study in other areas, and that it will favour integration with other projects of study of decline and conservation of Amphibians in Europe.

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Amphibians and reptiles of Groane Regional Park (Lombardy, NW Italy). First census and ecological notes

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Abstract: The author shows the preliminary results of Amphibians and Reptiles census in the Groane Regional Park. Eight Amphibian species (*Salamandra salamandra, Triturus carnifex, Triturus vulgaris, Bufo viridis, Rana* synklepton *"esculenta", Rana dalmatina, Rana latastei, Hyla arborea*) and nine reptiles species (*Trachemys scripta, Emys orbicularis, Anguis fragilis, Lacerta viridis, Podarcis muralis, Coluber viridiflavus, Coronella austriaca, Natrix natrix, Vipera aspis*) are present. Two species (*Bufo bufo* and *Elaphe longissima*) were pointed out some years ago, but recent sightings lack to confirm their presence. Remarkable populations of *T. vulgaris* and *R. latastei* are present in some non optimal habitats. The presence of a little *Vipera aspis* population is also reported; this population seems to be the nearest to Milan.

Key words: Amphibians, Reptiles, "Groane" Regional Park, heathland, ecology.

INTRODUCTION

The "Groane" Regional Park is located north of Milan and it was founded to protect heathland habitats, that are decreasing in the Po Valley. Heathland is characterised by a low diversity in plant communities, the most important of which is the ericoid shrub community. Italian heathlands are quite different from north-European ones, because of a greater diversity of plant communities and the tendency to reach a *climax* stadium typically of woodlands. Fire is probably the main factor allowing the maintenance of some heathlands in Italy.

Heathland herpetofauna of Italy has been a little studied and it seems to be generally poor (POZZI, 1980b). Some studies were made in northern Europe (SPELLERBERG, 1989; STRIJBOSCH, 1992; STUMPEL, 1992) and the importance of heathlands such a suitable habitat for reptiles was stressed. MASSA (1988: 48-53) studied the vertebrates of "Groane" Park and pointed out five amphibian species and eight reptiles species.

Clayey soil in "Groane" Park is waterproof and it enables the formation of many water pools in open zones and in woods (the typical "groane" canals). I analysed some sample zones, representative of most interesting habitats; these areas were located during surveys of the author and with the aid of forest warders. Some heathland zones ("Ca' del Re" [zone 5 in Fig. 1], west part of Cesate pine-wood [zone 7], heathland along the provincial road Monza-Saronno [zone 6], "Castellazzo di Bollate" [zone 10]), the most important wetlands ("Oasi di Lentate" [zone 1], "Foppa di San Dalmazio" [zone 4], pools near Ceriano Laghetto [zone 4], "Alzaia Villoresi" [zone 9]) and some wood

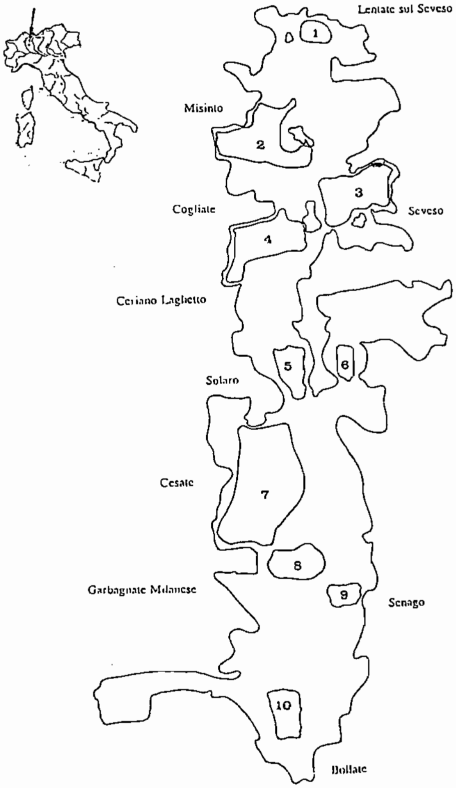

Figure 1: Map of Groane Regional Park. Numbered zones are the most interesting areas in the Park. Studied areas: 1: "Oasi di Lentate", 2: S. Andrea woods, 3: Seveso highland, 4: Cogliate and Ceriano Laghetto woods, 5: "Ca' del Re", 6: Limbiate heathland, 7: Cesate pine-wood, 8: Garbagnate Milanese Hospital Park, 9: "Alzaia Villoresi", 10: "Castellazzo di Bollate"

zones (Misinto [zone 2], Cogliate and Ceriano Laghetto [zone 4], Cesate Pine-wood [zone 7], Garbagnate Hospital [zone 8]) were analysed. *Pinus sylvestris*, *Betula pendula*, *Quercus robur* e *Robinia pseudacacia* are the best represented tree species. A large part of the Park is characterised by an high rate of human activities, such as agriculture, industry and building, in and out Park borders.

Area number	Main habitat
1, 9	Wet areas
2, 4, 8	Deciduous woods
3, 5, 6, 10	Heathlands
7	Heathlands and pine-woods

Table 1: Main habitats present (see legend of the studied areas in figure 1).

MATERIALS AND METHODS

Census was carried out starting from February to October, during 1991 and 1992. I made two or three excursions a week. In spring I privileged suitable habitats for the reproductions of the amphibians. Observations were made by direct sighting, using a binocular and capturing specimens. Anuran census was integrated by observations of breeding sites and mating calls. Literature and personal communications were also used.

RESULTS

Eight amphibian species (*Salamandra salamandra*, *Triturus carnifex*, *Triturus vulgaris*, *Bufo viridis*, *Rana* synklepton *"esculenta"*, *Rana dalmatina*, *Rana latastei*, *Hyla arborea*) and nine reptiles species (*Trachemys scripta*, *Emys orbicularis*, *Anguis fragilis*, *Lacerta viridis*, *Podarcis muralis*, *Coluber viridiflavus*, *Coronella austriaca*, *Natrix natrix*, *Vipera aspis*) were found (see Tab. 2).

Some of these species (*S. salamandra*, *E. orbicularis*, *A. fragilis*, *C. austriaca*) were not sighted, but they were pointed out by other authors or by local observators. *S. salamandra*, *R. dalmatina*, *A. fragilis*, *C. scripta*, *E. orbicularis*) were not listed by MASSA (1988).

Amphibia

Salamandra salamandra. It has been observed many times, in past years. Small populations of the spotted salamander are probably still present near the southern border of the Park, particularly in the neighbours of Bollate, where it reproduces in some canals, including "groane" canals.

Triturus carnifex. It's a very common species, its distribution being practically the same of that of *T. vulgaris*. Breeding habitat is characterised by pools in deciduous woods and in open

	ZONES									
SPECIES	1	2	3	4	5	6	7	8	9	10
S. salamandra	-	-	-	-	-	-	-	-	-	+
T. carnifex	-	-	-	+	+	-	+	+	-	+
T. vulgaris	-	-	-	+	+	-	+	+	-	+
B. viridis	-	-	+	+	-	-	-	-	-	-
R. dalmatina	-	+	-	+	+	+	+	-	-	+
R. latastei	-	+	-	+	+	+	+	-	-	+
R. synklepton *"esculenta"*	+	+	+	+	+	+	+	+	+	+
H. arborea	+	+	+	+	+	+	+	-	+	+
C. scripta	-	-	-	-	-	-	-	-	-	+
E. orbicularis	-	-	-	-	-	-	-	-	-	+
A. fragilis	-	-	+	-	-	-	-	-	-	-
L. viridis	+	+	+	+	+	+	+	-	+	+
P. muralis	+	+	+	+	+	+	+	+	+	+
C. viridiflavus	+	-	+	+	+	+	+	-	-	+
C. austriaca	-	-	+	-	-	-	-	-	-	-
N. natrix	+	-	-	+	+	-	-	-	+	-
V. aspis	-	-	-	-	-	-	-	-	-	-

Table 2: Presence of Amphibians and Reptiles in the studied areas. (+): Present, (-): Not present.

heathland areas (GIACOMA, 1988). *T. carnifex* is often present in the same places of *T. vulgaris*, but there is evidence that some micro habitat differences do exist (cf. DOLMEN, 1988).

Triturus vulgaris. It is one of the most widespread species; its distribution is almost the same of that of *T. carnifex*. There are large populations in central and southern parts of the Park. Some pools in the commune of Ceriano Laghetto [zone 4] are particularly interesting because of the presence of about 250 specimens of *T. vulgaris* during 1992 (SCALI, 1992). These pools were recently dredged and there was not aquatic vegetation at all.

Bufo viridis. This species has a great endurance capacity to habitat conditions altered by human activities. It is present in the central and southern part of the Park with a few specimens. Some breeding sites were found near Seveso [zone 3] and some specimens were found killed by cars on the roads, during their breeding migrations. Matings occur starting from the end of March.

Rana synklepton *"esculenta"*. It is the most widespread Anuran; it is present in every wet area. Its adaptability enables it to colonize very degradated habitats too. *R.* synklepton *"esculenta"* and *Hyla arborea* are the only two Amphibian species that use the greatest pools for deposition [zones 1 and 9]; these sites are often populated by fishes (*Cyprinus carpio*, *Ictalurus melas*, *Carassius carassius*). Mating season starts in April and it goes on during May.

Rana dalmatina. It is distributed in the central and southern parts, particularly in pools and "groane" canals of deciduous trees woodlands. It often cohabits with *R. latastei*, but there is a relatively apparent dominance of this or of the other species depending on different cases. For example in some pools in Ceriano woods [zone 4] the clutch ratio (*R. latastei*/*R. dalmatina*) was about 2:1 (about 100 egg laying of *R. latastei*/about 50 of *R. dalmatina*). On the other hand in "Foppa di San Dalmazio" [zone 4], where water is abundant, the clutch ratio was about 1:3 (SCALI, 1992). Mating season starts in March and it ends at the beginning of April. During 1992 the mating season lasted until the end of April, because of a long drought that prevented egg-laying. In this case the *R. dalmatina* mating season overlapped those of *H. arborea* and *R.* synklepton *"esculenta"*.

Rana latastei. It is another common species; it is one of the most important entity of the local herpetofauna. It is present in the same habitats of *R. dalmatina* and it has a central and southern distribution. It is commonly reported opinion that *R. latastei* is connected with plain woods, but in the Park it is well adapted to open habitats and I found eggs in heathland pools. In such cases the nearest woods were some hundreds meters far from. It is particularly interesting the colonization of the pools in Ceriano Laghetto [zone 4], where I sighted about one hundred of egg-layings (SCALI, 1992). Its mating period entirely overlap with that of *R. dalmatina*.

Hyla arborea. It is common all over the Park, being easily recorded by male calling from April to October. It is present in the same habitats of *R.* synklepton *"esculenta"* and it breeds either in woods or in heathlands. Sometimes matings occur in very little temporary pools for mating. An important population is present in the "Oasi di Lentate" [zone 1] near the northern border of the Park; this is one of the most important ponds of "Groane" Park. In this place I heard a chorus of some several hundreds of males in April and I found several hundreds of young tree frogs staying on *Typha* in July. Mating period starts in April and it ends at the beginning of May.

Reptilia

Trachemys scripta. This species was introduced in some areas of the Park, mainly near some sport fishing ponds. Four adult specimens were observed in a pond near "Castellazzo di Bollate" [zone 10], but informations concerning their acclimation and eventual reproduction in the Park are not available.

Emys orbicularis. The European pond turtle suffered a heavy throw-back in northern Italy, so its elective habitats should be protected. It is particularly interesting a capture of one specimen in "Castellazzo di Bollate" on 14 May 1983 [zone 10] (V. Ferri, pers. comm.), but the presence of this species at present must be verified.

Anguis fragilis. Though it is a very common species in the areas surrounding the Park, it is difficult to find it, because of its elusive behaviour. One specimen was found killed by a car in Seveso [zone 3] on 26 February 1993.

Lacerta viridis. It is very common, particularly in the heathlands in the central part. It lives where grass and shrubs are abundant and it is more strictly tied to this habitats than the other lacertid, *Podarcis muralis*. It is active beginning from the end of March to October.

Podarcis muralis. It is the most common lizard in the Park; it is present in any habitat: heathlands, wet areas and borders of woods. It is easy too to find near houses and burrows. It is active from March to October, but it is possible to find it in winter too, during hot days.

Coronella austriaca. Though it is a rather common species, it is very difficult to find, because it is active at dusk or at night. One dead specimen was found by a forest warder near Seveso [zone 3] (MASSA, 1988).

Coluber viridiflavus. It is the most common snake in the Park. It is present in every habitat, but it is particularly frequent in open zones, well exposed to sun, as heathlands; the termophily of this species is so confirmed. These habitats are particularly rich of lizards and little mammalians, that are often preyed by *C. viridiflavus*.

Natrix natrix. It is mostly distributed near big ponds, such as "Oasi di Lentate" and "Alzaia Villoresi", where its favourite preys, the Anurans, particularly *R.* synklepton *"esculenta"* are abundant. It is present in some woody areas in the central part too. Some specimens were observed while they were hunting catfishes (*Ictalurus melas*) trapped in pools during summer drought.

Vipera aspis. It is distributed in some areas, mostly in woods in the central part (Cogliate and Ceriano Laghetto [zone 4]). The population is probably small, but it is very interesting, likely being the nearest to Milan. In fact *V. aspis* went out in surrounding areas, because of human activities and it is present only northward, in the province of Varese. I sighted one specimen in Cogliate woods, near a cycle track on 24 July 1993.

CONCLUSIONS

The herpetofauna in "Groane" Park is rather differentiated, thank to the presence of some species considered good or excellent indicators, such as *S. salamandra*, *R. dalmatina*, *R. latastei*, *T. vulgaris meridionalis*, *V. aspis*. The presence of asp viper and of the spotted salamander is particularly valuable, the first because this area seems to be the nearest stand to Milan, the second being disappeared in the Po Valley about one hundred years ago. It is necessary to verify the presence of *E. orbicularis*, because it would be another relict entity, being extinct in the whole area of "Brianza" (FERRI, 1992: 61). On this subject Park authorities should control the letting in of some *Emydidae* foreign species, such as *Trachemys scripta*, because they are potential competitors of *E. orbicularis*.

The remarkable populations of *Rana latastei*, *R. dalmatina* and *Triturus vulgaris meridionalis* in the central part of the Park are interesting. In particular the two brown frog species showed a certain adaptability to different habitats, that enables them to reproduce in non optimal habitats too (cf. POZZI, 1980a); this is confirmed by the great number of eggs in pools far away from woods, that are still considered the elective habitats of these frogs (POZZI, 1976, 1980a, 1980b, 1982; DOLCE, LAPINI & STERGULC, 1982, ZUFFI, 1988). Smooth newt is reducing its distribution in northern Italy because of human activities, but here it is present with remarkable populations and it seems not to have surviving problems.

Bufo bufo and *Elaphe longissima* were not observed, but they are surely present in some areas surrounding the Park. The common toad may migrate in the northern part of the Park during mating period, mostly in "Oasi di Lentate". Aesculapian snake, instead, may not be found because of its elusiveness and its sylvan habits.

On the whole the herpetofauna of "Groane" Regional Park is rather rich and wide; some new species may add to the 17 till now observed, thank to other researches.

Comparing this number with the results of some researches conducted in other parks of north-western Italy we can see that the quantity of species is a little less than the 22 found by ZUFFI (1988) in the "Parco Lombardo della Valle del Ticino", but more than the 11 found by POZZI (1980b) in Rovasenda heathland and in "Bosco della Partecipanza" in Piemonte (POZZI, 1982) and by ZUFFI (1986) in "Bosco della Partecipanza". This result, contrasting with POZZI (1980b), may be justified by the great variety of habitats in the Park and by the particular soil conformation, that enables the formation of many little pools where Amphibians can reproduce. Furthermore some Reptile species take advantage by the presence of heathlands, where zones suitable for basking and hunting are in contact with shrubs that provide protection from predators (SPELLERBERG, 1989). On the contrary, wet woods are particularly suitable for the most terrestrial Anurans, such as brown frogs and green toad, and some Reptiles, such as asp viper, grass snake and, probably, aesculapian snake.

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Distribution and Status of *Hyla meridionalis* in Italy

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Abstract: The stripeless tree frog *Hyla meridionalis* reaches in Liguria (NW Italy) the eastern limit of its worldwide distribution. *H. meridionalis* lives along the ligurian coast, where the climate is sub-mediterranean, from sea level to about 600 m. Breeding sites are commonly found in agricoltural lands (46%), in natural or semi-natural habitats (38%), but this species can successfully reproduce in urban environments (16%). No areas of sympatry between *H. meridionalis* and *H. arborea* are known in Italy, although the nearest populations of these two species are separated by less than 15 km. The ecological status of the stripeless tree frog in Italy seems satisfying.

Key words: *Hyla meridionalis*, *Hyla arborea*, ecology, Italy

INTRODUCTION

The stripeless tree frog *Hyla meridionalis* (BOETTGER, 1874) is found in North Africa, South-West of the Iberian Peninsula, Catalonia, Southern France and North-West of the Italian peninsula (BARBADILLO ESCRIVA, 1987; LANZA, 1983; PAILLETTE, 1989; SALVADOR, 1985). Data on the distribution and ecology of *Hyla meridionalis* in Italy are lacking and no specific study on this species has ever been conducted.

The first Authors who suggested the presence of *Hyla meridionalis* (at that time *Hyla arborea meridionalis*) in North-western Italy were CAMERANO and LESSONA (1885), while PARONA (1902) confirmed this assumption after the examination of about 100 specimens from eastern Liguria. Later, CAPOCACCIA (1956) observed that in Tuscany only the green tree frog *Hyla arborea* (Linnaeus, 1758) was present, the exact range of the stripeless tree frog being unknown. Recently, LANZA (1983) reviewing the distribution of *Hyla meridionalis* and *Hyla arborea* in Italy, suggested that both species could be present in the eastern part of Liguria (NW Italy).

SOURCES OF RECORDS

All the available data on the stripeless tree frog in Italy were taken into consideration. Scientific literature (BOANO & DELMASTRO, 1989; BOLOGNA, 1972; CAMERANO & LESSONA, 1885; CAPOCACCIA, 1956; PARONA, 1902; TORCHIO, 1963), and the herpetological collections of the Museum of Natural History of Genova, of Firenze, of Torino and of Carmagnola yielded many historical records. Data posterior to 1980 were obtained from the Ligurian Herpetological Data Base (LHDB, a database implemented by the Museum of Natural History "G. Doria" of Genova) and from original field researches conducted by the Authors in 1990-1993.

THE STATUS OF *Hyla meridionalis* IN ITALY

In Italy, the stripeless tree frog is found exclusively in the north-western part of the peninsula, and all the known breeding sites fall within the limits of Liguria administrative region. The distribution of *H. meridionalis* is continuous along the Ligurian coast (fig. 1), and the eastern limit of the species worldwide distribution is located few kilometers East of the city of La Spezia (eastern Liguria, near Tuscany).

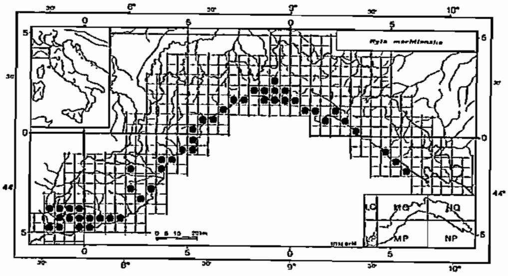

Figure 1: The distribution, in a 5 km UTM grid, of *Hyla meridionalis* (Boettger, 1874) in Italy.

This distribution is clearly related to a sub-mediterranean climate, since *H. meridionalis* is completely lacking above 600 m of altitude, both in the Maritime Alps and in the Apennines. The area in which *H. meridionalis* is more abundant is the western Riviera, where the climate is mediterranean (GENTILE, 1986).

About 40% of the records are located below 50 m, and only 8% above 300 m (fig. 2a). The highest known population lives at an elevation of about 570 m in the western part of Liguria.

Breeding sites are commonly found in agricoltural lands (in reservoirs located in vine-yards, olive-yards and near hot-houses) and in natural or semi-natural environments. Moreover, this species reproduces successfully in urban habitats, such as parks and domestic gardens (fig. 2b), where relatively large populations can be observed.

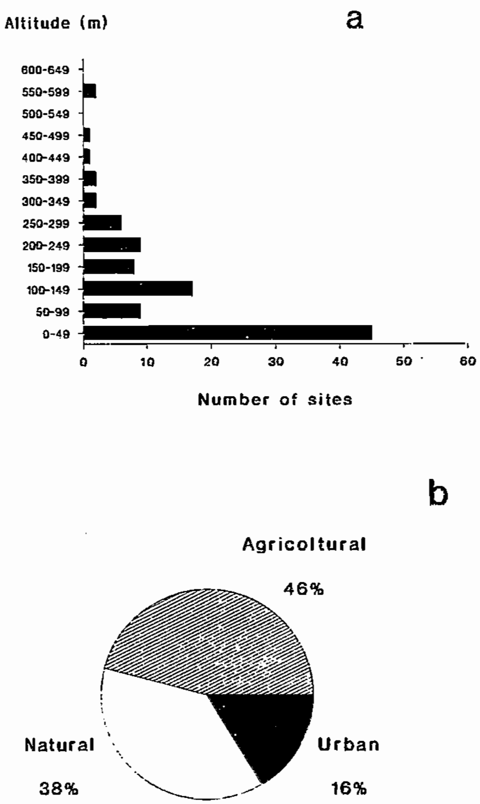

Figure 2: a) Altitudinal distribution of *Hyla meridionalis* breeding sites; b) habitats of *Hyla meridionalis* in NW Italy.

In Europe, *H. meridionalis* and *H. arborea* are largely parapatric and few areas of sympatry are known (OLIVERA *et al.*, 1991).

In the Italian peninsula, these two species are strictly parapatric, and no areas of sympatry have been found, even if extensive researches were conducted along the Magra river (eastern Liguria), where the nearest populations of *H.meridionalis* and *H. arborea* are separated by less than 15 km. No decline of the stripeless tree frog populations has been observed, as all the historical records have been reconfirmed by recent observations. In many areas, *H. meridionalis* is widespread and locally abundant and some breeding sites are included in Regional Nature Reserves, in which natural habitats and animal species are protected. On the other hand, *H. arborea* appears to be declining in the eastern part of Liguria, since populations are now sparse, and an historical record West of the Magra river has not been reconfirmed by field researches.

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Preliminary data on the Amphibians of a sand quarry in Northern Italy

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Abstract: Since February 1993 we have started a field study on Amphibian populations in a sand quarry near Milan. We have found the following species: *Rana* kl. *esculenta, Hyla arborea, Bufo viridis, Triturus carnifex.* The quarry environment is nowadays one of the few still exploitable by Amphibians in the surroundings of a large city like Milan, so we feel that it is particularly important to collect some more data about the Amphibians living and breeding in these places.

Key words: Quarry, Amphibians, Breeding site

INTRODUCTION

The water bodies belonging to an active quarry, even if they are not directly used in the extracting activities are, of course, far from being ideal for the Amphibians. There is always a heavy disturbance in terms of noise and vehicle transit near the water bodies. Moreover, all these little water bodies have a limited life (two or three years), and are normally drained or destroyed after this period.

However in the surroundings of Milan there are very few damp areas which Amphibians can exploit. Quarries are becoming more and more important just because other kinds of water bodies (such as the ones in the cultivated fields or in the cities) are even more disturbed: this is the main reason why we decided to investigate some aspects of the biology and ecology of Amphibians living in a quarry.

MATERIALS & METHODS

The field work was carried out from February to August 1993.

The SANNOVO S.N.C. sand quarry of Zibido S. Giacomo (Milan) was chosen because it had some very different water bodies in a relatively small area: in fact, in the spring of 1990, a project of environmental recovery was carried out in a marginal area to create an almost natural pond habitat which now is adjacent to the active part of the quarry. Therefore this quarry seemed suitable to examine and compare population size and structure, habitat preferences, movements to and from the water, sexual activities, feeding habits of the resident Amphibians, as well as the variation of these parameters over the years and the efficacy of this kind of environmental recovery for Amphibians. Of course these few months only allowed us to collect some preliminary data: the research will go on the next years to achieve more complete results.

The field work was carried out in the eight water bodies marked on the map and detailed later. We have found 4 species of Amphibians: the green frog (*Rana* kl. *esculenta*), the tree frog (*Hyla arborea*), the green toad (*Bufo viridis*) and the Italian crested newt (*Triturus carnifex*).

The Amphibians were detected by means of visual observation, calls recognition, netting in the water bodies. Since 22nd March we have placed a net surrounding the pond (site #4) and the decantation pool (site #6) with 30 fall traps for each site. The net was 40 cm high; the traps were cylindrical, 24 cm high with a diameter of 18 cm, and were placed on both sides of the net.

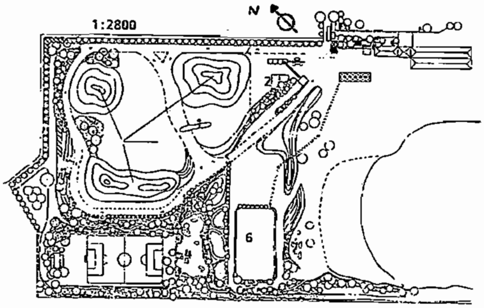

Figure 1: Map of the Sannovo Sand Quarry. Numbers 1 to 8 point out the position of the 8 investigated water bodies.

RESULTS

Here is a short description of the 8 investigated Sites (see Figure 1) with a list of the species found in each site.

SITE NO. 1: SMALL POOLS located among unused machineries. Vegetation is locally abundant, locally absent at all. Maximum depth is 20 cm. Breeding Amphibians: *Rana* kl. *esculenta, Hyla arborea, Bufo viridis.*

SITE NO. 2: LARGE SQUARE POOL, very deep, with vertical walls, no vegetation. Next year it will be covered and used to stock materials, but in 1993 it was filled by rain water and it was left undisturbed. Breeding Amphibians: *Rana* kl. *esculenta, Triturus carnifex.*

SITE NO. 3: CANAL communicating with the pond and also hosting fish. Vegetation is well-structured. Breeding Amphibians: *Rana* kl. *esculenta. Hyla arborea* is present but not breeding.

SITE NO. 4: POND in an almost natural state, because of the 1990 recovery project. It is surrounded by a green belt which separates it from the working area. It has an immissary and an emissary, and water is pumped in from the big quarry lake (simulating springs) to avoid overheating. Typical Italian hydrophytes were introduced to speed up the naturalization process; the only problem here is the presence of fish, also introduced with the same project. *Rana* kl. *esculenta, Hyla arborea* and *Triturus carnifex* are present but not breeding.

SITE NO. 5: SMALL POOL located near the pond and completely invaded by cane-brake so that there is no open water. *Rana* kl. *esculenta* and *Hyla arborea* are present but not breeding.

SITE NO. 6: DECANTATION POOL where the continuous pouring in of muddy water causes wide variations of the water level, temperature and transparency. Vegetation is present only on the banks. Breeding Amphibians: *Rana*kl. *esculenta* and *Hyla arborea; Bufo viridis* and *Triturus carnifex* are present but not breeding.

SITE NO. 7: SMALL POOLS near the lake from which materials are extracted. Water quantity is very variable; maximum depth is 20 cm; vegetation is very scarce. Breeding Amphibians: *Hyla arborea* and *Bufo viridis. Rana* kl. *esculenta* is present but not breeding.

SITE NO. 8: CANAL smaller than no.3, with less water and less vegetal cover, also communicating with the pond and hosting fish. *Hyla arborea* and *Rana* kl. *esculenta* are present but not breeding.

DISCUSSION

It is surely too early to draw conclusions about the ecology of the Amphibians living in the quarry; many other aspects of their activities have still to be analyzed, and will be in the next months.

However we can point out some observations from the data collected.

About the habitat preferences of the four species:

- *Rana* synklepton *esculenta* is the most abundant and widespread species. It can be found in all the eight water bodies, but it is not reproductive everywhere. Presence of fish could be a deterrent for reproduction (a reason appliable to sites #4 and #8; note also that only one egg clutch was laid in site #3, hosting fish), as well as water scarcity (appliable to sites #5 and #7).
- *Hyla arborea* is more abundant in the water bodies surrounded by cane-brake, which are better suited for the calling males.
- *Bufo viridis* seems to prefer the sites with no aquatic vegetation.
- *Triturus carnifex* seems to prefer the larger, deeper sites, with a lot of open water.

And in general:

- Water bodies with fish are rarely used by Amphibians.
- The reproductive activity is not continuous. There are some isolated reproductive episodes, which maybe can be related to the climate. The spawnings took place in the nights following very hot and damp days.
- The calling activity was very unfrequent and weak if compared to other areas. Maybe the loud noise coming from the machines of the quarry makes the calling too expensive and ineffective an activity to rely on.

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The Loggerhead distribution in the Western Mediterranean Sea as deduced from captures by the Spanish Long Line Fishery.

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Abstract: The loggerhead (*Caretta caretta*) is the most abundant sea turtle in the Mediterranean. Widely distributed in coastal tropical and subtropical waters around the world, this turtle is common in western Mediterranean sea, mainly in summer season. Mediterranean nesting beaches in Greece and north Africa coast and Florida nesting beaches in western Atlantic are probably the origin of the specimens captured by the Spanish Mediterranean long liners. Surface long line fisheries are very common in the Mediterranean countries. In Italy, Greece and Spain, a heterogeneous fleet around 1400 boats operating from more than 200 ports. In Spain some 145 long liners are target for swordfish (*Xiphias gladius*). Occasionally there are 100 others artisanal vessels, during the summer season, which could use the gear for swordfish in coastal waters. Captures reaching 1100 MT in 1989 and this capture are stabilized. Bluefin tuna and swordfish have important nursery grounds in different Mediterranean areas. Marine turtles, principally the loggerhead are incidentally captured during the fishing activity period. Inquiries in the main Spanish Mediterranean landing port (Alicante) during the 1988-1992 period have reported information on abundance and distribution of loggerhead, a non commercial and protected species by national and international laws. Geographical distribution present some important aspects: - The presence of the loggerhead in western Mediterranean waters is constant all around the year. - Waters around Balearic Island represent the main concentration area of juveniles loggerhead in eastern Mediterranean. - A migratory pattern in western Mediterranean could be conform by both eastern Mediterranean and western Atlantic specimens.

Key words: *Caretta caretta*, incidental captures, fisheries western Mediterranean, surface long line.

INTRODUCTION.

The Loggerhead turtle, (*Caretta caretta* L.), is a species belonging to the Cheloniidae family whose principal characteristics remind us that we find ourselves before a group whose ancestors were common in the Cretaceous, 130 million years ago (MARQUEZ, 1990). The long history of environmental changes, which should be collected in their genetic information, makes us think of organisms with extraordinary adaptive capacity. In spite of this fact the aspects that make them vulnerable, since before their birth, are numerous, such as the scarcity of laying beaches and the mortality due to predators going for eggs and young, on land and in the sea, in their initial phase. To this series of natural processes is added, since many millennia ago, fishing. For many centuries it has been a species with a clear target capture for its commercialization, although this species is actually protected by the Agreement of Commerce of Protected Species (CITES) and the signatory countries of the same are obliged to come up with the necessary legal means to avoid its illegal commerce and better the means of conservation of the species.

Mediterranean long line fishery.

The Mediterranean is a semi-closed sea with important areas of reproduction for large pelagic fishes (DICENTA, 1977; DICENTA & PICCINETTI, 1977; DUCLERC *et al*; RODRIGUEZ RODA & DICENTA,1981). Tuna and swordfish have important nursery grounds in different areas and marine turtles, principally the Loggerhead (*Caretta caretta* have breeding sites in Greece, Italy and other beaches in the Eastern and Southern Mediterranean (MARGARITOULIS, 1980; GELDIAY ,1981; ARGANO & BALDARI,1983). Surface long line fisheries are very common in the Western Mediterranean countries.

Information on by-catches in Mediterranean drifting long lines are very few. Some reports exist , mainly in Spain and Italy, on incidental catches of marine mammals, turtles, and in relation to sharks, but there isn't systematic information on this subject.

The surface long-line fisheries in the Mediterranean have the Swordfish (*Xiphias gladius*) and albacore (*Thunnus alalunga*) as the most important targets. Some other pelagic species are captured by the same gear, because there is space-food competence during some periods of the year. These incidental species could be separated as follows:

-species with commercial value and landed.

-species without commercial interest or protected.

The second group of species includes turtles, marine mammals and others. There is no collective information on the quantity of landings of the first group, nor captures and releases or discards of the second in the Mediterranean.

There doesn't exist in the Mediterranean a fishery directed to the capture of the Loggerhead, but our personal observations (CAMIÑAS, 1988; CAMIÑAS *et al*, 1992) and those of other authors in the Western Mediterranean(ARGANO & BALDARI, 1983; BRUNO, 1969; 1970; 1986 DE METRIO *et al*, 1983; LAURENT 1990a; 1991; and MAYOL, 1988; 1990) demonstrate that diverse fisheries affect this species. The fishing gear that have a more direct influence are the surface long-line (AGUILAR *et al*, 1992; CAMIÑAS *et al*, 1992; DEMETRIO & MEGALOFONOU, 1988; LAURENT 1990a; MAYOL *et al*, 1988), the bottom trawl (BRADAI, 1992; LAURENT, 1990a; LAURENT *et al*, 1990) and the gill nets (ARGANO *et al*, 1992; DE LA SERNA, 1991; LAURENT, 1991; PANOU *et al*, 1992).

In Italy, surface long-lines are used in several places along the coast, but mostly in Sicily, Puglia, Sardinia, Campania and Liguria by a very large fleet (more than 1500 vessels of various sizes). The use of this gear in the Sicilian seas dates back to 177 BC and it, in that time, was supplied with a little number of hooks. Around 1970 its nearby use began to increase and spread to all nearby seas, such as the Gulf of Taranto, South Adriatic to the Aegean sea. It is used principally for swordfish and albacore fishing. The gear used is supplied with a number of hooks from 250 to 3000 for the Swordfish fisheries and from 750 to 4000 for the Albacore. Swordfish juvenile gear, similar to Albacore, with some modifications is extensive in Italian areas too.

The majority of vessels target adult swordfish, with important by-catch components of sharks, other fishes, and marine turtles and sometimes, other protected species such as cetaceans .

In the Gulf of Taranto, the incidence of this fishing gear on some protected species such as *Caretta caretta* and *Dermochelys coriacea*, has been studied (De Metrio et al, 1983).

Surface long-lines are used in the Greek seas mainly for fishing Swordfish and to a far less extent for Albacore (Panou et al, 1992). Swordfish fishing is an expanding activity and it is estimated that 400 boats, operating from 70 different ports were involved in this fishery in 1991. Fishery is carried out from October to May throughout most of the Aegean sea, off the west coast of Greece in the Ionian Sea, and occasionally in the Levant sea towards Cyprus. Regular monitoring of incidental catches started in 1989 in the Ionian Sea and are regularly recorded for eighth vessels based in Kelfalonia island, Central Ionian Sea (Panou et al, 1992). The peak of the fishing season is from May to September and usually more than 70% of the total annual catch occurs during this time period. In the last five years the annual production of the Greek fleets has fluctuated from 900 to 2000 metric tons. The two main fishing fleets for Swordfish in Greece are based in Kalymos, in the south-eastern Aegean sea and in Chania, on the island of Crete. Both fleets are using identical fishing methods but they are working in different areas. Together they account for more than 50% of the total annual Greek production and more or less up to 80% of the production in the Aegean sea. Albacore fishing is less popular and is carried out in the Central and Northern part of the Aegean sea, mainly around the islands of the Sporades complex and the Chalkidiki peninsula. Fishing is carried out using either troll or long-line. It is estimated that 180 vessels are involved in the fishery and the annual production during the last five years has been approximately 500 metric tons.

In Spain, some 145 long-liners are targeting Swordfish. Occasionally there are 100 other artisanal vessels, during the Summer season, which could use the surface long-line for Swordfish in coastal waters. The Spanish Swordfish fishery covers the whole of the Western Mediterranean from 42°N to 8°E. Fishing is carried out all year round, with peak effort in Summer. In the Spanish Mediterranean long-line fishery, the non-targeted species represent 10% of the total landings in weight in the last years. The captures of *Caretta caretta* are not included in that percentage of landings

In France the drifting long-line fishery is little developed and there isn't a fleet interested in swordfish.

Spanish long-line fishery

The Spanish long-line fisheries traditionally exploit the swordfish (*Xiphias gladius*). Interest in this species has grown in the last years, due to the high price it has acquired on the market and the socio-economic importance that this activity represents in the Mediterranean fishing sector.

The capture of this species has fallen to less than 800 tons in 1992, from values of more than 1700 tons in 1988. The fishing effort, measured in thousands of hooks per year, has gone slowly down in the last years until reaching the figure of 6.9 x 10 hooks in 1992 (Figure 1). Effort

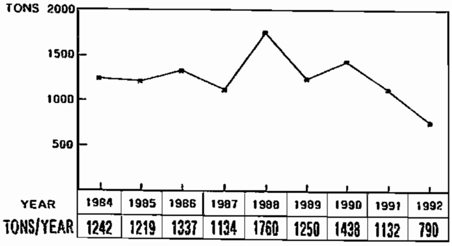

Figure 1: Spanish long line mediterranean captures. 1984-1992 Series.

variations through the months repeat themselves yearly, following the scheme of Figure 2 : the increase of effort takes place from the month of May, principally due to the increase of reproducers of the target species in the Summer months, that carries with it an increase of the number of hooks that work in the zone, as well as better meteorological and market conditions, and the concentrations of young in Autumn.

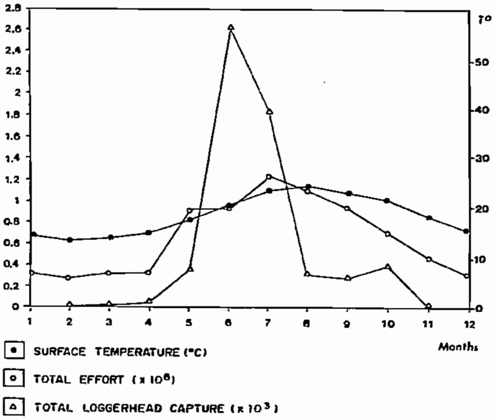

Figure 2: Monthly Loggerhead captures, spanish mediterranean long line total effort and surface mean temperature in Western Mediterranean.

The landings in the ports of Alicante, Palma de Majorca, Cartagena, and Aguilas represent approximately 60% of the Spanish captures in the Mediterranean. During the Summer and Autumn months about 70% of the yearly total effort is made.

The gear drafting surface long-line gear that the Spanish fleet use in the Mediterranean doesn't have standard measurements. Each long-line unit is composed of the following elements: mainline, branch line, filament and hook. The variety of each element not show any regional differences. In keeping with the characteristics of the vessel, capacity, season and environmental factors a determined size and number of hooks per vessel are released. In winter-spring between 90 and 115 long-lines (1000-1300 hooks) are released and between 130 and 160 in Summer-Autumn (1500-2000 hooks) (DE LA SERNA, 1991).

Fishing with surface long-line gear is regulated in Spain by order of the 18 of January of 1984, including in the same, the maximum length of the long-line, the number and size of the hooks.

The fishing of the loggerhead takes place when this species tries to eat the bait of the long-lines. Different species are used as, bait depending on the origin of the vessel. The most important are, mackerels (*Scomber scombrus*); cuttlefish (*Scomber japonicus*); sardinella (*Sardinella aurita*); (various species) the latter is frozen, also used are pieces of different species of sharks proceeding from previous catches.

The long-line is normally handled in the evening, the fishing lasts until the first hours of the night, and is brought in lever before dawn. Normally, when the long -line has been completely let out (between 2-3 hours depending on length and number of hooks), the vessel drifts, with the gear until it begins to set sail 6-8 hours later. The time taken to bring up the gear depends principally on the amount fished, approximately 3 hours. Once clean, preparation for handling the next day takes place.

The incidental catches of loggerhead in the Western Mediterranean have been put in the limelight on several occasions, as we have already mentioned. The IEO initiating their control by means of a network of information and sampling from 1986 (CAMIÑAS, 1988) even though MAYOL (1986) had studied the incidence of this species in the fleet that lands in Palma de Majorca, although originating partially on the peninsula.

MATERIALS AND METHODS

In order to obtain the information on long-line fishing the IEO counts on a network of observers in the principal Mediterranean ports. In this way facts on capture, efforts and fishing areas are obtained. More information is obtained by means of tagging campaigns. The systematic collection of data referring to the loggerhead is done when the fleet lands at the port of Alicante. This port represents a high percentage, as much in landing, as in efforts. The value of the effort of Alicante, with respect to the rest of the Spanish fleet in the Mediterranean in the period 1986-1991, varies between 33.2% and 42.7%. The dispersion of landings in more than 20 different ports of the region allows us to make sure these facts are representative of the Mediterranean. With the daily surveys of the skippers that land in Alicante, data of by-catches are elaborated with landings in this port, extrapolating the rest of the Mediterranean fleet (CAMIÑAS, 1986;1992).

Once the catch data from each vessel and for each landing have been obtained, a species distribution map is made in areas of one degree by one degree for the entire region. This process has been done monthly in the aforementioned period. From the geographical distribution of the captures, and the comparison of each month in the different years of observation, guide lines of distribution in the area are obtained, these have served to make some behavioral hypotheses.

RESULTS

Due to the type of survey done, no data on the size of specimen has been regularly obtained, although there are samples some occasionally been found. Even so, there is information obtained in the same region through tagging campaigns directed towards the loggerhead (AGUILAR *et al*, 1992; ANONYMOUS, 1991). This data, apart from those obtained from stranding, indicate that most of the specimens caught in the summer months belong to a sub-adult population whose carapace length varies between 27 cm and 75 cm, with modal values of 47,4 cm and 48,8 cm (AGUILAR *et al*, 1992).

Catches

Table 1 shows the historical series of by-catches of the long-line fleet that landed in Alicante in the period 1986 to 1992. Considering the yearly date, a slow increase from January leads to maximum catches in the Summer months (July- August). In Autumn the values are again lower to arrive at a Winter minimum. The catches have a clear correspondence to the efforts and surface temperature (Figure 3).

The number of specimens caught per year varies between 8389 in 1987 and 8308 in 1991 (Figure 4). This figure, is clearly different from the rest of the years, even though there had been a decrease in the number of outings of the fleet and those in which there had been accidental capture of the turtles (Table 2)

	1986	1987	1988	1989	1990	1991	1992	mean
Jan.	2	0	0	12	13	4	0	4
Feb.	4	1	1	5	1	0	3	2
Mar.	7	22	6	17	0	11	8	10
Apr.	12	115	18	46	8	6	0	29
May	308	739	253	355	140	60	72	275
Jun.	634	2732	709	2633	1253	171	34	1167
Jul.	3664	2906	1193	1842	3360	2409	194	2224
Aug.	2497	1438	2966	316	1619	698	269	1400
Sep.	219	385	267	281	724	343	86	329
Oct.	82	29	224	397	293	70	21	159
Nov.	49	21	36	23	57	28	3	31
Dec.	73	1	2	15	10	3	3	15
Tot.	7551	8389	5675	5942	7478	3803	693	5647

Table 1: Landings (in metric Tons) of Sword fish (*Xiphias gladius* L.) in the E.C. Mediterranean countries.

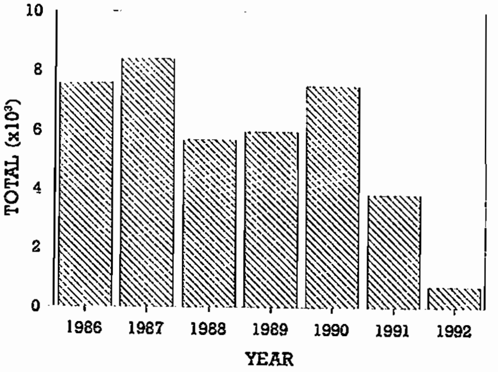

Figure 3: Total stimated incidental captures of Loggerhead from Alicante's data.

The highest levels of incidental catches were obtained in the years 1986 and 1987. 1990 was very similar to 1986 and the years 88 and 89 similar to each other, with values of between 5 and 6 thousand turtles, caught by the fleet that lands in Alicante. If we consider that the fishing and survey strategy have been similar during the whole series, it seems that there should exist other factors, different to long-line fishing, that condition the number of individuals in the area.

	N° Vessels	Fishing area	Landings (Tm) 1989	1990	1991
Italy	717 (1)	South Thyrrenian Sea	2989	4592	3629
Greece	400	Aegean and Ionian Sea	1120	1344	1904
Spain	245 (2)	Western Mediterranean	1250	1438	1132

(1) long-line and other gear included
(2) +/- 100 vessels occasionaly

Table 2: Incidental catches of *Caretta caretta* L. Alicante Spanish mediterranean longline fleet.

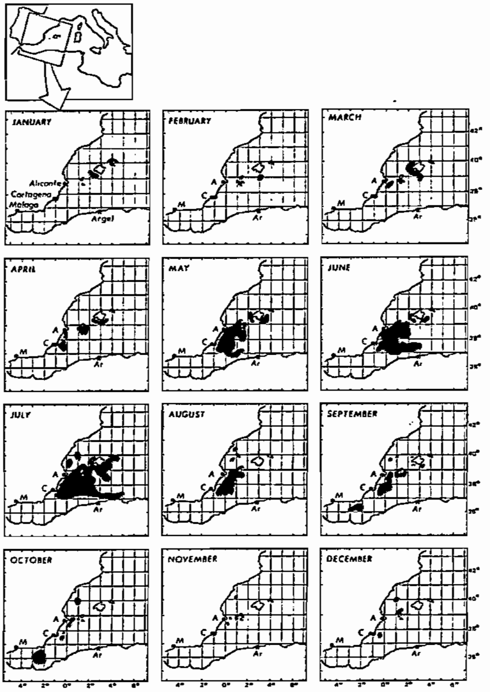

Figure 4: Loggerhead monthly distribution in Wstern Mediterranean

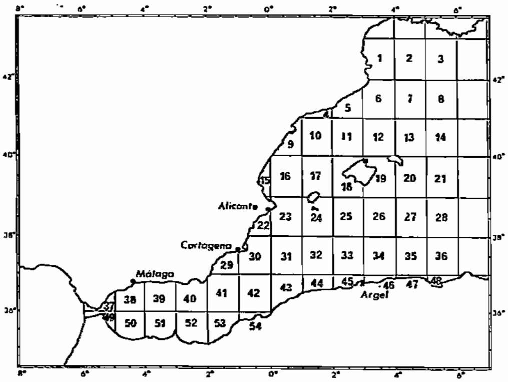

Figure 5: Fishing areas of the spanish longline fleet in the Mediterranean

Geographic distribution and fishing areas

The fishing area of the Spanish long-line fleet in the Mediterranean extends from the Straits of Gibraltar (5° W) to 7° E, near Cerdeña. In latitude it extends from 42° N to near the Algerian coast. (Figure 5).

The fleet moves, following the concentration of Swordfish and Red bluefin tuna reproducers, in June and July. During the autumn months fishing is done in more coastal areas, as much peninsular as insular (Balearic Islands, Columbretes and Chafarinas) catching smaller specimens of swordfish.

Because fishing is directed towards those two swordfish age groups, in certain areas, we cannot say that the incidental catch are precise estimations of the abundance of marine turtles.

The area around the Balearic Islands is known as an important laying area for large Pelagic. There are numerous bibliographic references that confirm this fact (DUCLERC *et al*, 1973; DICENTA, 1977; DICENTA & PICCINETTI, 1977; RODRIGUEZ RODA & DICENTA, 1981). Even so, the bibliography mentioned does not include the catching of swordfish larvae, but does of species such as bluefin tuna, *Thunnus thynnus*, albacore, *Thunnus alalunga*, frigate tuna, *Auxis thazard*, and Atlantic bonito, *Sarda sarda*. On the other hand the facts of the Spanish Mediterranean Swordfish (*Xiphias gladius*) fishery (DE LA SERNA, 1991) indicate an important abundance of young of this species in the peninsular coastal areas and the Balearic Islands, including an area where they are caught.

Undoubtedly, the loggerhead catching areas in the Western Mediterranean by the Spanish fleet, are very conditioned by the economic interests of the fleet and the biology of the target species (bluefin tuna and swordfish) and their reproductive behavior. For this reason the distribution areas that we propose would not be the only ones occupied by the species, although, as we have seen, the fishing area occupies a large part of the Western Mediterranean.

Distribution of the Loggerhead turtle in the Western Mediterranean

For the years 1986-1991, each of the months of the year has been analyzed, comparing the abundance of specimens and the distribution in the sea.

During the colder months (heeding the surface temperature of the Western Mediterranean) we find a reduced number of turtles in the region prospected by the long-line fleet. (Table 2). In those colder months (Miller, 1976) are included January, February, March, April and December with mean surface temperatures of less than 15,5°C.

The specimens were caught fundamentally to the South and SW of Ibiza and SW of Majorca, but some specimens were also caught to the North of Menorca (Figure 4). In this season it is not normal to catch around the Columbretes Islands, at approximately 40° N in front of Castellon. Occasionally, catches near the peninsular coast appear, as much in the proximity of Cabo de Palos, as Cabo de San Antonio. These specimens constitute a stable Winter residual population. The specimens caught in this period weigh between 15 and 30 kilos, but occasionally the fishermen tell us that they are much bigger, more than 50 kilos, which would correspond with adult specimens of a straight carapace length of more than 50 cm (MAYOL *et al* 1988).

In April not only is there an increase in the number of individuals caught, but also an increase in the distribution area, including the proximity of the Balearics and the peninsular coastal area between the delta of the Ebro and Cabo de Gata. In May this area increases towards the South, East and West, covering nearly all of the Western Mediterranean in June: between 41° and 36° N and from Cabo de Gata to the 5° meridian. In this last month, mean monthly values of 1116,5 individuals are reached with a maximum value corresponding to 1990 with 3360 individuals in the month of June.

This increase must be produced by the massive arrival of immature individuals proceeding from the Atlantic and the Central and Eastern Mediterranean.

If we heed the data of ARGANO *et al* (1992), a specimen tagged in May in Alicante, was recovered in July of the next year in the Alboran sea, which would indicate a migration of specimens of about 30 kilometers towards the W.

Another specimen tagged in Alicante was recaught in Ghar el Melh (Tunisia), in January of 1991. Which could indicate a migration towards those waters of specimens that passed the Summer in the Western Mediterranean, and the Winter in the warmer waters of the Central and Eastern Mediterranean, as is indicated by LAUREN *et al* (1990) that happens in the Gulf of Gabes.

In July the area of occupation continues enlarging, extending itself to the North African coast, and in the W to the sea of Alboran (2-3° W). In this month there is an important group in front of the Algerian coast, disappearing in the following months. These two groups could indicate movements of a part of the population towards Alboran and the Straits of Gibraltar, and of another group towards the Tunisian coast and farther East following the direction of the most important Atlantic surface water branch that flows near the North African coast, the denominated Algerian current, turbulent and with many eddies moving towards the East (LE VOURCH *et al* 1992).

The mean value in the month of July is high, 2224 individuals, that is the highest registered all year.

In August presence in front of the North African coast is rare. Only 12 specimens were caught in the area at the beginning of the month in 1988, so we could consider that the mentioned migration towards the East would end from that date.

From September, the number of specimens captured (mean = 329), begins to decrease, although the presence of the loggerhead in the Alboran sea and near the delta of the Ebro is common. The area of distribution is similar to the months before.

In October specimens continue appearing in the Alboran sea and the extension occupied by the turtles is maximum. From the delta of the Ebro to Alboran and from Menorca towards the South, arriving in 1988 to find 19 specimens in this month in front of the Algerian coast (3-4° E) 40 miles away from the coast.

In November the number decreases and only specimens in the area of the delta of the Ebro, around Ibiza and in the Ibizan channel near the peninsular coast, are left. They have disappeared from Alboran and also from the coast of Africa.

In view of these facts about the Spanish long-line fishery, we can propose the following hypothesis of distribution and migration of the Loggerhead turtle in the Mediterranean (Figure 6):

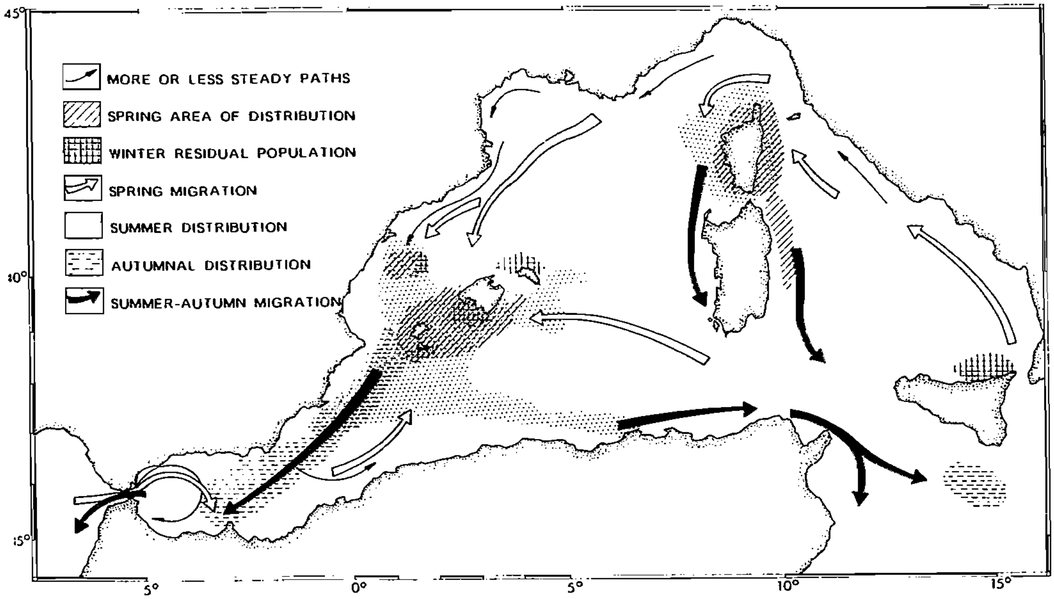

Figure 6: Loggerhead migration model in Western Mediterranean

-The population of the Western Mediterranean is formed principally of sub-adult and juvenile individuals, except in the Winter months in which the adults seem to take over, although by a small number.

-The population of the Western Mediterranean is composed of individuals proceeding from the Central and Eastern Mediterranean, and of a second group proceeding from the Atlantic. These two origins would be the population groups that, during the Spring, would occupy the region next to the Balearic Islands, principally between Mallorca and South of Ibiza.

-The Spring group grows more numerous until reaching a maximum population in the area during the month of July, to begin a descent from August. In this Summer period the population occupies practically the whole area situated to the West of 6°E and to the South from the 41°N parallel and the Alboran sea.

-From July the beginning of the return migration would be produced. A part of the population moves towards the Alboran sea, where they are caught until December when they disappear, and another part would move towards the East following the Algerian current to warmer waters.

-In the Autumn months small groups stay around the Balearic Islands, the delta of the Ebro and the Alboran sea.

-In Winter the population is reduced to few specimens, and possibly adults, to the South of Majorca, Ibiza and the Columbretes Islands.

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Attempt of spatial-temporal pattern distribution of Loggerhead Turtle in the Mediterranean

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Abstract: Loggerhead Turtles females still nest on some beaches of the Eastern Mediterranean coast, but nesting is very exceptional in the Western basin. However, many juveniles or subadults Loggerheads are regularly seen in Western Basin. Results of a genetic research, using mitochondrial DNA marker, indicate that the Mediterranean nesting female population is genetically isolated from the Atlantic population. Atlantic Loggerheads were found in Western Mediterranean basin, were they overlap the distribution area of Mediterranean Loggerheads. By its isolation, this Eastern Mediterranean population is more endangered than currently believed and has a very urgent need of and Action Plan for its Conservation.

Key words: *Caretta caretta*, Mitochondrial marker, dispersal, Mediterranean Sea, biological conservation.

INTRODUCTION

If we consider The range of the Sea turtle *Caretta caretta* in Mediterranean sea. Several questions arise. Is there one or several populations in Mediterranean? Is there a homogenous or heterogenous population? Are there differences between Western basin population and Eastern basin population and between Atlantic population and Mediterranean population? To answer at these questions, genetic studies of relationships between the possible different populations was done.

NESTING SITES IN MEDITERRANEAN

Caretta caretta females nest still on several beaches of the Eastern Mediterranean coasts. These females are smaller than other congenerics nesting in other parts of the world (MARGARITOULIS, 1982). The seven beaches of the Lagana bay in the Zakynthos island are the most important nesting site of the Mediterranean Sea (1480 nests by year in 1983-89). The other nesting beaches in Greece are in Kiparissia bay (517 nests in 1987 and 549 in 1988; MARGARITOULIS, 1988a) and Lakonikos gulf (128 nests by year) in Peloponesus, around Lixouri and Skala (particularly to Potamakia, 60 s/n, 80% of the island total), in Cephalonia, in west of Rethimnon (5 nests in 1985; MARGARITOULIS, comm. pers.) in Crete and in Rhodes (9 and 33 nests repectively in 1988 and 1989; STPS pers. comm. to Groombridge, 1990).

In Turkey, Loggerhead nesting occurs almost in all physically suitable beaches from the Dalyan region in the west to the Syrian border in the east. 17 beaches hold significant numbers of nesting loggerheads with a minimum total number of 1650 nests in 1988 for all Turkey (300 on Dalyan) (BARAN & KASPAREK, 1989; GROOMBRIDGE, 1990).

No nesting is known in Syria and Lebanon. Some nests are seen still in Israël. Around 150 females nest each year in Northern and Southern Cyprus, particularly to Akamas Peninsula. There are nestings between Alexandria and El-Salum in Egypt but nests are low and scattered without concentration of nesting on any special beach (KASPAREK, 1993).

In Libya, Nesting is known only from one site (Kouf national park in Cyrenaika) where 60-65 nests are recorded per season (SCHLEICH, 1987) but others sites and more nests are probably there. Loggerhead still lays eggs sporadically in Tunisia, some nesting beaches were delimited between Sousse and Sfax, but the nests are rare, only a few on each beach (LAURENT & *al.*, 1990).

There were several nesting sites in southern Italia (Ionian and Apulian coast) but we have no precise document. Single nests were located along the sicilian coasts from Marsala to Mazara del Vallo, Gela and Capo Passero in the last twenty years (COCCO & *al.*, 1988). One nest was observed in the last years respectively in Sinni River estuary and Isca Ionica in golfo di Tarento (Southern Italia) and Mazara del Vallo, Porto Palo di Menfi in Sicilia and four trails was seen in Granitola also in Sicilia (Argano and Gerosa, pers. comm. 12 oct. 1992). One laying eggs female around 1970 and four trails in 1990 were observed in Linosa and at least one nest is seen in Lampedusa (Pelagie Islands) each year (ARGANO & GEROSA, idem). Some Loggerheads layed eggs in the bay of Ramla-il-Hamra in Gozo island (Malta) until 1940.

Loggerhead nestings are very exceptional in Western Mediterranean basin. There are no nestings in Algeria, Mediterranean Morocco and Northern Tunisia (between Algerian frontier and Cap Bon) after recent prospectings by Laurent (LAURENT, 1990; LAURENT & *al.*, 1990). In September 1990, one hatchling was found dead in a beach of the South of Ebro Delta, it is the only nesting record for Spain (FILLELA, comm. Congrès Grenada 1992; LLORENTE & *al*, 1992/93).

Nesting was never observed in southern continental France but there were still nestings near Aleria in Corsica in 1935-40 (DELAUGERRE, 1987). No nesting is known from Western Italian Peninsula and Sardinia (Whitmore & *al.*, 1991). Finally, there is (truly or practicaly) no nesting population in the western basin.

After the estimations of the nests number per season (low: 5,200, high: 20,400) and the simulations of several models, the number of reproductive females in a theorical Mediterranean population is between 3,989 and 18628 (LAURENT, 1993).

LOGGERHEADS IN WESTERN BASIN

Many Loggerheads are regularly seen in western basin, particularly from May to September and around Baleares. 22-24000 are captured or recaptured accidentaly in Spain (AGUILAR & *al.*, 1992), 3500 in Morocco, 500 in Algeria, 100 in France (LAURENT, 1990, 1991), several hundreds in Western Italia, included Sardinia and Northern Sicilia (ARGANO & BALDARI, 1983). The large majority of these are not adults but juveniles (length of carapace between 32 and 51 cm) or subadults (LCC between 51 and 70 cm) (LAURENT, 1993).

Where these all Loggerheads come from? Atlantic or eastern basin? Because this large number of captures, an introduction of atlantic Loggerheads through Gibraltar straight is questionable.

TAGGING AND RECAPTURES

The best data on tagging and recapturing Loggerhead in Mediterranea was recently published by ARGANO & *al.* (1992). 1047 specimens, coming from accidental captures in open sea during fishing activities, have been tagged, 51 of them have been recaptured.

Analysis of results show that Loggerheads make broad movements all over the Mediterranean sea. There is an active passage between the eastern and the western basin, and vice-versa, and between Mediterranean and Atlantic, and vice-versa (ARGANO & *al.*, 1992). There are some areas (Peninsola Salentina, Gulf of Gabès) in central Mediterranea but in eastern basin more visited than others, probably for their trophic value. Some females tagged in Zakynthos were captured in Corsica, Sardinia, southern Italia, Sicilia and principaly the Gulf of Gabès in Tunisia (MARGARITOULIS, 1988b).

The majority of captured Loggerhead in the eastern Basin (eastern and southern Italia and Tunisia) are subadults (51-70 cm) or adults (more 70 cm) and therefore larger than specimens captured in western basin (LAURENT, 1993; LAURENT & LESCURE, 1994).

GENETIC STRUCTURE OF MEDITERRANEAN POPULATION(S)

Introduction

All these data raise two major questions: the origin of the numerous loggerheads captured in the western basin and the possible genetic isolation of the Mediterranean nesting population. We present the first results of a genetic research which use a mitochondrial marker analysed on Loggerheads (LAURENT & *al.*, 1993). The study with a molecular marker constitue a new approach in order to explore the pelagic movement and the functioning of a marine tetrapod population.

Material and methods

We used a mitochondrial DNA marker, a portion of the cytochrome b gene, analyzed on individuals from a double sampling. Samples were collected either from hatchling individuals and eggs on important nesting beaches, Zakynthos (Greece), Akamas (Cyprus) and East Florida, or from swimming turtles captured offshore. A *Lepidochelys kempii* was also included in the analysis as an outgroup. Total DNA was prepared from blood samples or from muscle tissues of animals found dead. For more details on amplification of DNA fragment, see LAURENT & *al.* (1993). The sequence polymorphisms were investigated on the individuals from the offshore sampling, by digesting amplified DNA by H*pa*II and separation of the resulting fragments on 3% Nusieve GTG agarose gel. DNA sequence haplotype frequencies were compared using an analysis of molecular variance (AMOVA, EXCOFFIER & *al.*, 1992). Restriction haplotypes frequencies were compared among samples using Fischer's exact bilateral permutation test.

Results

We found three DNA sequence haplotypes: A, B and C. Haplotype A differs from haplotype B by one, non-silent substitution (0.24% observed divergence). Haplotype C differs from haplotype A by four synonymous and one non-synonymous substitution (0.95% observed divergence). There are five differences between haplotype B and haplotype C (1.19% observed divergence).

Haplotype A is shared between Mediterranean and Atlantic nesting beaches. Haplotype C was not found in Mediterranean sites, whereas haplotype B was observed only in Mediterranean sites. Haplotype frequencies analysis demonstrates a genetic differentiation of the American female population from the Mediterranean female population.

Using H*pa*II digestions, we distinguished two restrictions sites haplotypes: H*pa*II- is obtained when digesting a type C sequence, and H*pa*II+ corresponds to a type A or B sequence. Haplotype H*pa*II- was observed on American nesting sites only, but, it was found also in 40% of the individuals sampled along the Atlantic French coasts and in 22% of the individuals sampled in the western Mediterranean basin.

Discussion

The high value of the genetic differentiation coefficient show that the nesting female population of the United States is genetically well differentiated fom the Mediterranean one. Spatial structure of the Mediterranean nesting female population is suspected because the presence of the haplotype B is only on the Cyprus nesting site.

Now, we have the proof that atlantic (juvenile) loggerheads migrate into the western Mediterranean basin. It is also evident, by captures and recaptures data, that Mediterranean Loggerheads (born in eastern basin) migrate from eastern basin into western basin and overlap the distribution area of Atlantic Loggerheads there.

Three elements allow us to believe that the number of Atlantic females which reproduce in the Mediterranean is very low.

1/ The first is the degree of genetic differentiation between the Mediterranean nesting population and the sample from East Florida nesting beaches. This rookery is numerically the most important in Atlantic Ocean. Furthermore, it is the nearest, and its position relative the Gulf stream is the most favorable for dispersal in the Mediterranean.

2/ The second is the significance of restriction haplotypes frequencies between the Mediterranean and the sample from the Atlantic French coasts, which is probably an area of pelagic dispersal for the individuals of the Atlantic nesting population.

3/ The third element is the fact that the genes HpaII- haplotype foreign to breeding Mediterranean population are moving in the western Mediterranean basin without being observed on the Mediterranean nesting sites. But, we don't know the sex of the specimens sampled in the western basin, so we are not totally sure that the mitochondrial genes are carried by females.

STATUS OF MEDITERRANEAN FEMALE POPULATION

Finally, our study demonstrates that the Mediterranean population female population is isolated from the Atlantic female population. Three explanations could be given.

1/ The first is that the Atlantic juvenile females show during their pelagic movement a strong natal homing when they are confronted with the option of the breeding to a non-natal region and should return to Florida.

2/ The second is that the Atlantic reproductive females don't reproduce in Mediterranean area and return in Atlantic ocean but don't reproduce in their natal region.

3/ the third is that the males participate alone to the Atlantic juvenile pelagic movement. We don't believe a such occurence, a such discrimination between the sexes of the immatures migrants or warrants.

However many Atlantic juvenile females die probably before their maturity because of the very strong fishing activities in all Mediterranean sea. 20000 to 35000 incidental captures of Loggerheads each year in Mediterranean Sea is an estimation, a lot of turtles are released but 20 to 30 % die, after AGUILAR & *al.* (1992).

CONCLUSION

The Mediterranean nesting population of the *Caretta caretta* is isolated from Atlantic population. The entry of Atlantic Loggerheads in the western basin don't strengthen the Mediterranean population reproducing actually restricted in the eastern basin. Therefore, its decreasing is not compensated by the contribution of foreign elements. By its isolation, this Mediterranean population is more endangered than currently believed, and has a very urgent need of a true Action Plan for its Conservation. After the recent population dynamic studies of LAURENT (1993), it must be given the priority to the protection of adults, the most sensitive stade for its dynamic population, particularly in eastern basin.

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Status of *Vipera aspis* and *Vipera latasti* (Viperidae, Reptilia) in Catalonia (NE Spain)

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ABSTRACT: The two species of the *Vipera* genus existing in Catalonia (NE Spain) are the only reptiles not protected by the prevailing legislation. Its unknown status and the suspicion of *V.latasti* regression justifies a questionnaire between forest guards, a collective wuich is distributed throughout all Catalan divisions. Starting from the questionnaire it has been possible to evaluate with some precision the distribution of both species and ti get a guiding idea of the abundance of these populations. According to the results, the distribution of *Vipera aspis* is homogeneus and covers all the Pyrenees and rainy east area of the country. It is stable in 70% of the Catalan territorial divisions and it is increasing by 25%. So it is not a threatened species. *Vipera latasti* however, has a fragmented distribution, and it is only present in the center and south of the country. The scarce population is considered to be in regression in 58% of the Catalan territorial division and stable in 37%. The progressive fragmentation and isolation of populations and their regressive tendency make this viper a threatened species. Possible causes of this regression (human development and forest fires) and future perspectives are discussed.

KEY WORDS: *Vipera*, status, distribution, trend, conservation.

INTRODUCTION

Vipera aspis is a viper which is present in the south of Europe but which, in the Iberian peninsula, does not extend any further south than the Pyrenees and the north of the Iberic system. They live in populations which are usually numerous and often found to be on the increase (BEA 1985, VIVES 1987:186, SAINT GIRONS 1980, DUGUY *et al.* 1979).

Vipera latasti only occupies the Iberian peninsula (except the Pyrennees and the Cantabric mountains) and the north of Africa. Catalonia is the north-easthern limit of this distribution. Various authors mention that their populations form isolated nuclei and that they seem to be found in regression (SAINT GIRONS 1980, VIVES 1987:188, DUGUY *et al.* 1979, BARBADILLO 1987:538).

In Catalonia, *V.aspis* seems to be the most abundant snake within its distribution area, while *V.latasti* the most scarce in its area, being one that many Catalan herpetologists have never seen. Because these two snakes are the only reptiles not protected by prevailing legislation, it was considered necessary to obtain more information about them in the event that the application of some means of protection became available, especially for *Vipera latasti.*

METHODS

With the intention of obtaining information which covered the whole of Catalonia, and as the vipers are well-known by the people who live in the countryside, a questionnaire was considered to be the most adequate method (Fig. 1). The questionnaire was addressed to local people with more experience in this field (shepherds, hunters, and farmers) through the forest rangers, who distributed them regularly all over the country. An identification brochure, a local topographic map (scale 1:250.000) and the

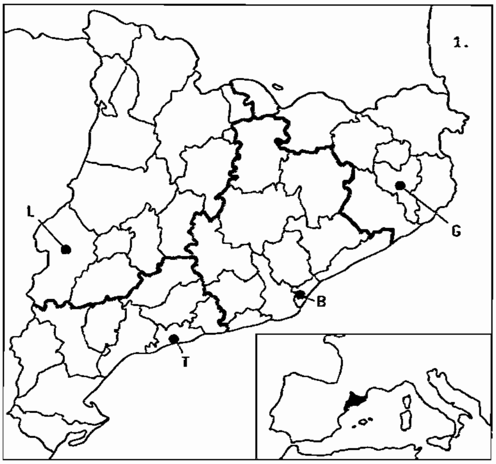

Figure 1- Territorial divisions of catalonia used in this Questionnaire. Wide line= provinces. Fine line="comarques". B=Barcelona, T= Tarragona, L= Lleida, G= Girona.

questionnaire paper with its comments was given to each ranger.

The estimation of the evolution of the viper populations was based on whether the people who were asked considered that they had increased, decreased, or if they were the same as twenty years ago. The abundance is calculated in terms of the number of vipers that one local person sees normally in one year: non-existent, exceptional observations (several years without observations), no more than one observation/year, and several observations/year.

The results have been surveyed first by our new personal consults to the rangers and local people, then by local herpetologists, and also compared with the Herpetological Atlas of Catalonia (in press.). A new local questionnaire was made with more experienced rangers of the National Game Reserve "Ports de Tortosa i Beseit", where *V.latasti* is found, to compare the results.

The main object of this study is to know the status of *Vipera latasti*. *Vipera aspis* was introduced into the questionnaire because people from the countryside do not know the difference between the two species, and also to make a comparison of the results. The answers to the questionnaire have been assigned to each of the species whose presence is known thanks to the Atlas information.

RESULTS

We have not obtained any surprising responses. The results of the distribution agree with the known observations, so local people obviously do not confuse the vipers with other more widely distributed snakes. Its presence in some areas where it is very scarce was not mentioned and this was the only defect that was observed. The cause of the error was probably due to the reduced number of people who were asked in those areas.

Distribution:

Vipera aspis is widely spread throughout the Pyrenees and the part of Catalonia which receives the most rain: its distribution area coincides with the isohyet of 700mm (see Fig.2).

Usually it is present in populated plains in scarce numbers. We don't know which species live in some places in the east of Catalonia (Gavarres), but it is probably V.aspis. On the southern edge of the Pre-Pyrenees, *V.latasti* is cited, but not confirmed in the Atlas information.

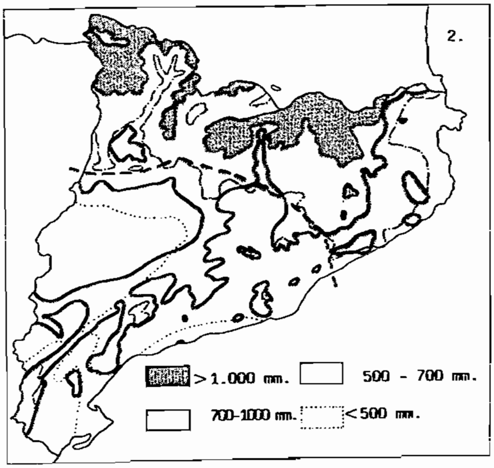

Figure 2- Distribution of vipers over annual rainfall (in milimetres).(from RIBA *et al.* 1979). Wide continuous line= distribution of vipers. Discontinuous line= probably limit between *Vipera aspis* (up) and *Vipera latasti* (below) distribution.

Vipera latasti is limited to Mediterranean rocky mountains, usually calcareous and over 400m a.s.l. (see Fig.3 and 4) , but in some places, they can be found near sea level. Its density seems to decrease on a parallel with the descent in altitude and rainfall, except perhaps in specific places (Almatret, near river Ebro). It is

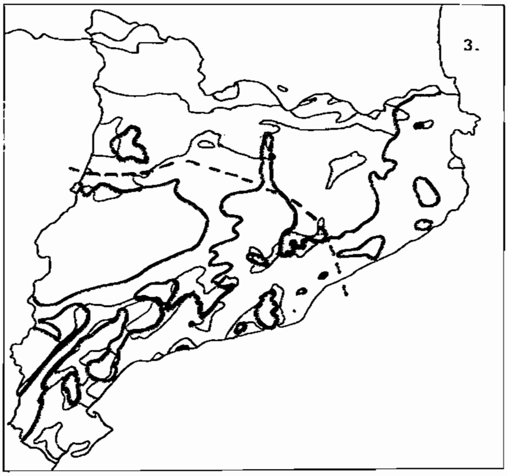

Figure 3- Distribution of vipers over karstic areas (from CERVELLO, 1992).

apparently absent in some favourable mountains of the south of Catalonia (the Montsia sierra and the siliceous mountains in the Priorat). Many populations near Barcelona city are completely isolated and progressively fragmented, and local extintion is possible in several places, also in the Tarragona province. Its presence in the Pre-Pyrenees and in the Girona province is not confirmed.

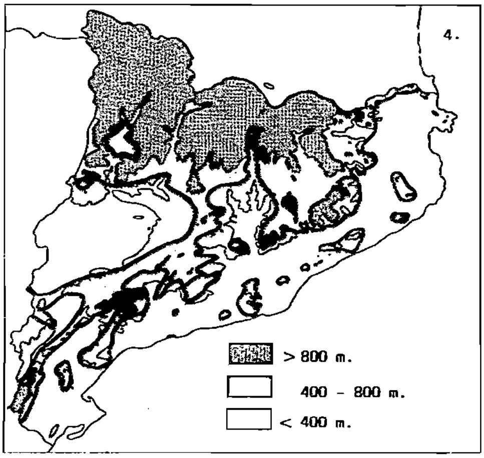

Figure 4- Distribution of vipers over altitude map.

Evolution of the populations (Table 1, Fig.5).

Vipera aspis: Increase responses are concentrated around the Oriental Pyrenees and Montseny. All of this area is the most heavily human-populated part of its range. Regression is only mentioned in peripheral areas, but the possible presence of *V.latasti* can affect this estimate. Stable responses cover the most part of its range.

	Vipera aspis	*Vipera latasti*
Increase	25%	5,2%
Stable	70%	36,8%
Regression	5%	57,8%

Table 1: Evolution of the populations. Percentage responses (n:67)

Vipera latasti: Only two increase responses (as the source of the river Foix, and Montsant Mountain). Stable responses only occur in the more mountainous and wildest areas of the Tarragona province. Regression responses cover all the Barcelona province (the most heavily human-populated part of its range), some areas of the Lleida province and the low mountains of Tarragona province. In the wild National Game Reserve "Ports de Tortosa i Beseit", rangers responses are not unanimous: 4 about regression, 3 about stability and 1 about increase.

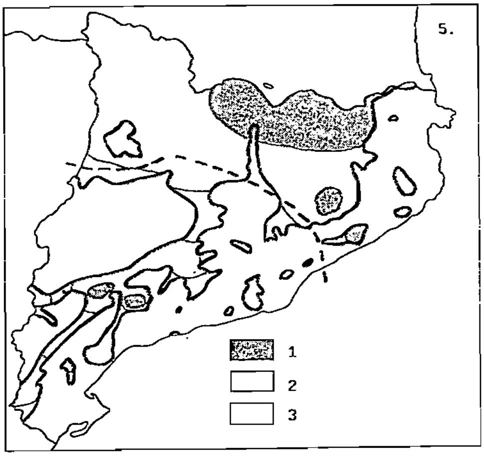

Figure 5- Evolution of the populations 1-Increase, 2-Stability, 3- Regression.

Abundance (see table-2 and Fig.6)

Vipera aspis: Two or more vipers/person/year are seen in the main part of its range. One or less vipers/person/year are seen only in the peripheral parts of its range and in populated plains.

	V.aspis	*V. latasti*
2 or + vipers/person/year	67,5%	30,9%
1 or - vipers/person/year	32,5%	69,1%

Table 2: Viper abundance. Number of vipers seen/person/year. Percentage responses (n:79).

Vipera latasti: Two or more vipers/person/year are seen in some parts of the Tarragona province and in specific parts of the province of Barcelona (usually the highest mountains of its range). In the rest of its range, observations are rare or they are an exception. According to the more detailed questionnaire made in the National Game Reserve "Ports de Tortosa i Beseit", each ranger usually sees around 7 vipers/year (rank:3-20).

Factors that affect the evolution of the population

According to the questionnaire, the causes of an increase in the viper population are mountain depopulation (n:2) and predator regression (n:1). The causes of regression are: Wild boar increase (n:9), forest fires (n:4), human persecution (n:2) and habitat destruction (n:2).

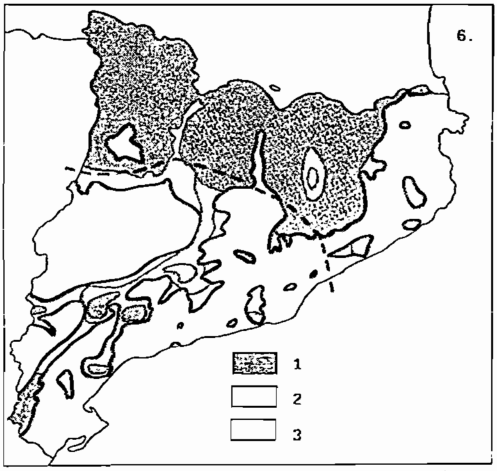

Figure 6: Abundance of vipers: 1= Two or more vipers seen by one person/year. 2= One or less vipers seen by one person/year. 3= Many years without observations.

DISCUSSION

According to these results, the previously suspected regression and scarcity of the *Vipera latasti* population in Catalonia is certain, but the factors mentioned like the possible causes of the different trends, need some comments:

In relation to the increase of vipers, we think that predator regression has not really been a factor in the last decades, because potential predators like the short-toed eagle (*Circaetus gallicus*) and wild boar (*Sus scrofa*) -from 500-1000 wild boar in 1960 to 35000-40000 at present- are on the increase (MUNTANER *et al.* 1981, DIRECCIO GENERAL DEL MEDI NATURAL 1993). On the other hand, mountain depopulation can be beneficial to the vipers -less persecution and an increase in vegetation covering-, but this reforestation of grasslands and fields can not be good if it is very intense (dense forest does not allow for basking places). Finally, other factors not mentioned in the questionnaires are: the evolution of vipers' prey populations in the last decades, and climate changes, both factors that can affect the body reserves -fat and liver- of females and so the frequency of the reproduction (SAINT GIRONS 1992, BONNET *et al* 1992). We have not information about these important factors.

In relation to vipers' regression, one of the factors mentioned -the increase in wild boar numbers-, should affect both species of viper, but regression does not affect the *V.aspis* population. Human persecution is probably decreasing due to mountain depopulation except in places of intense tourism, and this fact can explain the regression of *V.latasti* in some protected areas. *V.aspis* however, is not apparently affected by this factor (SAINT GIRONS 1978). Habitat destruction is more intense in the Mediterranean area, and *V.latasti* can be more affected. The area occupied by this viper is progressively fragmented by human development -road construction and urbanization-, and many populations are dangerously isolated. Forest fires have affected many regression areas, and we think that these, along with habitat destruction, are the two big problems for *V.latasti*. In its range, forest fires are very common and extensive: fires affecting several thousands of hectares are not exceptional, and can affect the same area some years later. It is possible that small forest fires will be beneficial to vipers -due to the opening of dense forest-, but very extensive forest fires make the wildlife recuperation from the edges difficult. Forest fire can kill many vipers, but it also eliminates the vegetation necessary for their thermoregulation -especially with high temperatures-, refuge and prey. Destruction of the vegetation cover is, according to SAINT GIRONS (1978), the only factor which could cause the decrease of *V.aspis*. So, areas inhabited by *V.aspis* are not greatly affected by forest fires (see Fig.7).

However, a low density in *V.latasti* populations due to natural factors can make this snake more vulnerable in the face of some of the previous negative factors mentioned which do not seem to affect *V.aspis* -an increase in wild boar or local tourism for example-. In the "garrigas" (dense scrubland typical of calcareous mountains in the Mediterranean area) and pine forests which spread accross the major part of the distribution area of *V.latasti*, the reduced density of existing small mammals (DUGUY *et al* 1979, GOSALBEZ 1987) could be the cause of the apparent low density of this viper. In these ecosystems, small mammals are concentrated on shady slopes, near rivers and streams, and in grasslands connected to the margins of now abandoned cultivated fields(GOSALBEZ 1987), a habitat which does not greatly coincide with the habitat where *V.latasti* is found. However, this viper seems to avoid the rich -in prey abundance- but humanized ecotones between field and forest or scrubland, which in some places characterize the habitat of *V.aspis* (NAULLEAU 1965). The refusal of human presence appears more important in habitat selection than prey abundance, and similar conclusions has found PLEGUEZUELOS (pers.com.) when the distribution of this viper is analised in Andalucia (south of Spain).

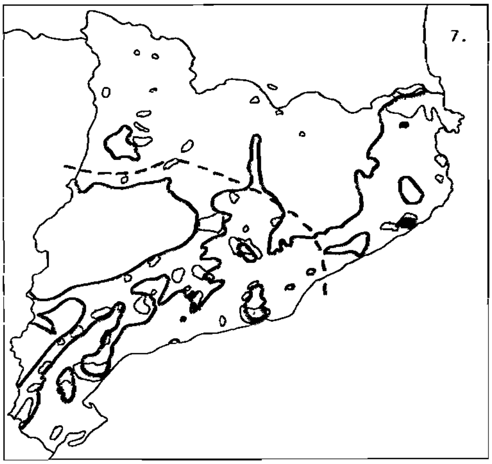

Figure 7: Distribution of vipers over areas affected by extensive Forest fires.

To go back to prey abundance, the increase observed in the abundance of *V.latasti* which is produced on a parallel with the increase in altitude and rainfall (as well as the attraction that places of elevated enviromental humidity have for this species in the more arid areas), could be related to an increase in the density of small mammals. On the other hand, in the Mediterranean area, the number of species (see Fig.8) and the average weight of these are much lower than in the Pyrenees: while seven common species of small mammals (whose weight is greater than 15 grammes) exist in the major part of the area occupied by *V.aspis* in Catalonia, in the area occupied by *V.latasti* there are only two (A*podemus sylvaticus* and *Microtus duodecimcostatus*) (GOSALBEZ 1987).

On the other hand, the density of these two species is much lower in the range of *V.latasti* than the density of the seven respective species in the range of *V.aspis*. This scarcity of small mammals is also shown in the number and density of species of predators which are specialists in their consumition presents in both areas. One of these, the common buzzard *Buteo buteo* for example, is abundant in the *V.aspis* range, and exceptional in the main part of the *V.latasti* range (TICO & PARELLADA 1984).

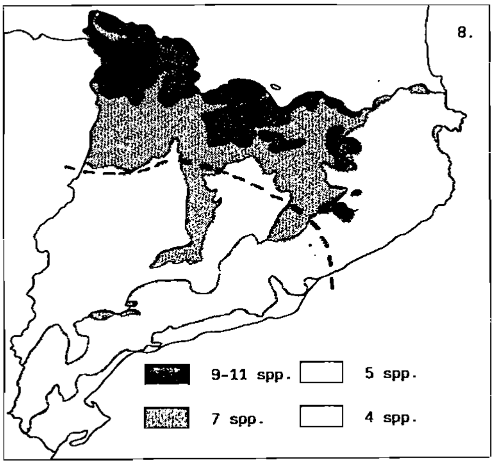

Figure 8: Number of species of small mammals (genus *Sorex*, *Suncus*, *Crocidura*, *Apodemus*, *Mus*, *Clethrionomys* and *Microtus*) presents in different natural areas of catalonia (from GOSALBEZ, 1987). Discontinuous line= probably limit between *V.aspis* (up) and *V.latasti* (below) distribution.

Also, the fact that, excluding *V.ursini* (which is basically an insectivore), *V.latasti* is the European viper whose diet has the least proportion of small mammals (only 59,0% from 92,2-97,7% in the diet of *V.aspis* for example), and which at the same time consumes a major proportion of Soricidae (55,0% of total small mammals, from 22,0-14,3% in the diet of *V.aspis*) (BEA *et al.* 1992), is a clear reflection of the scarcity of Muridae and Microtidae in its range.

If this hypothesis is correct, this fact along with the factors that affect *V.latasti* -especially forest fires and habitat destruction-, could be the causes of its regression.

Finally, one totally unknown aspect is the possible competition between both species where they are found together. According to SAINT GIRONS(1980), the limits of the distribution areas of European vipers, usually obey to competition. At present, we do not even know for sure (with one exception in the "Moianés"), the zones where both species make contact to evaluate this possibility. On the other hand, the actual non-existence of *V.latasti* in the places where it was cited many years ago inside the range of *V.aspis* (see revision in DUGUY *ET*

*AL.*1979), and according to these authors, seem to correspond more to a confusion in the identification than to its extintion.

CONCLUSION

It may be necessary to protect *V.latasti* by legislation, but it is not a popular idea and not easy to apply. Currently, to kill or capture vipers is prohibited in the Natural and National Parks, and in Natural Reserves. The other way to protect this species is to protect the places where they live, and to avoid forest fires. Fortunately, many of the places where *V.latasti* is found, are currently partially protected (habitat destruction) thanks to the "PEIN" agreement (Natural Interest Spaces Programme) (see Fig.9). The succesful aplication of the forest fire prevention programme by local government, has drastically reduced the annual surface area affected by forest fires: from 10.000-70.000 ha/year between 1979-86 (av.25.258ha), to 1.000-6.000 ha/year between 1987-93 (av.2.600ha).

We think that the indirect measures adopted for *V.latasti* protection, could however be of much assistance, and it is necessary to wait for its succes.

NOTE: The exceptional dry and hot spring and summer in 1994, has been followed by large forest fires in Catalonia (75.000 ha). Years like this last one could be fatal to Lataste's viper conservation.

Figure 9: Distribution of vipera latasti over protected areas. 1= Habitat and species protected, 2= Only habitat protected.

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Preliminary results of the herpetological expedition "ETHIOPIA'92"

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Abstract: A herpetological expedition has been undertaken in the March-July period of 1992 by three Czech biologists - Petr Nečas, Robert Lízler and Vladimír Trailin. The members of this expedition visited some very interesting localities in Ethiopia and collected a lot of herpetological material, which is deposited in ZFMK, Bonn. Especially in the Illubabor province some very poorly known reptiles and amphibians were found, f.e. *Dasypeltis atra* (first ethiopian record), *Pseudoboodon boehmei* (found earlier than the description-paper has been published), *Rhinotyphlops somalicus* (found largest specimen of the species - 67 cm), Lamprophis erlangeri (known only from the type), *Bitis parviocula* (seen only, collected within the next visit in 1993), *Conraua beccarii*, etc... The collection will be the topic of some further papers, the ethiopian herpetological research will be continued by further expeditions and bigger research- and conservation- projects.

Key Words : Ethiopia, faunistics.

INTRODUCTION

A group of Czech herpetologists (Petr Nečas, Robert Lízler and Vladimir Trailin) carried out an expedition to Ethiopia with mainly herpetological program from March to July 1992. Although Ethiopia gave a name to one of the zoogeographic regions, it still belongs to one of the worst explored African countries thanks to its long lasting political isolation and technically difficult accessibility. Despite many technical, material, and bureaucratic problems, the expedition succeeded to reach the target country and complete a lot of worth herpetological observations mainly in Illubabor province and in the Ethiopian part of Rift Valley at Langano lake. Most of the collected material is now in the deposition of "Zoologisches Forschungsinstitut und Museum A. Koenig, Bonn" and will be subject to following detailed studies (NEČAS & BÖHME in prep.).

Vast areas of wild Ethiopian nature are quickly being destroyed on the account of extensive agriculture and lack of protective measures. Along with vanishing biotops, the endemical flora and fauna gradually disappears. This alarming situation gave birth to the "Foundation for zoological research and conservation of the fauna in Ethiopia" in 1993. It should contribute to the protection and preservation of unique and endangered species and communities in Ethiopia on the base of zoological research.

PRELIMINARY RESULTS

During the expedition (mainly in Illubabor province), many species of commonly known amphibians and reptiles were found, which have been reported also from Ethiopia (e.g. *Bufo regularis*, *Mabuya striata*, *Varanus niloticus*, *Philotamnus battersbyi*, *Pseudoboodon lemniscatus*, *Crotaphopeltis hotamboiea* etc.). Aside from these, some less known and also unknown species were discovered.

One of the most interesting to be mentioned is the finding of the only eastafrican representative of the genus *Conraua* - *C. beccarii*. The analysis of the intraspecific variability of *Chamaeleo* (*Trioceros*) *affinis* preliminarily supports the conclusions of a German scientist OSKAR NEUMANN (1905) who stated that this species has probably some geographical forms. Exemplars from southeast Ethiopia are so much different from those of Addis Abeba region so that they should be regarded as a distinct subspecies (NEČAS in prep.).

The largest known specimen of *Rhinotyphlops somalicus* measuring 67 cm was also found (previous report - LARGEN 1978 - only 55 cm). Another finding of a immature *Dasypeltis atra* is shifting the teritorial boundary of this species significantly to the north. Exuvia of *Bitis arietans* was discovered in an atypical biotop (mountain rainforest) in the height of 2.200 m. It is upto now the highest documented elevation of occurrence in its range. One exemplar of the most rare species of the genus *Bitis* - *B.parviocula* was also sighted around the type locality. This species is known from only two exemplars (BÖHME 1977, 1990; NEČAS *et al.* 1993).

There were two species of snakes that could not be initially determined. Only an examination of a holotype of *Boodon erlangeri* Sternfeld, 1908 (upto now the only exemplar, described very superficially and with mistakes by Sternfeld) confirmed the fact that these exemplars belong to the mentioned species. Several exemplars of a species were found, that could be attributed to the genus *Pseudoboodon* (which has been considered monotypic - PARKER 1949). But, these specimens differed significantly from Pseudoboodon lemniscatus. A description publication of this new species - *Pseudoboodon boehmei* - was already in print (during the expedition) and it was issued in September of the same year (RASMUSSEN & LARGEN 1992).

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Ecological observations on some amphibians from a mountainous area near Xichang (Sichuan, P. R. China)

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Abstract: During an excursion in June 1992 to the province of Sichuan in southwest of China we investigated the ecology of *Bombina maxima* (BOULENGER, 1905) in an area close to Xichang (KOHNEL *et al.*, 1992). On a plateau at a level of 3000 m we found nine species of amphibians. Focussing on *Bufo andrewsi* SCHMIDT 1925, *Rana chaochiaoensis* LIU 1946, *Paa yunnanensis* (ANDERSON 1879), *and Calluelia yunnanensis* (BOULENGER 1919) ecological, morphometrical, micromorphological, and bioacustical data are presented in this study.

Key words: Amphibia, *Bufo andrewsi, Rana chaochiaoensis, Paa yunnanensis, Calluella yunnanensis,* micromorphology, morphometry, calls, ecology, Southwest China.

INTRODUCTION

The amphibian fauna of this territory is characterized by mountainous elements from the boreal Far East e. g. *Bufo gargarizans* CANTOR 1842, and from Southern Asia e. g. *Megophrys giganticus* LIU, HU & YANG 1960. On the poster we have presented fotographs of nine amphibian species, for example of *Paa yunnanensis* (ANDERSON 1879), *Batrachuperus karlschmidti* LIU 1950, *Tylototriton taliangensis* LIU 1950, and *Megophrys giganticus* LIU, HU & YANG 1960.

ECOLOGICAL DATA

The plateau is characterized by a humid climate (55% to 82 % relative humidity). During our visit in June 1992 air temperature ranged between 11°C and 16 °C. A very thin layer of soil covered the reddish-brown granite-like ground. In many cases a large erosion could be observed. No trees grew on the mountains. Only very small bushes were found along the runs (several species of *Rhododendron, Quercus, and Rosa omeyensis).* Four typical kinds of waters can be differentiated (KABISCH *et al.* 1994):

1. Fast running creek with a high speed and clear water (15 °C, pH 7. 1), the ground consisted of stones. At the border of these creeks bamboo, *Rhododendron, Quercus,* and a yellow *Iris* were found. Spawn, larvae and adults of *Bombitia maxima,* adults of *Batrachuperus karlschmidti,* and larvae of *Paa yunnanensis* were predominantly observed in these creeks.
2. Meandering creeks with slowly flowing water and with deepenings and overhanging borders. The depth of the water was 20 - 30 cm and the width about 1-2.5 m. In the mutual water temperatures of 15 - 17.5 °C and a pH of 5.5 were measured. The border vegetation consisted of *Primula, Guldenstaedtia, Pedicularis, Iris, Rhododendron, Juncus thomsonii* and *Caltha palustris.* In these creeks 33 adults and 2 subadults, larvae and spawn of *Bombina maxima,* adults and subadults of *Rana chaochiaoensis,* also larvae of *Calluella yunnanensis and Bufo andrewsi* were seen.
3. Standing waters ranging from of 80 to 135 m and a maximal depth of 25 cm. The ground consisted of mud. The reddish or muddy water had a temperature of 16 °C. Exept for some grass and rusk the borders of these pads were without vegetation. Big swarms of *Bufo andrewsi-tadpoles,* larvae, and some adults of *Bombina maxima* were observed.
4. Relatively clear standing waters without connections to creeks with a size of 700 and 800 m and a maximum depth of 50 cm. The temperature was 14.1 - 15.2 °C and the pH was 6.2 - 6.6. At the border *Rhododendron, Iris,* grass, bamboo, and rush were found. The submerge vegetation consisted of *Potamogeton.* In these waters adults and larvae of *Tylototriton taliangensis,* larvae of *Bufo andrewsi, Rana chaochiaoensis* and *Calluella yunnanensis* were found.

Adult specimens of *Megophrys giganticus, Bufo andrewsi, Bufo gargarizans, Rana chaochiaoensis,* and *Paa yunnanensis* mainly inhabited swampy areas near small mountain streams or ponds.

Figure1: Mouth of the tadpole of *Calluella yunnanensis*

Calluella yunnanensis (BOULENGER 1919)

The SEM-fotographs of the mouth of *Calluella yunnanensis*-larvae showed rows of thick warts on the ventral side. Laterally two big warts, which were banded to the inner side were seen on each side. Above the upper jaw a continuous row of denticles was developed. Below the mouth three of these rows of denticles were visible. Each denticle ended in five or seven tips. The inner margin of the jaw was sawed (Fig. 1).

The typical transparent, helicopter-like larvae of this microhylid were found in ponds and pools together with tadpoles of *Bufo andrewsi* and adults of *Tylototriton taliangensis* (KABISCH *et al.* 1994). They were seen to be swimming in the shallow zone under the surface of the water and showed a typical swarm behavior escaping into the deep water. *Calluella*-larvae were also observed in rice fields of lower regions. The maximal length of the tadpoles (n=30) was 32 mm, the width of the head was 8 mm.

Figure 2: Dorsal epithel of *Bufo andrewsi*

Bufo andrewsi SCHMIDT 1925

The SEM-fotograph of the dorsal epithel of *Bufo andrewsi* has shown, that its surface structure was not as rough as in many other closely related bufonids (for instance *Bufo gargarizans*; HERRMANN & KABISCH 1994, DATONG 1991; Fig. 2). The mouth part of the tadpole has showed the typical form of *Bufo*-larvae (Fig. 3). The number of denticle rows was extremly variable, but usually, one continuous and one discontinuous row above as well as two continuous and one discontinuous rows below the mouth were observed. The jaw is sawed.

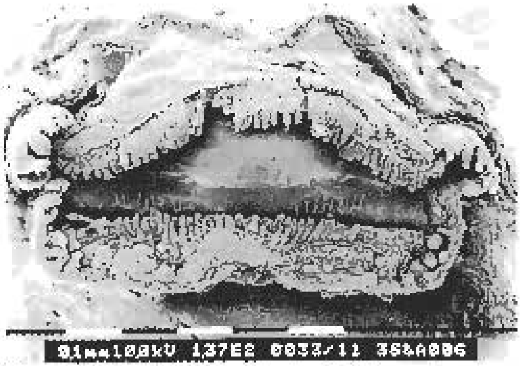

Figure 3: Mouth of the tadpole of *Bufo andrewsi*

Adults and juveniles of *Bufo andrewsi* were seen in all parts of the plateau. The larvae lived in pools and slowly running creeks together with tadpoles of *Calluella yunnanensis and Bombina maxima* (KOHNEL *et al.* 1992). They occurred mostly in large swarms on the muddy substrate.

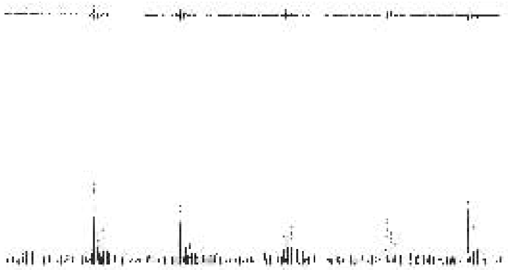

Figure 4: Sonagram of the distress call of a male *Bufo andrewsi*

The tadpoles attained a maximal length of 18 mm (n=20). Additionally the following data were collected: Snoutvent-length 69.8 mm and weight 52 g (one measured male), snout-vent-length 60.0 mm and weight 24 g (one measured female). The sonagramm of the distress call of the male (13,2 °C) showed 3 impulses per second and attained a maximum of 7 kHz (Fig. 4).

Figure 5: Micromorphology of the nuptial pads of *Rana chaochiaoensis*

Rana chaochiaoensis LIU 1946

The micromorphology of the nuptial pads showed in its tertiary structure relatively regularly arranged, semiblunt cones (Fig. 5). In contrast to *Rana japonica* these cones were not as distinctly pointed (HERRMANN 1992). In the secondary structure the fringed, very flat warts, which are developed in a irregular honeycomb-like pattern were visible. The macrostructure showed two fused epithelial wrinkles.

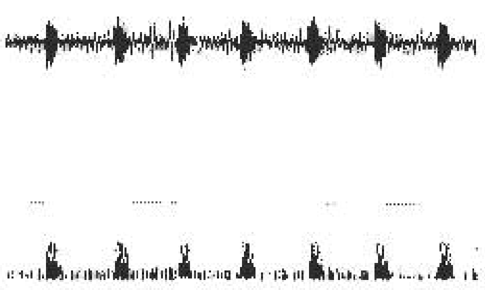

Figure 6: Sonagram of the distress call of a male *Rana chaochiaoensis*

Adults and subadults of *Rana chaochiaoensis* were seen in all humid parts of the plateau. They were very variable in pattern and colour. The snout-vent-length of eight adult males was 49.6 mm in average (maximum 55.5 mm, minimum 46.4 mm), and 57.8 - 58.6 mm in two adult females. The weight of the males was 15.1 g (maximum 19.5 g, minimum 10.5 g). The weight of the two females was 12.5 g and 19.7 g. The snout-vent-length of three subadult specimens was 26.5, 30.2, and 33.7 mm. The sonagram of the distress call of the males (13.2 °C) showed 4 impulses per second and attained a maximum of 4 kHz (Fig. 6).

Figure 7: Micromorphology of nuptial pads (thumb) of *Pau yunnanensis*

Figure 7. Micromorphology of nuptial pads (breast) of *Pau yunnanensis*

Pau yunnanensis (ANDERSON 1879)

In the males nuptial pads were developed on the breast and the thumb. The SEM-fotographs demonstrated, that the nuptial pads exhibited the same microstructure (thumb above, breast below; Figs 7-8). Epithelial structures with identical functions were formed independently in several areas of the ventral side.

Paa yunnanensis was one of the biggest anurans of China (LIU 1950, LIU & HU 1961). The males reached a snout-vent-length of 106.1 mm and a weight of 165 g. The black, single larvae were found in creeks under rocks. They lived on the ground and adhered to stones with their mouth. The tadpoles reached a total length of 4.2 mm in the maximum (n=15). In their mouthpart three continuous rows of denticles were developed below the sawed jaw as well as one continuous and one discontinuous row above (Fig. 9). Nine to ten thick warts were standing below the mouth.

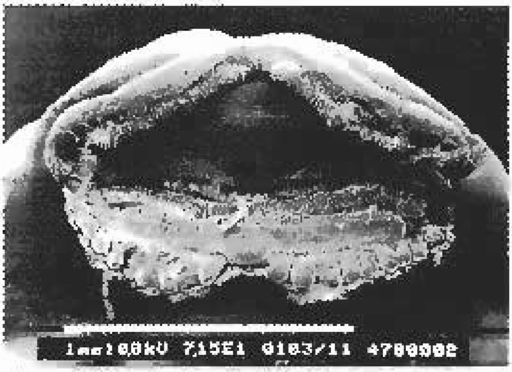

Figure 9: Mouth of a tadpole of *Paa yunnanensis*

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The herpetofauna of the World Heritage Site "Tsingy De Bemaraha" (Western Madagascar)

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Abstract: The World Heritage Site "Tsingy de Bemaraha" includes about 150.000 hectares of limestone karst massif covered with an unspoilt primary deciduous forest named "forêt de l'Antsingy". Water erosion on the rocks is responsable of creating a remarkable variety of microhabitats and consequently of forest *facies*. This area is sourrounded by grasslands which are burnt each year and are used for cattle grazing. The present study, dealing with herpetofauna species, has recorded 47 taxa (including some previous published and unpublished records). Such rich checklist, far to be definifive, is comparable to the extremely rich ones of Malagasy eastern rainforests and includes seven species which have been so far considered exclusive of these latter habitats. Two species of reptiles - the gecko *Lygodactylus klemmeri* and the dwarf chameleon *Brookesia perarmata* - have been recorded only in the Antsingy forest, therefore are likely to be endemic of this area.. Habitat preferences showed by herptile species and the importance of this World Heritage Site in terms of biodiversity are also discussed.

Key words: herpetofauna, checklist, Tsingy de Bemaraha, Madagascar.

INTRODUCTION

As any other aspect of wildlife in Madagascar, its herpetofauna is extraordinarily rich (about 150 amphibian and 270 reptile species have been recorded so far) and unique (respectively 99 % and 95 % are endemic to the Malagasy region).

The purpose of this study is to contribute to the knowledge of the herpetofauna of western Madagascar deciduous forests, in particular of Antsingy forest (Tsingy de Bemaraha massif) and of surrounding secondary formations. This forest seems to have a very high biogenetic value, but has so far received a poor attention - with the exception of a few isolated records and a recent unpublished study (HALLMANN *et al.*, 1990), mainly focused on reptiles - for what concerns its herpetofauna.

STUDY AREA

Physical background

The World Heritage Site "Tsingy de Bemaraha" -about 500,000 ha- is sited in the centre of western Madagascar (18°15'-19°20' S, 44°15'-45°05' E; fig. 1); it includes the Plateau of Bemaraha - whose western slopes are covered with the Antsingy forest -as well as the grasslands and the patches of forest delimitated by this to the east, by the Mozambique Channel to the west and by the rivers Soahany and Manambolo respectively to the north and to the south (fig.2). Most of the Antsingy forest - about 150,000 ha -constitutes the Strict Nature Reserve N° 9.

The climate is a typical tropical one, with marked contrast between the warm dry season

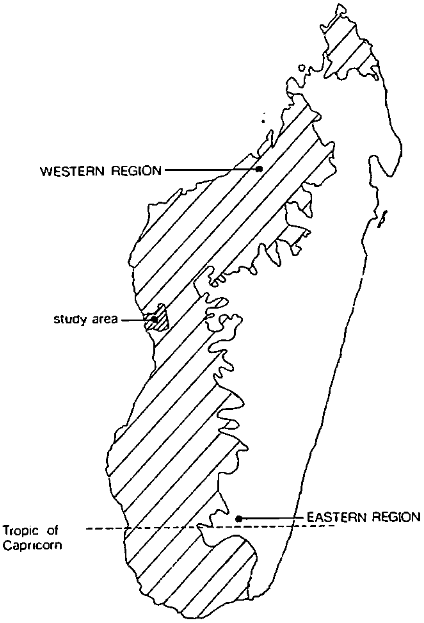

Figure 1: Map of Madagascar showing by spaced hatching the extent of Western region -whose potential dominant vegetation is the dry deciduous forest- and by fine hatching the site of the World Heritage Site "Tsingy de Bemaraha".

(May-October) and the hot rain season (November-April). The annual amount of rainfall is maximum in the Antsingy forest (1000-1500 mm); the mean annual temperature is 25-28°C.

From the geological point of view, the area is sited in the sedimentary region of Madagascar and, in particular, is part of the Morondava basin. The limestone massif which forms the Plateau of Bemaraha sedimentated during marine transgressions of the upper Jurassic and lies on Isalo clays of the early Jurassic. Reaching a maximum altitude of 934 m, it extends for more than 80 km north-south and has an average width of 20 km. Its inclination east-west guarantee a considerable supply of water to the region of Antsalova through the main rivers (Manambolo, Soahany, Beboka, Miharana, etc.).

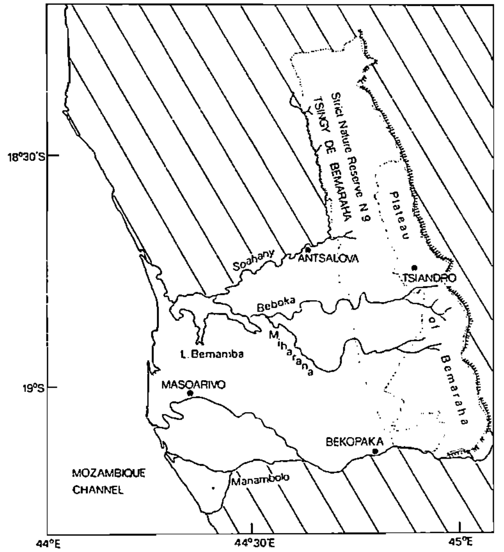

Figure 2: Map of the World Heritage Site "Tsingy de Bemaraha" showing main rivers, villages and the limits of the Strict Nature Reserve N°9.

The dissected relief of the plateau is markedly karstic and shows typical erosion shapes consisting in limestone blocks with sharp tips at the top (locally named "tsingy") interested by extensive cavern system. The abundance of springs and streams allows the presence of a flourishing vegetation which has never been affected by deforestation thanks to the inpenetrability of the habitat (BOUSQUET & RABETALIANA, 1992).

Vegetation and habitats

Western Madagascar native vegetation is mainly represented by dry deciduous forests, named also "tropophile forests". During the centuries forest destruction -which has so far eliminated the native woody plant cover on three quarters of the island- has converted 80 % of the original western forests in secondary formations, mainly grasslands which are burnt each year. These habitats, ranging from wooded savannahs to "pseudosteppes", are regularly used for cattle grazing.

The World Heritage Site "Tsingy de Bemaraha" includes at least 5 % of total western Madagascar dry deciduous forest cover.

The primary Antsingy forest , which grows on a karst limestone relief, shows an extraordinary variety of *facies* in relation to substratum nature, hydrography and exposition (BOUSQUET & RABETALIANA, 1992):

-A dense dry forest on sub-emerging calcareous plates (thin layer of humus).

-A xerophytic vegetation on the top of the tsingy.

-A dense sub-humid forest at the bottom of canyons (thick leaf litter).

-A dense sub-humid forest at the foot of Antsingy western slopes (sandstone substratum).

-A forest tunnel along the rivers (Beboka, Miharana, *etc...*).

-Spots of dense dry forest on the cones of the Plateau of Bemaraha.

Every *facies* shows a different flora, although there are plant species which can be found in most of them. As the name "tropophile" suggests, the forest presents marked variations in physionomy and ecology due to the deciduous character of many species which lose their leaves at the beginning of the dry season. Trees with persistent leaves are more common were humidity conditions are favourable enough, particularly in the dense sub-humid forest inhabiting the bottom of canyons.

Far to be sufficiently known, the extremely complex flora of the Antsingy was studied for the first time in the thirties by LEANDRI (1938) and is now object of a research carried on by bothanists of Paris University.

Many plant species, as *Diospyros perrieri* (the ebony of the west coast), *Musa perrieri* (the wild Malagasy banana) and species belonging to the genus *Delonix*, are exclusive of the associations found in the Antsingy. A good number of families, some of which including xerophytic species found only on the sharp rocks of the tsingy, is well represented in these habitats:

Flacourtiaceae, Orchidaceae, Leguminosae, Euphorbiaceae, Apocynaceae, Annonaceae, Bombacaceae and Moraceae (IUC/UNEP/WWF, 1987). The Antsingy forest is surrounded by large extensions of grasslands which have substituted the original dry deciduous forest cover. There are different kinds of savannahs in relation to rainfar nature and fertility of the soil and frequency of fires; the best pastures for cattles are represented by grasslands with *Hyparrenia rufa* and *Heteropogon contortus* found on the Plateau of Bemaraha.

		In the World Heritage Site "Tsingy de Bemaraha"		In Madagascar	
Families and species	Habits	Reference*	Habitats recorded	Distribution	Habitats recorded
Microhylidae Scaphiophryninae					
Scaphiophryne calcarata (Mocquard, 1985)	burrowing	2, 3	dense sub-humid forest	S, W, NW	temporary pools
Scaphiophryne marmorata Boulenger, 1882	bottom dwelling	1, 3	dense sub-humid forest	NE, W, E, SE	rain forest
Microhylidae Dyscophinae					
Dyscophus insularis Grandidier, 1872	burrowing	3	dense sub-humid forest	N, NW, W, SW	tropophile forest
Hyperoliidae					
Heterixalus betsileo (Grandidier, 1872)	treefrog	3	forest edges	widespread	secondary vegetations
Heterixalus tricolor (Boettger, 1881)	treefrog	3	cultivated areas	NW, N	secondary vegetations
Ranidae					
Tomopterna labrosa Cope, 1868	burrowing	1, 3	grasslands, forest edges	NW, W, SW, S	secondary vegetations
Ptychadena mascareniensis (Duméril & Bibron, 1841)	pool dwelling	1, 2, 3	temporary pools in cleared areas	widespread	stagnant water
Mantellidae					
Mantidactylus biporus (Boulenger, 1889)	pool dwelling	4	—	W, N, NE, E, SE, S	forests, cleared areas
Mantidactylus curtus (Boulenger, 1882)	brook dwelling	4	—	W, N, NE, E, SE, S	forests, cleared areas
Mantidactylus granulatus (Boettger, 1881)	bottom dwelling	4	—	W, N, NE, S	forests, sec. vegetations
Mantidactylus luteus Methuen & Hewitt, 1913	bottom dwelling	4	—	W, N, NE, E, SE	forests
Mantidactylus ulcerosus (Boettger, 1880)	brook dwelling	4	—	W, NW, N, NE, SE	forests, cleared areas
Mantidactylus wittei Guibé, 1974	bottom dwelling	3	dense sub-humid forest	NW, E	forests
Mantella betsileo (Grandidier, 1872)	bottom dwelling	3	dense forest, mango plantations	W, NW, N, NE, E	forests
Rhacophoridae					
Aglyptodactylus madagascariensis (Duméril, 1853)	bottom dwelling	1, 3	dense sub-humid forest	N, NW, E, SE	forests
Boophis albilabris (Boulenger, 1888)	treefrog	3	dense sub-humid forest	E	forests
Boophis tephraeomystax (Duméril, 1853)	treefrog	3	dense sub-humid forest	widespread	coastal cleared areas

Table 1: Amphibians recorded in the World Heritage Site "Tsingy de Bemaraha". (1): Hallmann et al. 1990. (2): Bloomers-Schlösser & Blanc 1991. (3): Present study. (4): Glaw & Vences 1992.

If compared with the soil of the Antsingy forest, which may have a 10 cm thick layer of humus, savannah soils show very poor quantities of organic matter. With the exceptions of coastal areas, widely covered by the sterile "sand-clay carapace", most of grasslands grow on red decalcified clays named "terra rossa" which resemble the lateritic clays found over the crystalline and metamorphic rocks of the Precambrian basement.

In relation to tree and bush cover, we can distinguish between "pseudosteppes" characterized by the total absence of trees and bushes and wooded savannahs with *Poupartia, Hyphaene, Medemia, etc...*

In vicinity of the villages, which are mostly sited along the rivers, the fertile alluvial soil allows the cultivation of manioc, maize, rise and fruit trees (coconut, banana, mango, papaya).

MATERIALS AND METHODS

The field work was carried on during two weeks at the beginning of the rain season (22/12/1992-05/01/1993); half of the time available was used for animal search in Antsingy forest, while the remaining 50 % was spent for the same purpose in cultivated zones around Antsalova village and in the grasslands. All sample areas were between 100 and 300 m above sea level. Forest habitats covered were all sited in the central part of Antsingy western slopes, close to the road which connects Antsalova to Tsiandro.

Herptiles (particularly amphibians) were mostly searched in the morning, in the evening and during the night, since they usually showed a drop in activity during the hottest part of the day. Rainshowers -which often occurred in the evening- were utilized for frog research. We used the search methods already described by RAXWORTHY (1988) and ANDREONE (1991).

RESULTS AND DISCUSSION

Herpetofauna species recorded

In the study area we were able to find 32 species of herpetofauna. With the contribute of previous published and unpublished records (NICOLL & LANGRAND, 1989; HALLMANN *et al.,*

		In the World Heritage Site "Tsingy de Bemaraha"		In Madagascar	
Families and species	Habits	Reference*	Habitats recorded	Distribution	Habitats recorded
TESTUDINATA					
Pelomedusidae					
Pelomedusa subrufa (Lacépède, 1788)	pond dwelling	3	temporary pools	widespread	stagnant water bodies
Pelusios subniger (Lacépède, 1788)	pond dwelling	2	—	E	slow-moving streams, lakes
LORICATA					
Crocodylidae					
Crocodylus niloticus (Laurenti, 1768)	river dwelling	3,4	rivers outside the tsingy	once widespread	rivers and lakes
OPHIDIA					
Boidae					
Acrantophis madagascariensis (Duméril & Bibron, 1844)	ground dwelling	1,2	—	E, NE, N, NW, W	forests, secondary vegetations
Sanzinia madagascariensis (Duméril & Bibron, 1844)	ground/tree dwelling	1,2	—	widespread	forests, secondary vegetations
Colubridae Boiginae					
Dromicodryas bernieri (Duméril & Bibron, 1854)	ground dwelling	2,3	dense sub-humid forest	widespread	forests, secondary vegetations
Dromicodryas quadrilineatus (Duméril & Bibron, 1854)	ground dwelling	3	dense sub-humid forest	widespread	forests, secondary vegetations
Langaha nasuta Shaw, 1790	tree dwelling	2	—	widespread	forests
Mimophis mahfalensis (Grandidier, 1867)	ground dwelling	1,2,3	grasslands	widespread	secondary vegetations
Colubridae Lycodontinae					
Lioheterodon madagascariensis Duméril & Bibron, 1854	ground dwelling	2,3	dense sub-humid forest	widespread	forests, secondary vegetations
Lioheterodon modestus (Günther, 1863)	ground dwelling	2	—	widespread	forests, secondary vegetations

Table 2: Chelonians, Crocodilians and Snakes recorded in the World Heritage Site "Tsingy de Bemaraha". (1): Nicoll & Lagrand 1989. (2): Hallmann et al. 1990. (3): Present study. (4): Bousquet & Rabetalia (1992).

		In the World Heritage Site "Tsingy de Bemaraha"		In Madagascar	
Families and species	Habits	Reference*	Habitats recorded	Distribution	Habitats recorded
Gekkonidae					
Hemidactylus sp.	—	3	village huts	—	—
Homopholis sakalava (Grandidier, 1867)	tree/rock dwelling	2	—	W, SW	tropophile forests
Lygodactylus klemmeri Pasteur, 1964	tree/rock dwelling	1,2,3	dense sub-humid forest	Antsingy forest	tropophile forests
Paroedura bastardi (Mocquard,1900)	rock dwelling	3	dense sub-humid forest	W, SW, S, SE	forests, secondary vegetations
Phelsuma abotti Steijneger, 1893	tree dwelling	2,3	dense sub-humid forest	N, NW	forests, secondary vegetations
Phelsuma dubia (Boettger, 1881)	tree/rock dwelling	2	—	NW, W, SW, S	secondary vegetations
Phelsuma lineata Gray, 1831	tree/bush dwelling	3	dense sub-humid forest	NW, NE, E, SE, S	forests, secondary vegetations
Phelsuma madagascariensis kochi Mertens, 1954	tree dwelling	2,3	dense sub-humid forest	NW, W	tropophile forests
Phelsuma mutabilis (Grandidier, 1869)	tree/rock dwelling	2,3	forest, tree savannah	NW, W, SW, S, SE	forests, secondary vegetations
Iguanidae					
Chalarodon madagascariensis Peters, 1854	ground dwelling	2,3	savannah, pseudosteppe	W, SW, S, SE	cleared areas
Oplurus cuvieri cuvieri (Gray), 1831	tree/rock dwelling	2,3	savannah, forest edges	NW, W	dry secondary vegetations
Chamaeleontidae					
Brookesia bonsi Ramanantsoa, 1980	leaf litter dwelling	2	—	NW	tropophile forests
Brookesia ebenaui (Boettger, 1880)	leaf litter dwelling	3	sub-humid and dry forests	NW, N, NE, E, S	forested areas
Brookesia perarmata (Angel, 1933)	leaf litter dwelling	1,2	dense sub-humid forest	Antsingy forest	tropophile forests
Furcifer oustaleti (Mocquard, 1894)	tree/bush dwelling	1,2,3	forest edges	SW, NW, N, NE	forests, secondary vegetations
Furcifer verrucosus (Cuvier, 1829)	tree/bush dwelling	2,3	sub-humid and dry forests, edges	widespread	forests, secondary vegetations
Scincidae					
Mabuya elegans (Peters, 1854)	ground dwelling	1,2,3	sub humid and dry forests	widespread	forested and cleared areas
Mabuya gravenhorsti (Duméril & Bibron, 1839)	ground dwelling	1,2	—	widespread	forested and cleared areas
Gerrhosauridae					
Zonosaurus laticaudatus (Grandidier, 1869)	ground dwelling	3	forest and cultivated areas	widespread	forests, secondary vegetations

Table 3: Lizards recorded in the World Heritage Site "Tsingy de Bemaraha". (1): Nicoll & Langrand 1989. (2): Hallmann *et al.*, 1990. (3): Present study.

1990; BLOMMERS-SCHLÖSSER & BLANC, 1991; BOUSQUET & RABETALIANA, 1992; GLAW & VENCES, 1992) a total of 47 species -17 amphibians and 30 reptiles- is obtained. They are listed in tables 1 to 3 together with some data regarding their habits, habitats recorded in the study area (only for the species recorded by the present study), distribution and habitats recorded in Madagascar.

Although probably far to be definitive, this list shows that Bemaraha tropophile forest and adjacent cleared areas host a very rich herpetofauna which is comparable to the extremely rich ones of the eastern rainforest areas. It may be interesting, in this respect, to compare these figures with ones regarding other

Malagasy forest areas reported by IUCN/UNEP/WWF (1987) and NICOLL & LANGRAND (1989); it must anyway be kept in mind that these areas have received much more attention than Tsingy de Bemaraha:

-Montagne d'Ambre (rainforest): 7 amphibians, 16 reptiles.
-Zahamena (rainforest): 14 amphibians, 9 reptiles.
-Lokobe (rainforest): 8 amphibians, 35 reptiles.
-Marojeyi (rainforest): 17 amphibians, 22 reptiles.
-Perinet-Analamazoatra (rainforest): 25 amphibians, 29 reptiles.
-Kirindy (tropophile forest): 1 amphibian, 32 reptiles.
-Ankarafantsika (tropophile forest): 5 amphibians, 38 reptiles.

More recent field studies carried out in eastern areas show an even richer herpetofauna: for instance, according to Andreone (pers. comm.) Ranomafana National Park -as well as other rainforest sites- is inhabited by 40-50 species of frogs.

It is nevertheless likely that future studies sampling other portions of Antsingy forest during the peak of the rain season will lead to enlarge, especially for what regards the amphibians, the list proposed in this paper.

Herpetofauna presence in different habitats

Fig. 3 shows schematically the main terrestrial habitats found in the World Heritage Site "Tsingy de Bemaraha" together with the number of herptile species that we recorded.

The villages and the cultivated areas seem to host mostly euritopic species which are widespread in Madagascar. The bottom dwelling frogs *Ptychadena mascareniensis, Tomopterna labrosa and Mantella betsileo* were commonly seen on the ground of mango plantations; the treefrog *Heterixalus tricolor* inhabits tall grasses along village edges. *Phelsuma mutabilis* and *Hemidactylus* sp. (probably *H. frenatus)* occur inside village huts and on surrounding vegetation. The large lizard *Zonosaurus laticaudatus* was found on the ground in cultivated areas, while shells of the pond terrapin *Pelomedusa subrufa* were collected in the same habitat.

The savannah is characterized by a very low presence of bushes and trees. The two euritopic amphibians *Ptychadena mascareniensis* and *Tomopterna labrosa* were found only in shady humid areas. The snake *Mimophis mahfalensis,* the iguanid *Chalarodon madagascariensis* and the skink *Mabuya clans* were the only herptiles seen in pseudosteppe habitats, while *Phelsuma mutabilis* and *Oplurus cuvieri cuvieri* could been found on bushes and trees.

The treefrogs *Heterixalus betsileo* and *Boophis tephraeomystax* and a few other amphibians *(Ptichadena mascareniensis, Tomopterna labrosa* and *Mantella betsileo)* were commonly found along the edges of the dense sub-humid tropophile forest. Also three geckoes (*Lygodactylus klemmeri, Phelsuma abotti* and *P. mutabilis*), two chameleons (*Furcifer oustaleti* and *F. verrucosus*), one iguanid (*Oplurus cuvieri cuvieri)* and one gerrhosaurid lizard (*Zonosaurus laticaudatus*) inhabit these ecotone habitats.

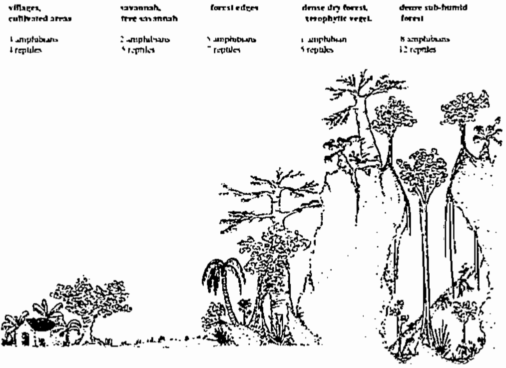

Figure 3: Herpetofauna species presence in different habitats within the World Heritage Site "Tsingy de Bemaraha".

The dry forest growing on top of the tsingy was occasionally visited because of inaccessible problems. In this habitat we found *Mantella betsileo* and some reptiles: *Lygodactylus klemmeri, Phelsuma mutabilis, Mabuya elegans, Furcifer verrucosus* and the dwarf bottom-dwelling chameleon *Brookesia ebenaui.*

As expected, most of the species were found in the dense sub-humid forest. For what regards the amphibians, three microhylids (*Scaphiophryne calcarata, S. marmorata* and *Dyscophus insularis*), two mantellids (*Mantidactylus wittei* and *Mantella betsileo)* and three rhacophorids (*Aglyptodactylus madagascariensis, Boophis albilabris* and *B. tephraeomystax*). Among the reptiles, three snakes (*Dromicodryas bernieri, D. quadrilinealus* and *Lioheterodon madagascariensis*) and eight lizards (*Lygodactylus klemmeri, Paroedura bastardi, Phelsuma abotti, P. lineata, P. madagascariensis kochi, P. mutabilis,* *Brookesia ebenaui, Furcifer verrucosus* and *Mabuya elegans*).

It was thus evident that some species seem to inhabit exclusively degradated areas (villages areas and grasslands): it is the case of *Heterixalus tricolor, Tomopterna labrosa* and *Ptychadena mascareniensis*, for whar regards amphibians; among the reptiles, *Hemidactylus* sp., *Chalarodon madagascariensis* and *Furcifer oustaleti*.

Other species, some of which highly stenotopic, were found only in the tropophile forest: the amphibians *Scaphiophryne calcarata, S. marmorata, Dyscophus insularis, Mantidactylus wittei, Aglyptodactylus madagascariensis, Boophis albilabris* and the reptiles *Dromicodryas bernieri, D. quadrilinealus, Lioheterodon madagascariensis, Lygodactylus klemmeri, Paroedura bastardi, Phelsuma lineata, Phelsuma madagascariensis kochi, Brookesia ebenaui*.

In other parts of Madagascar we observed that some of these latter species commonly occur also in degradated areas (for instance *Dromicodryas* species and *Lioheterodon madagascariensis*). Their apparent absence in the grasslands around the tsingy might be explained considering that an incomparably more advantageous situation is found inside the forest habitat.

At least one amphibian *(Mantelia betsileo)* and one reptile *(Phelsuma mutabilis)* can be chosen as examples of species inhabiting all kinds of primary and secondary habitats.

Distribution in Madagascar of Bemaraha herptiles

In relation to climate and vegetation cover, Madagascar can be divided into two main regions (fig. l): the Eastern is more humid, richer floristically and has as predominant potential kind of vegetation the rainforest, while the Western has a long dry season, is less rich in species and is characterized by deciduous kinds of vegetation.

In the areogram of fig. 4 every portion of Madagascar -split in accordance with the cardinal points- is represented by a sector whose width is proportional to the number of herptile species shared with our study area. It is evident that a relatively high part of the amphibians and the reptiles found in the World Heritage Site "Tsingy de Bemaraha" occur in areas which are not included in the Western Region. In particular, some of these species seem to have an unusual distribution which covers only Eastern Region localities, with the exception of the Antsingy forest itself. It is the case of the amphibians *Scaphiophryne marmorata, Mantidactylus biporus, Mantidactylus granulatus, Mantidactylus luteus, Boophis albilabris*, of the freshwater turtle *Pelusios subniger* and of the day gecko *Phelsuma lineata*.

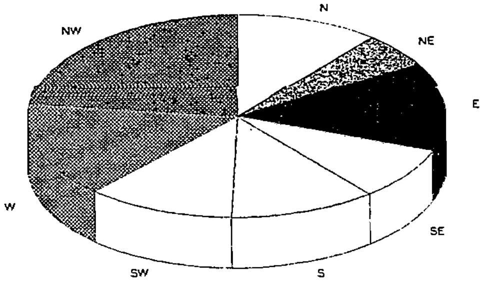

Figure 4: Aerogram showing the relative number of herpetofauna species shared with the WHS "Tsingy de Bemaraha" by the different portions of Madagascar (split in accordance with the cardinal points).

The presence of these seven species, which are considered typical of the eastern rainforest and/or corresponding secondary formations, in the Antsingy forest may be explained on an ecological basis, by the unusually high permanent humidity of some parts of the tropophile forest due to the relief geomorfology and to the good amount of rainfall (1000-1500 mm). Actually these habitats, in which both deciduous and evergreen species of plants live, resemble the rainforest for what regards the richness of epiphytes, plants which require high air humidity, and the presence of many streams.

Biogeographically, these eastern species may have reached the Antsingy forest -through the Central Highlands- when karst formations occupied a wider surface and primary forests covered most of Madagascar. The relatively recent deforestation of the Plateau may have caused the extinction of the central populations and therefore the actual disjunct distributions. An alternative theory maintaining a spread southwards from the Sambirano region for some of these species (which seems to apply very well to explain the presence of *Mantella betsileo* in the Antsingy forest) does not appear convincing unless they are recorded in NW forest areas such as, for instance, Tsingy de Namoroka and Ankarafantsika. On the other hand it should not be underestimated the importance that these peculiar habitats are likely to have as centres of origin of species: for instance, for what regards the chameleons, *Furcifer petteri* is endemic to Ankarana, *Furcifer angeli* and *Brookesia decaryi* to Ankarafantsika, *Brookesia bonsi* to Tsingy de Namoroka and *Brookesia perarmata* to Tsingy de Bemaraha. Also the gecko *Lygodactylus klemmeri* is, as far as we know, restricted to the Antsingy forest of the Tsingy de Bemaraha.

CONCLUSIONS

Despite the scarce efforts dedicated by naturalists to the quite inaccessible Tsingy de Bemaraha massif, this area is likely to have a considerably high biodiversity value. First, because of the peculiar high variety of habitats found in the area. Beside this, more than 300 species of plants (including some endemic ones, as *Musa perrieri*, the only wild banana in Madagascar), 16 mammals, 77 birds (BOUSQUET & RABETALIANA, 1992), 30 reptiles and 17 amphibians have been found in this site.

The herpetofauna checklist proposed in the present study must be regarded as a preliminary one, but nevertheless reveals some interesting aspects related with the biogenetic values of the study area which can be summarized by the following considerations:

-The number of species of herptiles recorded (47) is remarkably high -especially for what regards amphibians, usually poorly represented in deciduous forests- and comparable with the figures concerning Madagascan eastern rainforest.

-At least 13 species and one subspecies probably behave as stenotopic since they have been recorded only in primary forest habitats.

-As far as we know 7 species show an unusual distribution which includes only Eastern Region localities - whose potential dominant vegetation is the rainforest - except for a unique deciduous habitat: the Antsingy forest.

-At least 2 species of reptiles are likely to be endemic to the Antsingy forest.

It is to be hoped that further field surveys will lead to increase our knowledge of the herpetofauna of the Tsingy de Bemaraha massif, not only for what regards the taxa represented, but also their ecological niche and their distribution within the area compared with the rainfall gradient (the mean annual rainfall, the length of the rain season and the rate of plant species with persistent leaves decrease going southwards).

Dealing with conservation aspects, it must be remarked that, while the grasslands suffer problems related with fires, overgrazing and erosion, the Antsingy forest is threatened only at a very small scale.

Forest edges are affected by illegal logging and cattle trampling, but all forest habitats are interested by a small scale illegal collecting of lemurs, herptiles, insects and waterplants (especially *Aponogeton madagascariensis*).

A UNESCO eco-development programme is planning to improve standards of living of local people, reduce soil erosion and create ecotourism facilities within the most spectacular parts of the Tsingy (BOUSQUET & RABETAHANA, 1992).

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A fast environmental impact assessment method for the evaluation of road construction effects on amphibian communities

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Abstract: During the coming decade 22.000 km motorway is planned to be built in Europe. As in many cases there is not enough time for a detailed survey there is a growing need for fast and effective herpetological impact assessment methods. In April, 1992 the Toad Action Group was contracted to make an environmental impact assessment on the planned M3 motorway. Our aim was to produce an easy to handle report for decision- makers. Data were transferred into a score system. We took only three paramcters, the species and individual number of amphibians and the distance between the breeding sites and the planned route into consideration, which resulted in an eight point system. Other parameters recorded (e.g. habitat characteristics) were only indirectly involved in the evaluation with the exception of the presence of other protected species. Altogether 43 possible breeding sites were found and investigated. 40 of them were threatened to a greater or a lesser extent by the would-be construction along the 120 km long route. In cases when the water body belonged to the most threatened category the alteration of the route was recommended. When they were ranked 2 or 3 the building of toad tunnels and fences was proposed. In case of 4, 5 and 6 it was recommended that material should be exploited and transported and material depots should be selected carefully. The system worked well in the case study as the categories seemed to be "digestible" for decision-makers. Three of the four suggested route alterations were accepted by the planners of the M3 motorway. As very often environmental impact assessments are to be made in a relatively short amount of time there is a need for a similarly fast reptile survey as well.

Key words: amphibians, environmental impact assessment, road planning, the effects of motorways, toads

INTRODUCTION

During the coming decade a 12.000 km motorway network is planned for Western Europe (BOWERS, 1993) and 10.000 km for Central and Eastern Europe. The longest will be the Southern Motorway,running from Lisbon to Kiev (Figure 1).

Roads that are not carefully enough planned and / or constructed considerably burden local plant and animal communities through their direct effects and genetic isolation (REH,1989).

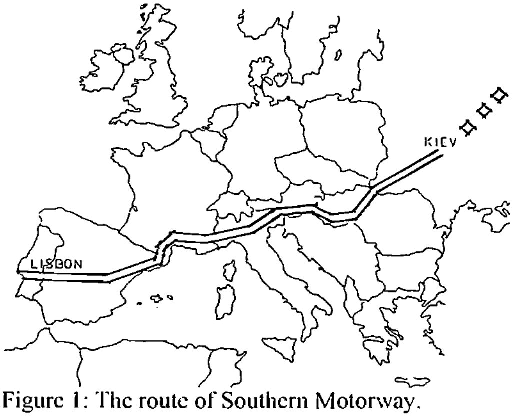

Figure 1: The route of Southern Motorway.

Among vertebrates amphibians are the most susceptible to the direct effect, nine out of ten vertebrates killed on roads were from that class in a recent Hungarian survey (FENYVES, 1989). Environmental impact assessment, which has become obligatory at large constructions in Hungary nowadays (86/1993 (VI.4) Korm. rendelet), can be an important tool to lower the pressure caused by roads. However in many cases there is not enough time to carry out detailed surveys. This factor underlines the importance of fast and effective herpetological impact assessments as a necessary tool to avoid e.g. *Vipera ursinii* site destruction, which happened in Hungary last year.

In April, 1992 the Toad Action Group was contracted to make an environmental impact assessment with the Hungarian Ministry for the Environment on the planned M3 motorway.

Our aim was to produce an easy-to-handle report for decision-makers.

SITE AND METHOD

The M3 motorway runs 120 kms from Gyongyos to Polgar in the eastern part of Hungary (Figure 2). It is mainly a lowland area with intensive agricultural use, but there are valuable wetlands with a number of protected species there, especially along the Tisza river

Figure 2: Map of the M3 Motorway.

and large fish ponds with extensive reed belts.

In the survey we used a modified data sheet from our previous investigation along the M0 ringroad (Table 1) (PUKY & KECSKES, 1992). Because of a very limited amount of time (six weeks after the breeding season of terrestrial amphibians) we could mainly focus only on (possible) breeding sites.

01. Serial number:
02. Location (km):
03. Name:
04. Distance from the road:
05. Side:
06. Water type:
07. Water surface:
08. The estimated area of reed belt:
09. Ratio of areas with 30-50 cm of water cover:
10. Water depth: (max): (average):
11. The ratio of forested areas around the water:
12. List and estimated number of amphibians:
13. Other protected species:
14. Other remarks:
15. Rank (1-8):

Table 1: Data sheet of the M3 Motorway.

Data were transferred into a score system. We took only three parameters, the species and individual number of amphibians and the distance between the breeding sites and the planned route into consideration, which resulted in an eight point system (Table 2). The number of amphibians were divided into groups according to the genetical requirements for a healthy population.

Other parameters recorded (e.g. habitat characteristics) were only indirectly involved in the evaluation with the exception of the presence of protected species other than amphibians.

N° of amphibians	threat for the site	score
more than 200	high	1
51-200	high	2
1-50	high	3
more than 200	medium	4
51-200	medium	5
1-50	medium	6
any	no	7
none	any	8

Table 2: The score system of the environmental impact assessment.

RESULTS

Altogether 43 possible breeding sites were found and investigated. 40 of them were threatened to a greater or a lesser extent by the would-be construction along the 120 km long route. The frequency of the different categories can be seen in Figure 3. In cases when the water body belonged to the first category the alteration of the route was recommended. When they were ranked 2. or 3. the building of toad tunnels and fences was proposed. In case of 4., 5. and 6.it was recommended that material should be exploited and transported and material depots should be selected carefully so as not to destroy the aquatic habitat of amphibians. It was necessary to note whether the construction itself could also cause serious damage in neighbouring habitats.

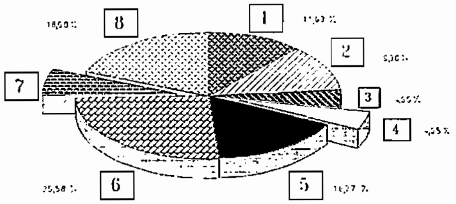

Figure 3: Frequency of differents categories (1-8) along the M3 Motorway.

Continuous consultation with environmental experts is also needed during the process. The creation of new artificial ponds was an option but was not suggested because of the lack of time for a more detailed investigation. These may also be suitable solutions for other cases.

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Conservation of an amphibian community in the marsh of Charvas (Isère, France).

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Abstract: Consecutive to the construction of a new motorway plus railway line, the Marsh of Charvas (Isère, France) has been separated in two parts . To preserve amphibian populations and to compensate the cut effect on the amphibian migratory axes, several substitute ponds have been established.
Key words : Amphibian, Conservation, Substitute pond.

INTRODUCTION

The Marsh of Charvas is located about 20 kilometres eastern Lyon. This is a natural area interesting for its flora and fauna classified as Z.N.I.E.F.F. (Zone Naturelle d'Intérêt Ecologique Floristique et Faunistique). Eight species of Amphibians have been found there : - 2 Salamanders: *Triturus helveticus, Triturus cristatus.* 6 Anurans: *Bufo bufo, Bufo calamita, Hyla arborea, Rana dalmatina, Rana ridibunda and Rana kl. esculenta.*

Every year, Amphibians are moving through the Marsh from their winter area to the natural ponds where they use to reproduce. Since 1991, a new railway line (T.G.V.) and a motorway (A432) separate the Marsh in two isolated parts (figure 1).

MATERIAL AND METHODS

In 1990, the S.N.C.F. (Société Nationale des Chemins de fer Français) contacted the S.B.F. (Société Batrachologique de France) to study the different impacts of the new railway line and the motor way on the amphibian populations (S.B.F., 1990). Two types of impacts have been found:

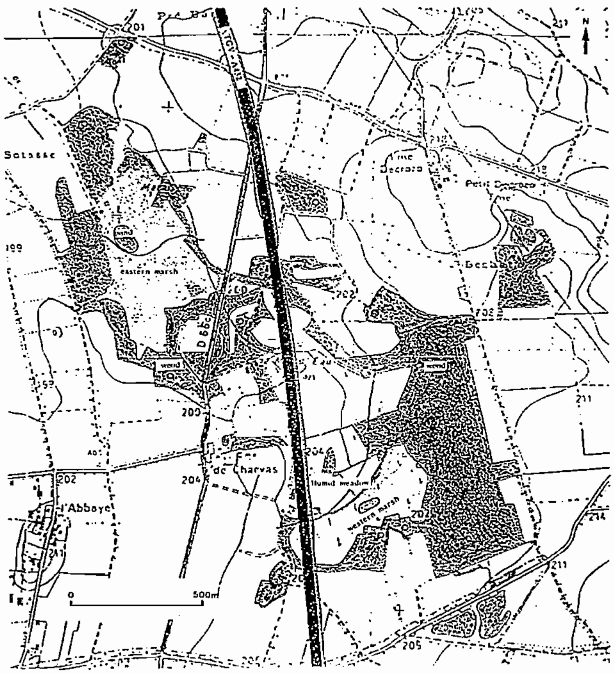

Figure 1: Situation of the Marsh of Charvas.

The direct impacts

The road system prevents the annual migration of the Amphibians which are now unable to reach their breeding sites. The Amphibians and other animals cannot cross the new road: they have no chance to attain the other side of road without being run over by cars.

Migrations of common toad (*Bufo bufo*) and agile frog (*Rana dalmatina*) have been observed in the Marsh of Charvas. So these species have been a lot affected by the partition. Several migratory axes have been found from the western part towards the eastern part of the Marsh.

The new railway line and the motorway have caused an isolation of the south-western part of the Marsh, becoming too small to allow survival of Amphibians. The last direct impacts are sound and car pollutions caused by the traffic. Several hedges will be planted along the road system to reduce the sound pollution.

The indirect impacts

Modifications in agricultural methods have been observed because of re-allocations of land. New paths have been opened, hedges destroyed, ponds filled in. The Marsh has been reduced of 10 hectares and cut in two parts, breaking the complementary effect of the different biotops. The risk for the Marsh to be drained and farming methods to be changed (i.e. turning a humid meadow into a maize field) was very great and had to be prevented.

RESULTS AND DISCUSSION

To prevent the decreasing of the Marsh biologic value, the S.B.F. studied various actions of conservation in relation with the different species (LEMAN, 1993). One measure would have been to settle toad tunnels to allow Amphibians to cross the road plus railway line. This measure has not been chosen because the T.G.V. line and the motorway were about 80 meters wide. It was too long to be efficient. Moreover, the common toad (*Bufo bufo*) and the agile frog (*Rana dalmatina*) populations were not large enough. Toad tunnels are not so efficient and profitable for small populations. Other measures, in the present case, are better to protect amphibian populations. As it is necessary to take away the Amphibians from the road system, these measures consist in creating substitute ponds (figure 2 a,b) and then transferring eggs and endangered adults in those protected biotops. Three substitute ponds have been already dug in the eastern part of the Marsh. Two ponds have still to be dug in the western part. Aquatic plants have been pricked out. Eggs and adults have been transported to these new ponds. An enclosure has been built in the pond to prevent adults to escape before egg laying (figure 2). At the end of the reproductive period, the enclosures have been opened. According to their homing behaviour to breeding sites, the new generations of Amphibians are supposed to come back to their birth pond to reproduce. They should not try to cross the road and railway line.

To be efficient, this work of conservation had to be performed several years by visiting the ponds and overseeing the populations of Amphibians. In this way, since the construction of the new breeding sites, students regularly go to the Marsh to control the evolution of ponds and populations of Amphibians. The new ponds being now stabilised are progressively repopulated.

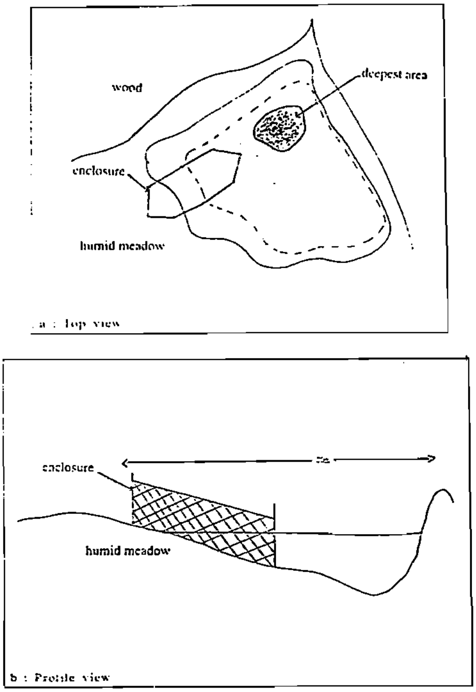

Figure 2: Schema of the substitute pond.

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A Natural Reserve for the "Ferreret" (*Alytes muletensis*) (Sanchiz & Adrover 1977) (Anura, Discoglossidae).

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Abstract: In the last few years four populations of *Alytes muletensis* have been discovered in precise biotopes, outside of the karstic canyons (the characteristic habitat of the species). The importance of these population nuclei is numerically limited, given that in all cases they depend on an isolated spot of water, and relatively far from the nearest canyon with stable population of the species. These spots of water are related in three cases by transformations or adaptations undertaken by man in the environment for the capture and conservation of rain water for the herds. The importance of these nuclei is very relevant from the conservation viewpoint, as the potential habitat is more extensive than was supossed. One of the mentioned populations is found in a quiet valley on the south slope of the *Tramuntana* mountains. Of the four discovered populations, this is the furthest from a canyon inhabited by *A. muletensis*, Which gives it a greates biogeographical interest. This valley houses an old abandoned agricultural aperation. The *Conselleria d'Agricultura i Pesca* (Regional Authority for Conservation) has initiated the process of acquiring the property to create a Natural Reserve as a special conservation area. The management of the Reserve will be carried out under the Ordination Plan of the *Tramuntana* mountains (now being prepared). Some of the ecological characteristics of the locality are described here.

Key words: Balearic Islands, *Alytes muletensis*, Predators, canyon, open-water.

INTRODUCTION

The "Ferreret" (species of comunity interest) is currently considered a relictual, insular, endemic species (its distribution area is limited to a few points of The "Tramuntana" mountains in the N.W. of the island of Majorca). Its precarious conservation status has been caused mainly by the historic introduction of foreign predators, specially snakes (*Natrix maura, Macroprotodon cucullatus*). These species have erradicated the discoglossid from most of the island. Other largely human-related factors such as biotopus modification, pollutant wastes, depletion of aquiferouses, and drought have contributed to the present situation of extinction danger.

To start with, after the discovery of the first populations it was presumed that this species would have strict habitat requirements (ALCOVER *et al* 1984). However, we can actually speak of the existence of two types of habitat occupied by *Alytes muletensis*:

a) Karstic canyons, excavated in the limestones rocks, whose physical characteristics rend the carnivores presence difficult. In these canyons the "Ferreret" populations, although quite restricted, sustain a relatively high density.

b) Open-waters: Points of permanent water in open areas (e.g. isolated ponds, cisterns and drinking places for cattle) where its main predators are not present. This habitat probably its more representative for the species, but in most cases has lost its capacity for maintaining "Ferreret" populations. However, in the last three years four populations with these characterisces have been detected (ALOMAR & REYNES, 1993), (ROMÁN, 1993). These habitats are quite different from the relictual conditions initially described for the species (Streams with stepped and "canyon-like" morphology).

NEW POPULATIONS

The numeric importance of the population nuclei in open habitats is limited in most cases, since they depend on isolated water bodies. The adition of these populations involves a 23.3% increase of the total known population of *Alytes muletesis* (Fig. 1). Yet, except for the last population discovered (july 1993), in the remaining three cases the presence of permanent water year-round is related to environmental transformations undertaken long ago by man in order to capture and conserve rain water. Such habitat alterations were essential to ensure the presence of herds in areas subjects to long summer droughts.

Location and importance

Open-water populations are biogeographically not very important, since all are in the already know distribution area. One of them is the highest (775 m) among the known populations. Three of them are situated in a drainage that includes a canyon with a stable population for the "Ferreret". However, the importance of open-water habitats is quite relevant from the conservation viewpoint -- the potential habitat for the species is more extensive than was supossed at first. We can affirm that the "Ferreret" can indeed live outside the canyons, but only in the absence of snakes. In few cases of human constructions, their architectonic characteristics rend difficult the predators entry. This open new expectations for the *A. muletensis* management offering models for old constructions restoration or new constructions.

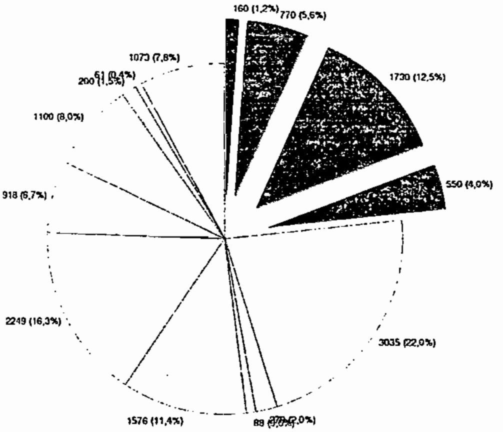

Figure 1: Population distribution of *Alytes muletensis* in Majorca (number and percentage of larvaes of each locality). The open-area populations (23.3%) are shadowed in dark, whereas canyon populations are shown in light.

The fourth open-water locality has a great biogeographical interest. It is found in the drainage of a temporal stream where the species is represented only by an unestable, small nucleus. The nearest inhabited canyon is four km. away from the stream course. The open-water population lives around an isolated semiartificial pond at an altitude of 480 m., in an oak forest located in a valley inaccessible on wheels. This valley is in a very good preservation state, houses an old abandoned agricultural operation, and it´s very quiet. It is a typical karstic valley with caves and deep sinkholes that preclude the formation of a superficial drainage. The oak wood occupies an extension of only 6 ha. In the remaining part of the valley, where the wood extended long ago, we find brushwood substitution comunities, characterised by *Eryca multiflora, Cistus monspeliensis, Ampelodesma mauritanica, Smilax aspera var. balearica, Pistacia lentiscus*, with a few oak wood fragments. The rocky hillsides that close the valley on the north, present a brushwood vegetation typical of calcareous litosole with predominance of *phyllirea latifolia* and *Buxus balearica*. The pond is 5m. x 2m. and its maximum depth is 2m. No other anphibians breed in the pond. The only potential predator detected for *A. muletensis* larvae are a few water beetles (*Meladema coriacea*). Predation on adult toads is exceptional. Only one frog (*Rana perezi*) was observed predating on an adult toad. Another potential predator detected in the area is *Macroprotodon cucullatus*.

This population has been studied by Criado, Bush and Román in the setting of the Recovery Plan of the "Ferreret" (CRIADO, *et al*, 1991). Annual census have been carried out and some productivity data has been obtained (CRIADO, 1991; ROMÁN, 1992; ROMÁN, 1993; BUSH, in press) testing an individual identification method based on the cutaneous spot design (CRIADO, 1991).

CONSERVATION MEASURES

The property that includes the open-water locality described is on sale, with prospect of opening an access road. This act would involve a definitive threat for the *A. muletensis* population placed in the valley. In view of this danger, the researchers working on the preservation, prepared and acquisition proposal, which was promoted by the "Direcció General d'Estructures Agraries i Medi Natural". At this moment the acquisition process is very advanced. This land is adjacent to three public properties. This purchase will result in a larger public area permitting a more effective preservation. The proposed legal figure for this area is Natural Reserve. This reserve will include other interesting areas in surrounding lands. The reserve creation will develop an agreement between the Spanish Conservation Office (ICONA) and the Local Balearic Governement. The "Ferreret" is a priority species in the Habitats Directive of the E.C. Such things oblige the creation of natural reserves. The creation of reserves that include canyons is planned. With the creation of the described reserve both types of habitat will be protected. Economical support of the E.C. is expected to create this reserve.

GLOBAL SCHEME OF THE NATURAL RESERVE PLANNING AND MANAGEMENT

(*) Realized measures.
(**) Initiated measures.

- **Preliminary information**
  - Species distribution in the zone (*)
  - Current demographic state (*)
  - Potential demographic state (**)
  - Threat factors (*)
  - Faunistic catalogue (**)
  - Floristic catalogue (**)
  - Soil classification
- **Habitat protection**
  - Vigilance
  - Specific Action and Management Plan
  - Fauna and Flora protection (*)
  - Vegetal cover and soil protection
- **Species protection**

  Measures for the preservation of existing population and its numerical increase:
  - Protection of the pond from the sun with a branch shelter (*)
  - Restoration or adaptation of old cisterns or similar constructions.
  - Creation of new ponds in suitable places.
  - Placing and quantification of nearest predator (and possible competitor) populations (**)
  - Control of nearest predator (and possible competitor) populations (**)
- **Complementary measures**
  - Population tracking and census (*)
  - Genetic study of the population (all living populations)

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Modern situation and perspective of conservation of the herpetofauna in the Western Transcaucasia.

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Abstract: The Caucasian Black Sea coast region are characterized by four assemblies of amphibians and reptiles: an European group entering the area from the west, north of the Black Sea, during the end of Tertiary, an East Mediterranean group entering the area from the west, south of the Black Sea, a Caucasian group and a Colchis group. We strongly recommend the formation of four Reserves for the protection of the diverse herpetofauna which includes several species and subspecies submitted to actual or potential threats. These areas are 1. The upper Mzymta river valley, from the Aishkha mountain range up to lake Kardyvach for the protection of a unique polymorphic population of *Vipera dinniki*. This region will be seriously affected by a construction of roads, hotels, ski pists etc. for planned Olympic Winter Games. 2. The Novorossiysk Reserve which includes habitat for taxa like *Bufo verrucosissimus circassicus*, *Triturus cristatus karelini*, *Testudo graeca nikolskii*, *Lacerta saxicola sherbaki*, *Lacerta media*, *Elaphe quatuorlineata sauromates*, *Vipera renardi*, and *Vipera kaznakovi*. 3. The Gagrinskiy Reserve for protection of species like *Lacerta saxicola darevskii*, *Bufo verrucosissima*, *Hyla arborea schelkownikovi*, *Lacerta derjugini*, *Vipera kaznakovi*, *Triturus vulgaris lantzi*, *Triturus vittatus*, *Natrix megalocephal*, *Pelodytes caucasicus*, *Lacerta media* and *Emys orbicularis*. 4. The Lasistan-Shavsketian Reserve on the Turkish-Georgian border, with species like *Mertensiella caucasica*, *Hyla arborea schelkowníkovi*, *Lacerta rudis*, *Natrix megalocephala*, *Natrix natrix (scutata)*, *Vipera kaznakovi*, *Lacerta mixta*, *Lacerta parvula*, *Lacerta clarkorum*.

Key words: Conservation, herpetofauna, Caucasus, Transcaucasus

INTRODUCTION

The Caucasian Black Sea coast area has a Geological and Biological History that differ from most other regions of the former USSR. The warm and humid flora and fauna during Miocene remained as a refugium almost without changes in the central and southern-east parts of Colchis, which are the lowland areas and Black Sea shores west of the Caucasian mountains where Georgia and adjacent parts of Russia and Turkey are today. During the Holocene the north-western part of the Caucasian sea shore region changed into a condition more similar to past Mediterranean conditions. Further the modern biological struture of the high-mountain belts of the western Transcaucasia was formed during pleistocene.

The Caucasian Black Sea coast region are characterized by four assemblies of amphibians and reptiles with special ecological and geographical affinity, and dispersal history (TUNIYEV, 1990). The herpetofauna can be refered to as an European group entering the area from the west north of the Black Sea during the end of Tertiary, an East Mediterranean group entering the area from the west south of the Black Sea, a Caucasian group and a Colchis group.

Seven of the species in these groups have been included in the Red Data Book of former Soviet Union. Within each group the amphibians and reptiles can be classified into common and rare species.

The red data book species have had some official protection. However, in some cases the non-protected species have a much more restricted distribution than those listed in the Red Data Book. These the more 'common ones' have in several cases only local distribution within protected areas and they may therefore disappear inconspicuously in the region as a whole.

There are two endangered reptiles amongst the European species as here discussed, and these are *Emys orbicularis* and *Vipera renardi*. All other species are quite common, and the following species can be included in this group: *Rana ridibunda*, *Bufo viridis*, *Anguis fragilis*, *Natrix natrix*, *Elaphe longissima* and *Coronella austriaca*.

Among the East Mediterranean species some, like *Lacerta praticola pontica*, *Natrix tesselata*, and *Ophisaurus (Pseudapis) apodus* have a comparatively good situation. All the other species from the east Mediterranean complex are in a more problematic situation or close to become extinct. In this group we can find *Triturus cristatus karelinii*, *Testudo graeca* *nikolskii*, *Lacerta media*, *L. strigata*, *Elaphe quatuorlineata sauromates*, *Coluber najadum*, *Coluber caspius*.

It must be pointed out that no taxon within this east mediterranean fauna group have presently enough protection. Some of the species are locally protected in the Pitzunda-Mussera Reserve, situated at river Pzyb in Abchazia, Georgia, but this reserve is much to small to efficiently protect any of these populations.

The Caucasian complex is then much better protected, mainly because of the existence of some large Nature reserves, (such as the Caucasian, the Ritza, the Gumista and the Kintrish reserves,) and within which good populations of these Caucasian species can be found. Species of this group are *Hyla arborea shelkownikowi*, *Rana macrocnemis*, *Lacerta alpina*, *Lacerta rudis* and *Vipera dinniki*.

The species of the east Mediterranean, the Caucasian, and the European groups have comparatively large distributions and can, of course, be preserved outside this region of Western Transcaucasia, as here discussed, but the taxa belonging to the Colchis complex can naturally only be protected within the Colchis region, where they have their main distribution.

Species and subspecies more or less endemic to this region as a whole are *Triturus vittatus ophryticus*, *Triturus vulgaris lantzi*, *Mertensiella caucasica*, *Pelodytes caucasicus*, *Bufo verrucosissimus*, *Lacerta saxicola*, *Lacerta clarkorum*, *Lacerta mixta*, *Lacerta parvula*, *Lacerta derjugini*, *Lacerta agilis grusinica*, *Natrix megalocephala*, and *Vipera kaznakovi*.

The modern Colchis forest area is a region that can be characterized by a rather stable climate corresponding to a subtropical climate with evergreen subtropical forests in the lower mountain belt. Typical with a high diversity of tree and plant species with no dominance of any single species, but today only smaller fractions of this original forest remains as undisturbed.

The herpetofauna of the higher elevations of west Caucasus has presently a good protection in the Western Caucasus Biosphere Reserve, an area of 232.000 hectars and covering the upper parts on both sides of the main range. This Reserve area was created in 1882, and it covered originally 0.5 million hectar. To give you some idea of the uniqueness of this Reserve it inhabits 26 species of Red Data Book plants. Further in Caucasus as a whole where this Reserve is a key area there are 1.600 higher plants, 1.600 lower plants, 20% endemics, 17% relicts. 820 species of the plants are found at higher altitudes and 278 (34%) of these are endemic. 32 of the plants are in the Red Data Book.

There are 19 families of mammals, with 75 species and 81 subspecies, and of which 34 are endemic (mainly rodents). There are 18 species of fish and 10 species of amphibians, and of which 67 % are endemic. Further 18 species of reptiles, with 31 % endemic, and 7 species of reptiles and amphibians in the Red Data Book. Further there are at least 10.000 insect species, 100 molluscs, 222 species of birds of which 44 are endemic. Amphibians and reptiles characteristic for this reserve are *Rana macrocnemis*, *Lacerta alpina* and *Vipera dinniki*.

However, a great problem is that this Reserve has been reduced in size all the time, and now there are planes to construct stations for skiing, by a Hong Kong firm!. At the first event 2.000 hectars of the upper Mzymta river valley, near lake Kardyvach, will be included in this project.

The Upper Mzymta river valley, from the Kardyvach lake region down to Aishka mountain range is a unique valley in the western border of the Reserve. This part is a holocene refugie with *Abies nordmaniana*. But this particular region is presently not protected and there are planes to apply for the Olympic Winter Games to this region and to reconstruct this particular area, together with parts of the Nature reserve, to a sporting center.

Now, one can also put an historical-political perspective on the whole situation. During the old Soviet Union system certain regions were declared as Nature Reserves. The former much more closed society also made poaching and collecting of wildlife a more difficult task. Today, with the old system breaking down and before a functional new system is operating in the former USSR, one consequence is that protection of some of the Nature Reserves has changed. The situation for wildlife, and herpetofauna, has become much severe in several regions. Economical forces in a free democratic system can be very powerfull and nature may suffer from commercial interests. Therefore we believe that speciall concern must be considered for the protection of the herpetofauna in this region as a whole. A considerable work must of course be done on the local basis by biologists and conservationists. Opinions of the international public and of international societies can be important as tools for conservation. We think one possible way is to put international preassure on local and regional authorities. International opinions might have a positive effect on conservation of nature, and the SEH will actively, through its Conservation Committee, participate in this work. This is what we have been trying to realize earlier by focusing on the Olympic Game buisness at Sochi. International WWF is presently actively following up this problem.

To our knowledge it must be strongly recommended to create four areas in west Caucasus and Georgia as future Nature reserves due to their unique animal communities, and which is clearly expressed in herpetofauna diversity. Within these four areas representatives of the east Mediterranean-, the Colchis- and the Caucasian reptile and amphibian complexes are found in comparatively good numbers. These four regions, essential for protection of the different faunal assemblies are (Figure 1):

Figure 1: The location of the four suggested Nature Reserves in the Western Transcaucasus (stippled, at arrows). The suggested Mzymta River Reserve is surrounded by the large Caucasian State Biosphere Reserve (dark stippled), which as well is submitted to future changes of borders due to planned Olympic Winter Games. The proposed Shavsheto-lazistanskiy Reserve on the Georgian border towards Turkey would be a continuation of the planned Turkish Reserve around the town Hopa on the Turkish side of the border.

First the upper Mzymta river valley, from the Aishkha mountain range up to lake Kardyvach, and to incorporate it with the Caucasian Reserve for protection of the unique polymorphic population of *Vipera dinniki* found in this particular valley (NILSON *et al.*, 1994). This is one region, together with parts of the large Caucasian Nature Reserve, that will be seriously affected by a constuction of roads, hotels, ski pists etc. for the planned Olympic Winter Games.

Second, we strongly recommend formation of the Novorossiysk Reserve (which goes from cape Utrishi to mountain Papay and village Dzhubga), and which includes habitat for taxa like *Bufo verrucosissimus circassicus*, *Triturus cristatus karelini*, *Testudo graeca nikolskii*, *Lacerta saxicola sherbaki*, *Lacerta media*, *Elaphe quatuorlineata sauromates*, *Vipera renardi*, and *Vipera kaznakovi*.

Third, we strongly recommend formation of the Gagrinskiy Reserve, from the Russian-Georgian border area of river Psou and village Salme to river Bzyb in Abshasia. It would be separated from the already existing small coastral reserve Pitzunda-Mussera by only a 15 km wide cultivated zone. This region includes narrow gorges of the SW slope of the Gagrinskiy range with part of the Colchis flora in gorges and decidious forests of the Mediterranean type on the slopes, from sea-level to about 900 m altitude. This is for protection of species like *Elaphe longissima*, *Natrix tesselata*, *Anguis fragilis*, *Lacerta saxicola darevskii*, *Bufo verrucosissima*, *Hyla arborea*, *Lacerta derjugini*, *Vipera kaznakovi*, *Triturus vulgaris lantzi*, *Triturus vittatus*, *Natrix megalocephala*. Further also *Pelodytes caucasicus*, *Lacerta media* and *Emys orbicularis*.

Finally, we strongly recommend the formation of the Lasistan-Shavsketian Reserve in Georgia. This suggested Reserve is on the Turkish-Georgian border, and the same habitat and faunal structure continues along the Pontic range into northeastern Turkish Anatolia, as is well known from the Hopa town area. In this last case we are presently working, through the SEH Conservation Committee and the Council of Europe, together with Turkish Environmental Authorities to define the important key area in the Turkish part of the Lazistan region, mainly in the surroundings of the town Hopa. This is the well known, and up to now the single known locality outside the former USSR for the attractive viper, *Vipera kaznakovi*. For the protection of this taxon, as well as the rest of the fauna, a Turkish reserve will be created around Hopa in cooperation with SEH, CooE and Turkish authorities. What is important is if the Georgian part could as well be protected a comparatively large and faunistically most important region could be created in this border area. Such a Reserve would protect species like *Mertensiella caucasica*, *Hyla arborea*, *Lacerta rudis*, *Anguis fragilis*, *Natrix megalocephala*, *Natrix natrix (scutata)*, *Vipera kaznakovi*, beside species like *Elaphe longissima*, *Lacerta mixta*, *Lacerta parvula adjarica*, *Lacerta clarkorum*.

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The restoration of habitats for Amphibians by the Roer and Overmaas Waterboard with special attention for the restoration of the most important breeding biotope of the tree frog (*Hyla arborea* L.) in the Dutch province of Limburg.

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Abstract: The Roer and Overmaas Waterboard is charged with the care of surface waters from a quantitative point of view in the southernmost part of the Netherlands. Measures are taken to ensure that excess water is drained adequately. In addition, priority is given to (re)developing the ecological function of streams and rivers. The Waterboard fulfils its tasks by constructing, managing and maintaining hydraulic engineering structures. In the context of the (re)development of the beneficial aspects of (semi)aquatic sites, measures are taken which include the creation of pool/bog sites, the construction of pools, the elimination of culverts in natural waterways and the restoration of springs and seepage zones. This is of immediate benefit to groups of animals such as the amphibians. A recently executed project concerns the restoration of the most important breeding water of the tree frog (*Hyla arborea* L.) in the province of Limburg. This water located in the state nature reserve "De Doort" was in direct contact with the water table. As a result of a drop in the groundwater level from the Seventies onwards, problems arose in maintaining the water level essential to amphibian breeding. By the end of the Eighties the drying out effects were so severe that the breeding success of the tree frogs came under immense pressure. The survival of the population became endangered. A decision was taken to make the breeding water independent of the groundwater. This was done by lining the base of the pond with HDPE sheets. The pool now holds water permanently. The development of the tree frog population will be carefully monitored.

Key words: waterboard, restoration of habitats, tree frog

THE ROER AND OVERMAAS WATERBOARD: A SHORT INTRODUCTION

The Roer and Overmaas Waterboard serves the southern part of the Province of Limburg, the southernmost province in the Netherlands (see Figure 1). It is responsible for an area covering about 95,000 hectares. The region is characterised by catchment areas of four main streams: the Roer, the Vlootbeek, the Geleenbeek and the Geul. The Waterboard manages and maintains more than 700 kilometres of waterways. This includes not only slow flowing lowland streams but also fast flowing hillside streams and a few small rivers. All of the streams have been assigned both an ecological function and a so called human oriented function.

The Waterboard is charged with the care of surface waters from a quantitative point of view, taking account of morphological as well as hydrological aspects. Among other things, this means that it must ensure that excess water in its area is adequately drained via rivers and streams. It also means that, where necessary, it encourages the (re)development of the ecological function of water systems. The Waterboard builds, equips, manages and maintains hydraulic engineering structures in pursuit of its aims.

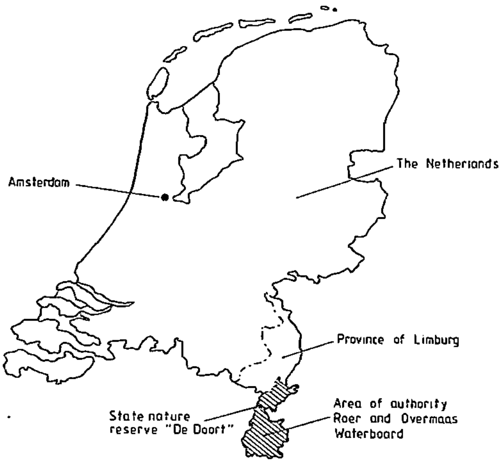

Figure 1: Location of the area managed by the Roer and Overmaas Waterboard in the Netherlands. The state nature reserve "De Doort" is located in this area.

In the context of the ecological development of stream systems the Roer and Overmaas Waterboard supports the restoration of biotopes for amphibians in stream valleys where such biotopes originally existed. This article deals in greater detail with the way in which the Roer and Overmaas Waterboard is attempting to do this. Special attention is devoted to the restoration of the most important breeding biotope of the tree frog in the province of Limburg.

THE RESTORATION OF HABITATS FOR AMPHIBIANS

In the context of restoring and maintaining waterways, special emphasis is placed on creating conditions that encourage the conservation and (spontaneous) development of the beneficial aspects of natural (semi-aquatic) sites. For example, when "standardised" streams are restored to a less artificial state, with a greater or lesser degree of natural meander, consideration is given not only to the stream itself but also to the associated habitats. The creation of pool/bog sites, the construction of pools and even the (re)generation of marshes are often integral aspects of the reconstruction of a stream. In this context, a marsh was created in the valley of the Geleenbeek when the topsoil was removed from a previously raised meadow until the original soil layer was exposed (river clay with local residues of peat), and the drainage system was removed. Within one year the resultant pools and marsh were populated by the common frog (*Rana temporaria* Linnaeus 1758), the green frog (*Rana esculenta* synklepton) and the common toad (*Bufo bufo* Linnaeus 1758). The smooth newt (*Triturus vulgaris* Linnaeus 1758) and the Alpine newt (*Triturus alpestris* Laurenti 1768) are expected to follow quickly. Another field of action is the elimination of culverts. Especially in the southern part of Limburg, many kilometres of natural waterways run through pipes. These pipes are now being removed in order to restore the ecological relevance of these enclosed stretches of water. Species found near streams, such as the fire salamander (*Salamandra salamandra* Linnaeus 1758), profit from this in particular. Recently the protection and restoration of springs and seepage zones has received additional attention. Intact, ecologically valuable springs are protected and neglected springs are restored.

Some examples of restoration measures are the removal of rubble and rubbish from filled up springs, the repair of damage caused by the trampling of livestock, and the implementation of hydrological measures to prevent drying out. Amphibious species which are found in spring and seepage habitats, e.g. the fire salamander, common frog and Alpine newt, benefit from this.

RESTORATION OF THE BREEDING BIOTOPE OF THE TREE FROG

At this moment in time the tree frog is a seriously endangered species in the Netherlands (BERGMANS & ZUIDERWIJK, 1986). There are only 5 areas left with tree frog populations. Most of the populations are small and are continuing to decline (VERGOOSSEN, 1991). In the Dutch province of Limburg the tree frog is exclusively found in the "De Doort" state nature reserve in the municipality of Echt.

The "De Doort" reserve covers some 50 hectares, of which 26 are deciduous forest and 15 are water and marshland. The remaining area is made up of undergrowth and wet grasslands. The upkeep of "De Doort" is the responsibility of the Forestry Commission. The area is part of the old Maas landscape. The presence of old clay soils resulted in the growth of the tile and brick industry in this area between 1920 and 1960. Excavation of the clay resulted in the formation of so called "tile pits". A number of these pits developed into water biotopes suitable for tree frogs. The tree frogs indigenous to the region very quickly populated the new manmade habitat. Tree frogs have been sighted in "De Doort" since 1946 (VERGOOSSEN, 1991). In the Sixties the breeding biotope which is the subject of this article was dug. In the mid-Seventies this biotope actually became the last remaining breeding biotope in "De Doort" and also in the province of Limburg. A relatively large, stable population of tree frogs developed. However, during the Seventies there was a major decline in the population. Destruction of land biotopes (bramble thickets), together with land accretion and a drop in the groundwater level were responsible. In 1977 no more than 12 individuals were counted in a day, whereas in 1966 the number had been 250 (VERGOOSSEN, 1991). Thanks to a range of conservation measures taken by the Forestry Commission from 1980 onwards, including the construction of various new meadow pools, the population has grown to a reasonable size once more. Moreover, there has been a spontaneous expansion of the population over a wider area (Vergoossen, 1991).

Despite the favourable recovery of the tree frog population in "De Doort" described here, there are still no guarantees that the population will survive. The breeding biotope is dependent on groundwater, and threatens to fall a victim to a continuous process of drying out. When the drying out problem became apparent in the Seventies, artificial measures were taken in an attempt to solve the problem of the lowering of the groundwater level as a consequence of groundwater extraction, etc. Initially it was possible to supply water from a nearby stream (Middelsgraaf) to maintain a reasonable water level in the breeding biotope during the larval development period of the tree frog. However, from 1989 a succession of dry years caused the regional groundwater level to drop dramatically in spring and early summer. Topping up from the stream became impossible, because the water level there had dropped so far that lowering it any further would have caused ecological damage both in and alongside the stream. The survival of the tree frog population became again endangered. In 1992 it was agreed with the Forestry Commission that the breeding biotope would be made independent of groundwater by sealing its base. A number of local and higher level public authorities provided surety for the financial consequences.

In mid 1992 an engineering company was commissioned to carry out a survey of the soil and hydrology of the breeding biotope site and to determine the best method of sealing off the pond. The results of the survey showed that the base of the breeding biotope was composed of very permeable sand, covered by a variably thick layer (from a few centimetres to around 0.5 metres) of moderately to very permeable material made up of sand and clay. During the survey (September 1992) the groundwater level was at a depth of 0.5 to 1.30 m below ground level. The vertical resistance of the covering layer was so minimal that a great deal of water seeped away underground.

Four suitable methods of sealing the site were considered:

-sealing with clay.
-sealing with bentonite matting.
-sealing by injecting bentonite (in situ).
-sealing with HDPE sheets.

The main criteria for the definitive decision were the needs of the tree frog biotope, the durability of the seal and the financial consequences of the project. The final choice was for HDPE sheets of 1 mm thick polyethylene.

The project commenced in early December 1992. The most important criteria for the various measures that had to be carried out were as follows:

-To start at a time favourable to the tree frog (and other amphibian species present), i.e. late in the year.
-Lining with HDPE sheets. These sheets are resistant to the root growth of marsh and water plants and the digging activities of muskrats (*Ondatra zibethicus* Linnaeus).
-The area to be sealed was approx. 5000 m^2;
-The sheets had to be welded in dry conditions;
-The sheets had to be covered by a minimum of 0.5 m soil. The water and soil must be able to resist a possible rise in the groundwater level in winter and early spring.
-The northern side of the pond, on which the sun shines, was given a slight slope (1:15, 20 m wide, 80 m long). This was to provide optimal spawning conditions for the tree frog.
-The depth of the water decreases slowly from south to north from roughly 0.8 to 0 m.
-An adjustable link was constructed between the pond and the stream (Middelsgraaf) mentioned earlier. This link will allow water level fluctuations caused by precipitation and evaporation to be controlled.

In mid-December 1992, heavy rainfall and high groundwater levels made it necessary to stop work. Work was resumed and completed in September 1993.

The purpose of creating a breeding biotope with a permanent volume of water is to try to maintain a core habitat for the tree frog. In the long term, however, a habitat of this nature provides no guarantee for the survival of the species (BORGULA 1993). For this reason efforts are being made to recolonise areas previously populated by tree frogs. This depends not only on the availability of suitable water and land biotopes; the presence of suitable migration paths is also an essential factor. The construction of pools and the (spontaneous) development of undergrowth, mainly in stream valleys, are being used to try to link the core habitat with suitable tree frog habitats in the area (WERKGROEP DE DOORT, 1993). The first spontaneous migrations have already occurred. It is hoped and expected that all of these efforts will result in the current status of the species in Limburg, i.e. seriously endangered (VERGOOSSEN, 1992), being favourably altered by natural means. The Roer and Overmaas Waterboard will provide all the support it can to promote these ends. In the coming years the development of the tree frog population will be carefully monitored.

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Herpetofauna conservation in Liguria (NW Italy)

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Abstract: Liguria is a NW Italian region in which about 50% of Italian herpetofauna species is present. A database, containing more than 2500 records posterior to 1980, has been realised at the Museo Civico di Storia Naturale of Genova to obtain the actual status of all the Ligurian species of Amphibians and Reptiles. These data have been used both in education and in scientific researches. In addition, they provided the basical information to select the most interesting herpetological sites that will be proposed to become protected areas, according to the regional act on the "Conservation of Amphibians, Reptiles and Freshwater Crustaceans".

Key words: conservation, education, herpetofauna, Liguria, Italy.

INTRODUCTION

Liguria is a NW Italian administrative region with a surface of about 5400 km^2 (Fig. 1). This region is bordered by the Mediterranean Sea, the Apennines Mountains and the Maritime Alps; thus different bioclimatic areas are present: a mediterranean climate is found along the western coast, a mountain climate in the Ligurian Alps and a submediterranean climate in the remaining parts of the region (GENTILE, 1986).

Figure 1: Location of Liguria, NW Italy administrative region.

The herpetological interest of Liguria has already been evidenced (CAPOCACCIA, 1956): infact the coexistence of Italian endemic taxa (*Salamandrina terdigitata, Speleomantes ambrosii, Speleomantes italicus, Rana italica, Chalcides chalcides* and *Podarcis sicula*) and of typical west-mediterranean species (*Hyla meridionalis, Lacerta lepida, Chalcides striatus, Natrix maura* and *Elaphe scalaris*) that reach this region from Spain through Southern France increases the herpetological diversity of the region. For these reasons, Ligurian herpetofauna is particularly rich: 18 species of Amphibians are present (being 53% of Italian Amphibian species) and 21 species of Reptiles (being 55% of Italian Reptile species).

METHODS AND OBJECTIVES

To obtain data on the actual distribution and the status of Ligurian herpetofauna, the Museum of Natural History of Genova has been collecting, from June 1989, all available herpetological records posterior to 1980. These records were obtained from scientific publications, zoological collections and especially from original field researches by more than 100 collaborators.

These data have constituted the Ligurian Herpetological Data-Base (LHDB) containing 2535 records. The data-base has been used for different purposes (fig. 2):

a) **Education.** An illustrated Regional Atlas has been edited (DORIA & SALVIDIO, 1994). The aim of this Atlas was to spread local herpetological knowledge from high school students and teachers to scientists, attempting to increase curiosity and interest upon these poorly-known animals. For this reason the regional Atlas provides distribution maps and dichotomic keys (both textual and figured) to allow an easy specific determination and possibly to stimulate more field researches in the future.

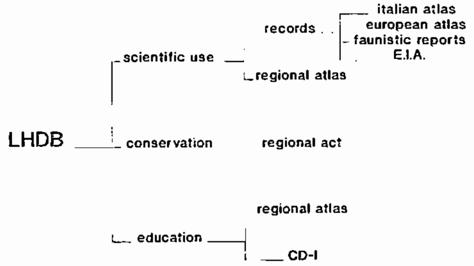

Figure 2: Different purposes of Ligurian Herpetological Data-Base (LHDB).

In addition, a compact disk-interactive (CD-I) about Ligurian herpetofauna has been developed. CD-I is a new multi-media technology that offers a combination of video, images, audio and text (Fig. 3). Interactivity means that exists a dialogue between the user and the program: the user infact can choose the type and the level of the contents. The most interesting aspect of CD-I is that all information is stored on only one compact disc, is easy and quick to obtain and is presented with high quality sound and images. Moreover, the CD-I player is connected with a standard television.

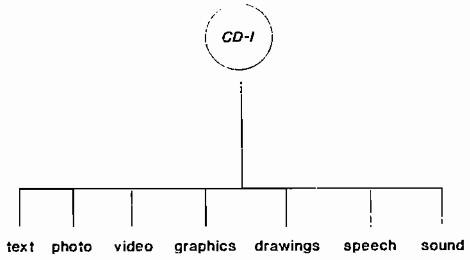

Figure 3: Compact disc-interactive (CD-I) is a multimedial system that offers a combination of text, photo, audio and video.

b) **Scientific.** All the data of the LHDB are available to the scientific community; they were send to the Coordinators of the European Herpetological Atlas, and they will contribute to the Italian Atlas. In addition, they have already been used in some faunistic studies and in few environment impact assessment (E.I.A) reports.

c) **Conservation.** The results obtained from the LHDB records are of great interest in local herpetofauna conservation. They allowed to individuate the areas with the highest number of Amphibian and Reptile species. Moreover, it was possible to estimate the status of each species plotting on a graph its distribution (expressed as the percentage of the territory in which the species is found) and its relative density (expressed as the mean number of observations per unity of territory) (Fig. 4).

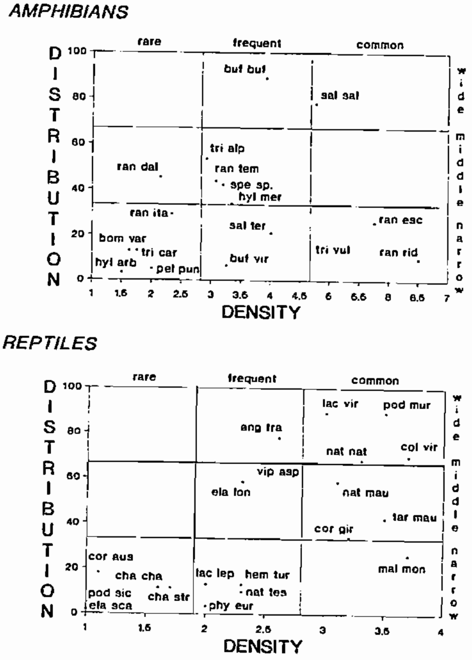

Figure 4: Status (distribution and relative density) of Amphibian and Reptile species of Liguria. The distribution is expressed as the percentage of territory in which the species have been founded, the density as the mean number of observations per unit of territory.

In this way the species that are actually declining and/or are most endangered in Liguria were evidenced: *Rana italica, Bombina variegata, Triturus carnifex, Hyla arborea* and *Pelodytes punctatus* for the Amphibians; *Coronella austraica, Chalcides chalcides, Chalcides striatus, Podarcis sicula* and *Elaphe* *scalaris* for the Reptiles. It is important to observe that *Lacerta lepida* and *Phyllodactylus europaeus* are overestimated and are to considered as endangered species. Moreover, the pond terrapin *Emys orbicularis*, which was present in Western Liguria until the 1970s, is now probably extinct.

These final results have a practical impact on the herpetological conservation policy in Liguria, as in 1992 a regional act on the "Conservation of Amphibians, Reptiles and Freshwater Crustaceans" have been promulgated. This act protects all the species of Amphibians and Reptiles and states the conservation of the most important herpetological sites in the region. It is worthwhile to note that this is the first case in Italy of a regional act stating not only the conservation of herptiles, but extending the protection to their habitats.

To select the most interesting herpetological sites a two-year scientific program has been funded by Ligurian regional authorities: in the first year about 150 herpetological sites will be visited and censused; in the second year the most interesting sites will be intensively studied and proposed to become fully protected areas. The criteria that will be taken into consideration in selecting such sites will be: the presence of endangered species, of a high herpetofauna diversity, of well preserved natural ecosystems, and the possibility of using these sites as scientific or educational stations.

Actually, this scientific program is in its first year, and it will be ended in 1995 when the selected sites will be proposed to become regional protected areas.

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The "Progetto Rospi" of Lombardy (Northern Italy)

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Abstract: Since 1990 the Centro Studi Erpetologici "Emys" (of the Societá Italiana di Scienze Naturali) and the Acquario Civico di Milano have started a project directed to the protection of toads and other Amphibians in Lombardy (Northern Italy). The two main objectives are: the safeguard of the remaining populations of *Bufo viridis* in the town of Milano and the rescue of *Bufo bufo* and other species from the traffic along the roads during the breeding period. In addition to these protective operations a census of the Amphibians'populations and of their breeding sites is being carried out. These efforts, which since 1992 are conducted in a more integrated way, are backed by the Regione Lombardia (Department of Ecology) and are made possible by the work of the Guardie Ecologiche Volontarie (voluntary wardens), whose activity is guided by the project coordinators. Tbe success obtained by this initiative and the interest it has raised led to the organization of the First Italian Meeting on the safeguard of Amphibians, held in November 1992 at the Museo Civico di Storia Naturale of Milano.

Key words: Conservation, Lombardy, Italy, Amphibians.

INTRODUCTION

Since some decades the naturalists of Lombardy have been denouncing, generally with little impact on public opinion, the increasingly critical situation of this region's Amphibians.

Starting from the seventies, an increasing attention toward the Amphibians' protection led to identifie some situations that needed urgent interventions.The major worry concerned then the Italian agile frog (*Rana latastei*), whose populations seemed to be isolate and strongly endangered by environmental deterioration (Pozzi, 1976, 1980). First actions were addressed to the protection of the breeding migrations of the Common Toads (*Bufo bufo spinosus*): the first tunnels for toads in Italy were set up on the riparian road of the Idro Lake (Brescia), while the volunteers of the "Gruppo Naturalistico della Brianza" started an intervention based on the use of fences on the riparian road of the Segrino Lake (Como).

Thanks to the perseverance of some naturalists, in 1977 the Regione Lombardia promulgated the first Italian regional law addressed to the protection of Amphibians (Legge regionale N.33, 27/7/1977).

During the following decade the main Amphibians' safeguard interventions concerned the Spadefoot Toad (Pelobates fuscus insubricus). The species was then little known and it was considered in extinction on the whole Po valley. In 1986 a number of specialists in the study of this subspecies formed a study group, while WWF Italia started a project named "Progetto Pelobate". The planned interventions were the following: search of new populations; rescues of eggs and tadpoles from upset places and manteinance in captivity in order to obtain successful reproduction; reintroduction of bred specimens into protected areas located within the historical range; safeguard of the environments in which Pelobates still lives.

In 1986 the Lombardy regional census of Amphibians and Reptiles officially started with the coordination of Centro Studi Erpetologici (C.S.E.) of SocietÓ Italiana di Scienze Naturali.

This census has enriched the knowledge of the faunistic situation of the region with the discovery of populations of Triturus alpestris apuanus (Zuffi, 1983), Salamandrina terdigitata and Rana italica (Zuffi & Barbieri, 1988). Moreover more details have been added to our knowledge of the distribution of some rare species and subspecies such as *Salamandra atra atra*, *Triturus alpestris apuanus*, *Speleomantes ambrosii*, *Bombina variegata variegata* and *Rana latastei* (Agapito, 1987; Ferri 1992).

The last decade has been the richest in initiatives in favor of the Amphibians of Lombardy. In 1990 the C.S.E. and the Acquario Civico have started a census of the places considered at risk for the migrations of toads and other Amphibians and have begun operations of safeguard of the reproduction of Bufo viridis viridis in the town of Milan. Thanks to the spontaneous help given by the Guardie Eologiche Volontarie (G.E.V., voluntary wardens directed by the Regione Lombardia), the Department of Ecology of the Regione Lombardia accepted to sponsor a regional project for the protection of Amphibians, named **Progetto Rospi Lombardia**.

The scientific guidance remained to C.S.E.

and the Acquario Civico, while the interventions are guaranteed by the G.E.V. and by volunteers of WWF Lombardia and Lega Anti Caccia (L.A.C.).

PROGETTO ROSPI LOMBARDIA

The birth of **Progetto Rospi** coincides with the publication of the book "Anfibi e Rettili in Lombardia" (Ferri, 1990) by the WWF Lombardia and the activation, at the Museum of Natural History of Milan, of a telephone number devoted to problems of Amphibians' populations living in Milan. The project is coordinated by C.S.E. and Acquario Civico di Milano and it is aimed to the following subjects:

Vulgarization, census, amphibians on roads, amphibians in town, rare amphibians

1. Vulgarization

During the first three years we tried to promote all the initiatives that could make Amphibians better known and stimulate their safeguard. To this end, the project coordinators and the Department of Ecology of the Regione Lombardia organized general and specific courses on herpetofauna and its conservation problems and prepared papers and other material addressed to schools.

Press releases and articles have been sent to the most widespread newspapers and magazines and the same subjects have been also covered by some TV networks.

The C.S.E. and the Acquario Civico adopted also a definite position by sending detailed statements of facts, letters and suggestions and operating advices to the Municipalities in order to correct environmental changes that could directly or indirectly damage aquatic or terrestrial lesser fauna.

2. Census

The regional census of the herpetofauna has been continued: the data collection has been improved and a data base has been prepared.

Prearranged forms for all the necessary observation have been distributed to operators and we tried to stimulate detailed census (at province and district level). To this aim, the Dep. of Ecology appropriated a sum for small provincial nature museums (such as the Mus. of Nat. Hist. of Morbegno for the province of Sondrio, the Naturalistic Mus. of Induno Olona for the province of Varese and the Mus. of Nat. Hist. of Brescia). This sum is needed to organize and coordinate the census of small Vertebrates which are carried out by the G.E.V. By the end of 1992 the results of a decade of these census were delivered to the Dep. of Ecology (FERRI *ET AL.*, 1992) together with suggestions for the management and the safeguard of the populations.

3. Amphibians on roads

In the pre-alpine part of Lombardy, near the insubric lakes, the populations of Common Toad (*Bufo bufo*) and other Amphibians are cut down in spring by the road traffic while migrating from the winter sites to the breeding sites. Many populations of Common Toad of Lombardy have already completely disappeared in this way.

For each interested area, the points of major transit and with the highest number of dead animals were found. According to the kind of road and to the environmental conditions of the area around the road, for each site it was chosen the best way to perform the rescue. For example, in those places where it was impossible to set up fences on the road side due to the slope steepness (or to the presence of dense vegetation), the volunteers should walk along the road and pick up the animals one by one.

In order to save the most endangered populations left, the G.E.V. have been briefed about the technics of rescue of Amphibians on roads (set up of fences, needed materials and so on) and also about the problems that the rescue on roads can trigger on the reproductive biology and behavior of these animals. A correct transport methodology was defined n order to avoid a negative impact by recuers' actions on migrations development; for instance it was particularly stressed the relevance of a well coordinated team activity: each rescuer should operate only along his own road section to strictly monitor each toad in or near it, to clearly assess the direction of its movement. By this way, it was avoided the risk to take a toad on the wrong side of the road.

The involvement of the G.E.V. for the rescue of Common Toads and other Amphibians on roads was decisive. Indeed, for activities like this it's needed a big number of persons and a coordination which is easier to find in an official volunteers' corps.

4. Amphibians in town

The concern of naturalists and protectionists in the animal communities living in big cities has conducted to the discovery that five species of Amphibians live in uncultivated or suburban areas of the city of Milan.

The future of these animals depends only on the decisions of the city rulers and on people sensibility; for this reason, while on one hand we are trying to give these "survivors" a right habitat, on the other hand we are trying to make these animals better known to the people, particularly to the youngsters.

An example of this kind of management is the activity conducted since 1988 till today in favor of the populations of Green Toad (*Bufo viridis viridis*) in Milan. The breeding sites are generally located either in public gardens and parks or in cultivated and uncultivated suburban areas, where the toads spawn in rainwater ponds or in fountains. Yet the ponds often dry up before the metamorphosis and the fountains are often damaged by people.

Since 1990 until now the **Progetto Rospi** in Milan has taken a census of the areas frequented by toads and of their breeding sites in order to know the actual distribution and the state of Bufo viridis in Milan. Moreover we made efforts to assure the reproduction of these animals also by moving eggs and tadpoles from endangered places to safer ones. In some public parks artificial ponds have been purposely built.

5. Rare amphibians

The **Progetto Rospi** works with the same care of the previously described activities also for the study of the Lombard rarest Amphibians species.

At a WWF request, the **Progetto Rospi** has begun a monitoring and management of two populations of *Rana latastei* living in the Monticchie Nature Reserve (at Somaglia, province of Milano) and Le Bine Nature Reserve (at Calvatone, province of Cremona). In both reserves the preexisting wet areas, which had almost completely dried, have been restored (AGAPITO LUDOVICI & SCHIAVO, 1992).

Anyway it was verified that *Rana latastei* is now distributed along all the major rivers of the Po valley, even if not in a continuous way.

On the contrary, the situation of Pelobates fuscus insubricus in Lombardy is more and more critical in spite of the big efforts carried out during the last decade. Attempts to rear tadpoles taken from threatened sites did not obtain the hoped results. Moreover, field researches are confirming the hypothesis that this animal may disappear from Lombardy in the near future. The presence of this subspecies in documented in our region only in four sites, which are unfortunately situated in four different provinces. Furthermore, the environmental conditions of all these sites are critical and it is difficult to safeguard them. So long the numerous denunciations and requests to protect such important areas have given no results.

CONCLUSIONS

After three years of actions in favor of the Amphibians of Lombardy, we can say we are satisfied of how the **Progetto Rospi** has worked.

We consider very positively the cooperation achieved among public structures (Department of Ecology of the Regione Lombardia), scientific structures (C.S.E., Acquario Civico di Milano and the nature museums that joined the project) and protectionists (WWF Lombardia, L.A.C., Lega per l'Ambiente). The number of volunteers working at the project increased from about ten for the first year to more then two hundred in the last spring. With such a number of volunteers we were able to improve the local organization of the spring rescues.

The good results obtained and the interest caused by these activities drove us to organize the first national meeting on the safeguard of Amphibians in Italy. It was kept the 19-20 of November 1992 at the Museo di Storia Naturale of Milan. More people than expected participated to the meeting, coming from all over Italy and from Switzerland.

The next years will surely be very important for the conservation of natural environments and the small fauna. We must exploit this moment in which both common people and public structures are interested in such kind of problems. The fate of Amphibians will depend from specific projects of safeguard. Thanks to the zeal of many volunteers, the **Progetto Rospi Lombardia** is trying to invert the decremental trend of the Lombardy populations of Amphibians.

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Table. 1.- Volunteers involved in the activity of census and rescue.

1 Guardie ecologiche volontarie
COMUNITA' MONTANA ALTO LARIO OCCIDENTALE
Palazzo Gallio. Gravedona (COMO). cens./rescue.
2 Guardie ecologiche volontarie
COMUNITA' MONTANA ALPI LEPONTINE
Via Garibaldi, 62. PORLEZZA (COMO). cens./rescue.
3 Guardie ecologiche volontarie
COMUNITA' MONTANA LARIO INTELVESE
Via Roma, 1. S.FEDELE INTELVI (COMO).cens./rescue.
4 Guardie ecologiche volontarie
C.M. VALTELLINA DI SONDRIO
Via N.Sauro, 33. SONDRIO cens.
5 Guardie ecologiche volontarie
C.M. VALTELLINA DI TIRANO
Via Pedrotti, 24. TIRANO (SONDRIO). cens./rescue.
6 Guardie ecologiche volontarie
C.M. VALCHIAVENNA
Piazza Castello, 10. CHIAVENNA (SONDRIO). cens./rescue.
7 Guardie ecologiche volontarie
C.M. VALTELLINA DI MORBEGNO
Piazza M.E.Bossi, 2. MORBEGNO (SONDRIO).cens.
8 Guardie ecologiche volontarie
C.M. VALLE SAN MARTINO
Via E.De Amicis, 2. CALOLZIOCORTE (BERGAMO). cens./rescue.
9 Guardie ecologiche volontarie
C.M. VALLE SERIANA
Via Libertà, 21. ALBINO (BERGAMO).cens./rescue.
10 Guardie ecologiche volontarie
C.M. VALLE CAVALLINA
Via Don L.Zinetti. CASAZZA (BERGAMO) cens./rescue.
11 Guardie ecologiche volontarie
C.M. VALLE BREMBANA
Via Roma, 50. PIAZZA BREMBANA (BERGAMO) rescue.
12 Guardie ecologiche volontarie
C.M. ALTO SEBINO
Via Marconi, 23. LOVERE (BERGAMO).rescue.
13 Guardie ecologiche volontarie
C.M. VALCERESIO
Via Matteotti, 18. ARCISATE (VARESE).cens./rescue.
14 Guardie ecologiche volontarie
C.M. VALCUVIA
Piazza Marconi, 1. CUVEGLIO (VARESE).cens./rescue.
15 Guardie ecologiche volontarie
C.M. VALGANNA E VALMARCHIROLO
Via Municipio, 2. CADEGIANO VICONAGO (VARESE). cens.
16 Guardie ecologiche volontarie
C.M. VALLI DEL LUINESE
Via Collodi, 4. LUINO (VARESE) cens./rescue.
17 Guardie ecologiche volontarie
C.M. VALLE SABBIA
Via Reverberi, 2. NOZZA (BRESCIA).cens./rescue.
18 Guardie ecologiche volontarie
C.M. VALLE CAMONICA
Via A.Moro. BRENO (BRESCIA).cens.
19 Guardie ecologiche volontarie
C.M. SEBINO BRESCIANO
Via Roma, 41. SALE MARASINO (BRESCIA). cens./rescue.
20 Guardie ecologiche volontarie
C.M. VALLE TROMPIA
Via Matteotti, 327. GARDONE (BRESCIA). cens.
21 Guardie ecologiche volontarie
AMMINISTR. PROVINCIALE DI MILANO
Corso di Porta Vittoria, 27. MILANO.cens./rescue.
22 Guardie ecologiche volontarie
AMMINISTR. PROVINCIALE DI COMO
Via Borgovico, 148. COMO.cens.
23 Guardie ecologiche volontarie
AMMINISTR. PROVINCIALE DI CREMONA
Corso V.Emanuele, 17. CREMONA. cens.
24 Guardie ecologiche volontarie
AMMINISTR. PROVINCIALE DI VARESE
Piazza Libertà, 1. VARESE. cens.
25 Guardie ecologiche volontarie
AMMINISTR. PROVINCIALE DI BRESCIA
Via Milano. BRESCIA. cens.
26 Guardie ecologiche volontarie
PARCO NORD MILANO

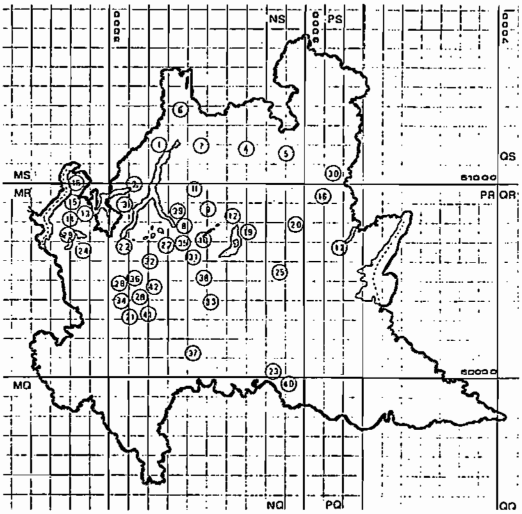

Figure 1: Localization of volunteers.

Via Manin, 2. MILANO. cens./rescue.
27 Guardie ecologiche volontarie
PARCO DI MONTEVECCHIA E DELLA VALLE DEL CURONE
c/o Munic. di Montevecchia. (COMO). cens./rescue.
28 Guardie ecologiche volontarie
PARCO DELLE GROANE
Via Piave, 5. CESATE (MILANO. cens./rescue.
29 Guardie ecologiche volontarie
PARCO DEL CAMPO DEI FIORI
Munic. di LUVINATE (VARESE).cens./rescue.
30 Guardie ecologiche volontarie
PARCO DELL'ADAMELLO BRESCIANO
Via Mazzini, 8. BRENO (BRESCIA)cens.
31 Guardie ecologiche volontarie
PARCO DEI COLLI DI BERGAMO
Via Pradello, 12. BERGAMO. cens./rescue.
32 Guardie ecologiche volontarie
PARCO DELLA VALLE DEL LAMBRO
Via Indipendenza. TRIUGGIO (MILANO). cens.
33 Guardie ecologiche volontarie
PARCO DEL SERIO
Piazza Rocca, 1. ROMANO DI LOMB. (BERGAMO).cens.
34 Volontari
DELEGAZ. WWF LOMBARDIA
Via Donatello 5/B. MILANOrescue.
35 Volontari
SEZIONE W.W.F. BERGAMO
Via Pignolo, 73. BERGAMO.rescue.
36 Volontari
SEZIONE W.W.F. GROANE
Palazzo Novelli. COGLIATE (MILANO). cens./rescue.
37 Volontari
SEZIONE W.W.F. BASSO LODIGIANO
Via Zoncada, 75. CODOGNO (MILANO).rescue.
38 Volontari
SEZIONE W.W.F. ADDA MILANESE
Piazza Matteotti. CASSANO D'ADDA (MILANO). rescue.
39 Volontari
SEZIONE W.W.F. LARIO ORIENTALE
Viale Dante, 21. LECCO. rescue.
40 Volontari
SEZIONE W.W.F. CREMONA
Via Gioconda, 3. CREMONA.rescue.
41 Volontari
SEZIONE W.W.F. SUD MILANESE
Via C. Battisti, 19. S.DONATO MIL.SE (MILANO). rescue.
42 Volontari
LEGA ABOLIZIONE CACCIA LOMBARDIA
MILANO.rescue.

First report about conservation status of herpetofauna in the Skadar Lake Region (Montenegro): Current situation and perspectives

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Abstract: Lake Skadar is a large, shallow, mainly oligotrophic lake in southwestern Balkan peninsula. Herpetofauna of this area include some rare species as *Typhlops vermicularis*, *Algyroides nigropunctatus* and *Elaphe quatrolineata* and many endemic ones for this part of Europe (*Rana shqiperica*, *Lacerta trilineata*, *Lacerta oxycephala*, *Podarcis melisellensis*, *Ophisaurus apodus*, *Coluber laurenti*). No complete list of amphibians and reptiles of Lake Skadar exists, but according to the previous results we can suppose that there is a high biological richness associated with the herpetofauna inhabiting coastal region as well as islands of the lake. Beside report on (non-) undertaken measures and lows of protection, we refer about reasons for proclaiming the region of Lake Skadar as one of European Biogenetic Reserves.

Key words: Skkadar Lake Region, herpetofauna, Conservation status, Center of Diversity, European Biogenetic Reserve.

INTRODUCTION

Skadar Lake Region, situated in southern part of Republic of Montenegro, (Figure 1.) presents interesting area from several points of view. By geographical position it belongs to the Mediterranean region, the wast intercontinental zone comprising the Mediterranean Sea. This zone separates the Euriasiatic from the Indoafrican continental block and is a meeting point of two different climatic and biogeographic regions. The geology of Lake Skadar is very closely connected with the geological history of the Balkan Peninsula. The Lake Skadar basin and its drainage area are situated at the feet of the last members of the Dinaric Alps which are parallel to the Adriatic coast and in the foothills of the Prokletije massif. It is the zone of so-called "Medovian bend", where Dinaric Alps change its direction from NW-SE to N-S. Also, Dinaric mountain system there collide with the Pindus-Shar mountain system.

Proposed study area spatially can be defined inside the coordinates: 42°-42°35'North latitude and 18°45'-20°05' East longitude. Border line follows mountain ranges (from SE to NE in the clockwise direction): Tarabosh, Rumija, Sutorman, Sozina, Lovcen, Stavor, Garac, Prekornica, Zijevo, Prokletije massif through Grbaja and Plav valleys to Bogicevica) and along the frontier line with Albania. Total surface area covers about 2600 km^2. The Lake Skadar lies in a tectonic basin of karst topography, on 6 m above sea level, while the highest point on mountainous country, Bogicevica, reachs 2501 m above sea level.

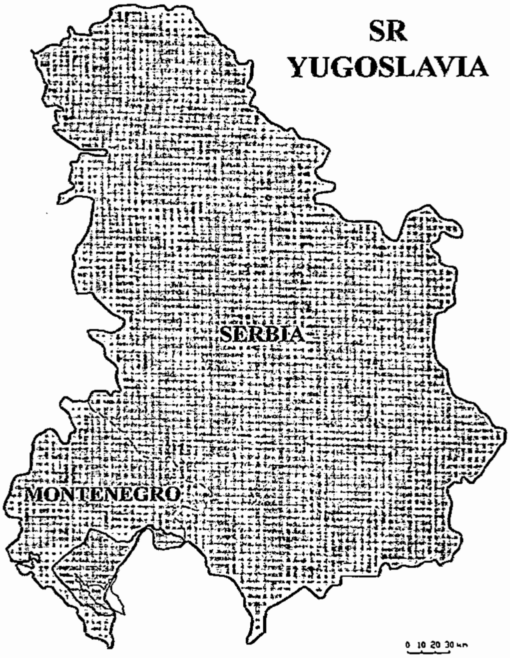


Figure 1: Location of proposed Skadar Lake Region (shaded area).

The natural, historic and present day ecological specificities of Region are reflected in the most striking way in the wealth of flora and the diversity of vegetation. On proposed territory biogeographist Matvejev and fitocenologist Puncer (MATVEJEV AND PUNCER, 1989) have separated 6 landscape types or

Biome	Sign	Ecotonic biomes	Characteristic vegetational units(by JOVANOVIC B. ET AL., 1986)	sign
Biome of evergreen Mediterraneanmaritime woodlands and maquis	(1)		*Orno-Quercetum ilicis*	*(3.)*
Biome of Submediterranean Adriatic mostly Oak woodlands	(2)		*Querco-Carpinetum orientaliss.lat.*	*(5.)*
		2_1	*Quercetum trojanae s.lat.*	*(10.)*
		2_3	*Ostryo-Quercetum pubescentis*	*(11.)*
			Quercetum frainetto adriaticum	*(15.)*
Biome of South European, mostly deciduous woodlands	(3)	3_2	*Fagetum montanum s.lat.*	*(32.)*
		3_{21}	*Seslerio-Fagetum s.lat.*	*(34.)*
		3_7	*Periploco-Quercetum roboris s.lat.*	*(25.)*
Biome of European, mostly coniferous boreal type woodlands	(4)		*Picaetum excelsae montanum s.lat*	*(45.)*
			Pinetum mugi s.lat.	*(51.)*
		4_3	*Abieti-Fagetum s.lat.*	*(38.)*
			Fagetum subalpinum s.lat	*(39.)*
		4_7	*Pinetum peucis s.lat.*	*(52.)*
			Pinetum heldreichii s.lat.	*(53.)*
Biome of Alpine and High Nordic rock-grounds, pastures and snow patches	(5)			
Biome of rocky grounds, pastures and woodlands on rocky grounds of (oro) Mediterranean mountains	(7)		*Oxytropidion dinariaceae*	*(64.)*

Table 1: Landscape types (biomes) which characteristic habitats are distributed in the Skadar Lake Region (by MATVEJEV AND PUNCER, 1989).

biomes, together with 7 ecotonic biomes (Table 1.). For the comparison, there are only 3 landscape types in Northern Europe and 4 in Central Europe. According to JOVANOVIC et al (1986), 15 different communities of natural potential vegetation are distributed within the Region, each of them being characteristic for certain basic or ecotonic biome.

The combinations of above mentioned main biomes were presented on a geographical map divided in sectors - UTM squares of 10 x 10 km in size (Figure 2.). Originally, 31 different combinations of main and ecotonic landscape types were noticed inside UTM squares whose correspond to surface of Skadar Lake Region. The whole area is characterized with presence of geographically and geomorphologically caused oromediterranean existence conditions on appropriate mountain peaks and in gorges. They differ essentially from so called alpine-high-nordic conditions by strong radiation and insolation throughout the year (MATVEJEV AND PUNCER, 1989). Many preglacial plant and animal species have survived there and formed communities abundant in relics and endemics.

HERPETOFAUNA

Complete list of amphibians and reptiles of this Region is still under the work. Of the 20 amphibian and 36 reptilian species which are known for the present territory of Yugoslavia, 14 amphibian and 28 reptilian species were noticed in the study area (Table 2.). Their founding sites within the Region are presented on the following maps (Figs. 3.-14.).

Among these were four stenoendemic taxa: *Triturus alpestris montenegrinus* (RADOVANOVIC, 1951), *Triturus alpestris piperianus, Rana shqiperica* (HOTZ et al, 1987), *Podarcis muralis albanica*; one south-dinaridian endemic taxon: *Salamandra atra (?) prenjensis*; three dinaridian endemic taxa: *Lacerta mosorensis, Lacerta oxycephala* and *Podarcis melisellensis fiumana*; one taxon endemic for western Balkan peninsula: *Vipera ammodytes illyrica*; and eight taxa endemic for Balkan peninsula: *Triturus vulgaris graecus, Bombina variegata scabra, Rana graeca, Algyroides nigropunctatus, Lacerta agilis bosnica, Coluber laurenti, Vipera berus bosniensis* and *Vipera ursinii macrops*.

The following threatened taxa were listed according to the categories of threat assigned by SEH: Category 2: *Vipera ursinii macrops*. Category 3:*Emys orbicularis, Algyroides nigropunctatus, Podarcis melisellensis* and *Coluber najadum*.

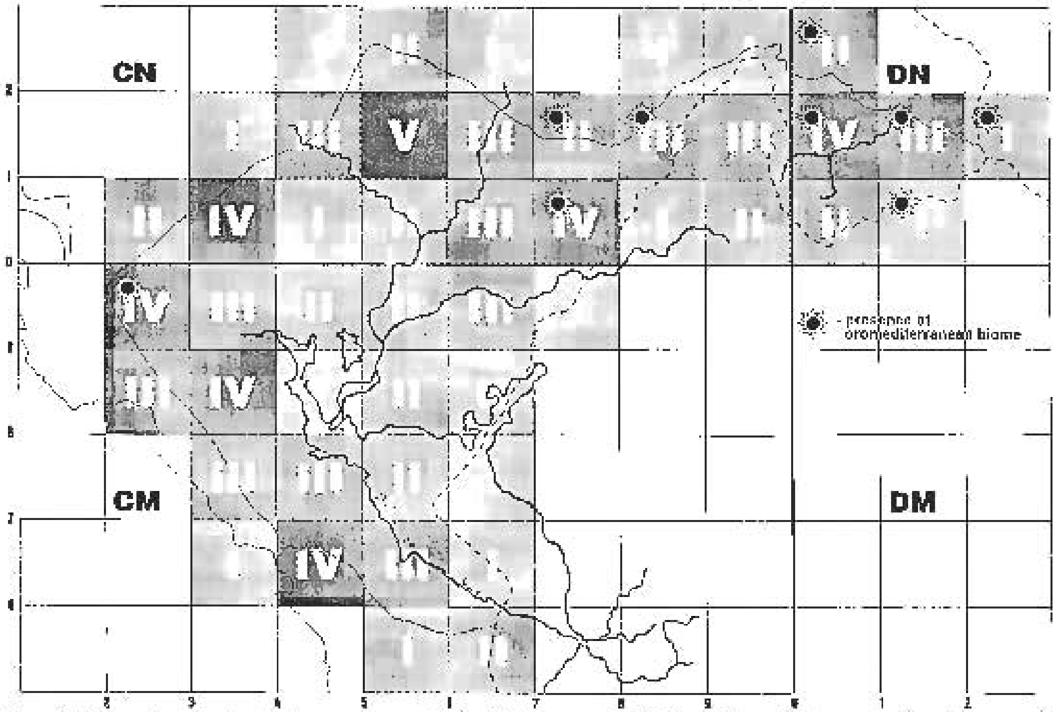


Figure 2: Diversity of main landscape types within UTM squares of 10 x 10 kms. I-elements of only one main biom presented; II-elements of two main biomes presented; III-elements of three main biomes presented; IV-elements of four main biomes presented; V-elements of five main biomes presented.

The following taxa were under the Berne protection: *Triturus carnifex*, *Bombina variegata*, *Bufo viridis*, *Hyla arborea*, *Rana dalmatina*, *Testudo hermanni*, *Emys orbicularis*, *Algyroides nigropunctatus*, *Lacerta viridis*, *Lacerta trilineata*, *Lacerta agilis*, *Podarcis muralis*, *Podarcis melisellensis*, *Ophisaurus apodus*, *Coluber najadum*, *Elaphe situla*, *Elaphe quatuorlineata*, *Elaphe longissima*, *Natrix tesselata*, *Coronella austriaca*, *Telescopus fallax*, *Vipera ammodytes* and *Vipera ursinii macrops*.

The species not found in the Skadar Lake Region but known for the adjacent areas, include: *Mauremys caspica* (under the Berne protection), *Tarentola mauritanica*, *Podarcis sicula* (under the Berne protection) and *Coluber caspius* (listed in the Category 3 of threat).

Also, coastal area of Lake Skadar is inhabited by rare serpent species *Typhlops vermicularis* (RADOVANOVIĆ, 1964; GRILLITSCH AND GRILLITSCH, 1986).

Until now, the highest number of recorded species per UTM square reached 9 for amphibians and 18 for reptilians (Figure 15.), with bearing in mind that many UTM squares were not at all or were very poorly observed. Anyway, even those preliminary data pointed out to important richness and diversity of herpetofauna distributed throughout this area. Those taxa belong to the different biogeographical entities, and for some of them Skadar Lake Region presents edge of the total distribution area or contact zone of different subspecies.

THREATS AND PROTECTION

Complex investigations of Lake Skadar whose were done during the period from 1972 to 1977 pointed out on importance of serious protection of whole area. In dubious study of biota and limnology of Lake Skadar, KARAMAN AND BEETON (1981) concluded that the lake is especially important for its fishery and as a nature preserve, as well as water supply for the villages in the vicinity of the lake and for the cities and villages along the coast of the Adriatic

			Skadar Lake Region						
Groups	n. species in Skadar Lake Reg.	n. species SRY	Steno endems.	South Dinar. endems.	Dinaridiam endems.	Westwern Balkans endems.	Balkan endems.	SEH** cat.: 1 2 3	Berne*** prot.
Tailed Amph.	5	(7)	2*	1	-	-	1	- - -	1
Tailess Amph.	9	(13)	1	-	-	-	2	- - -	4
Tortoises & terrapines	2	(4)	-	-	-	-	-	- - 1	2
Lizards	12	(17)	1	-	3	-	2	- - 2	8
Snakes	14	(15)	-	-	-	1	3	- 1 1	9

Table 2. Herpetofauna of the Skadar Lake Region. * Two subspecies of *T. alpestris*.** SEH categories of threatened species. *** Species under the Berne protection.

Sea for transportation, waste disposal and tourism. As a most preserved parts of the lake the authors mentioned Podhum Bay near Yugoslavian-Albanian border, the single nesting place for the most rare birds.There was not so much words about conservation status of Lake Skadar herpetofauna, but in the earlier study of DIESNER (1966) exist quite detailed survey of amphibian and reptilian fauna of Montenegro. Also, DZUKIC (1977), after his first investigations of the Lake Skadar, correctly perceived the great diversity and complexness of amphibian and reptilian fauna in the Region.

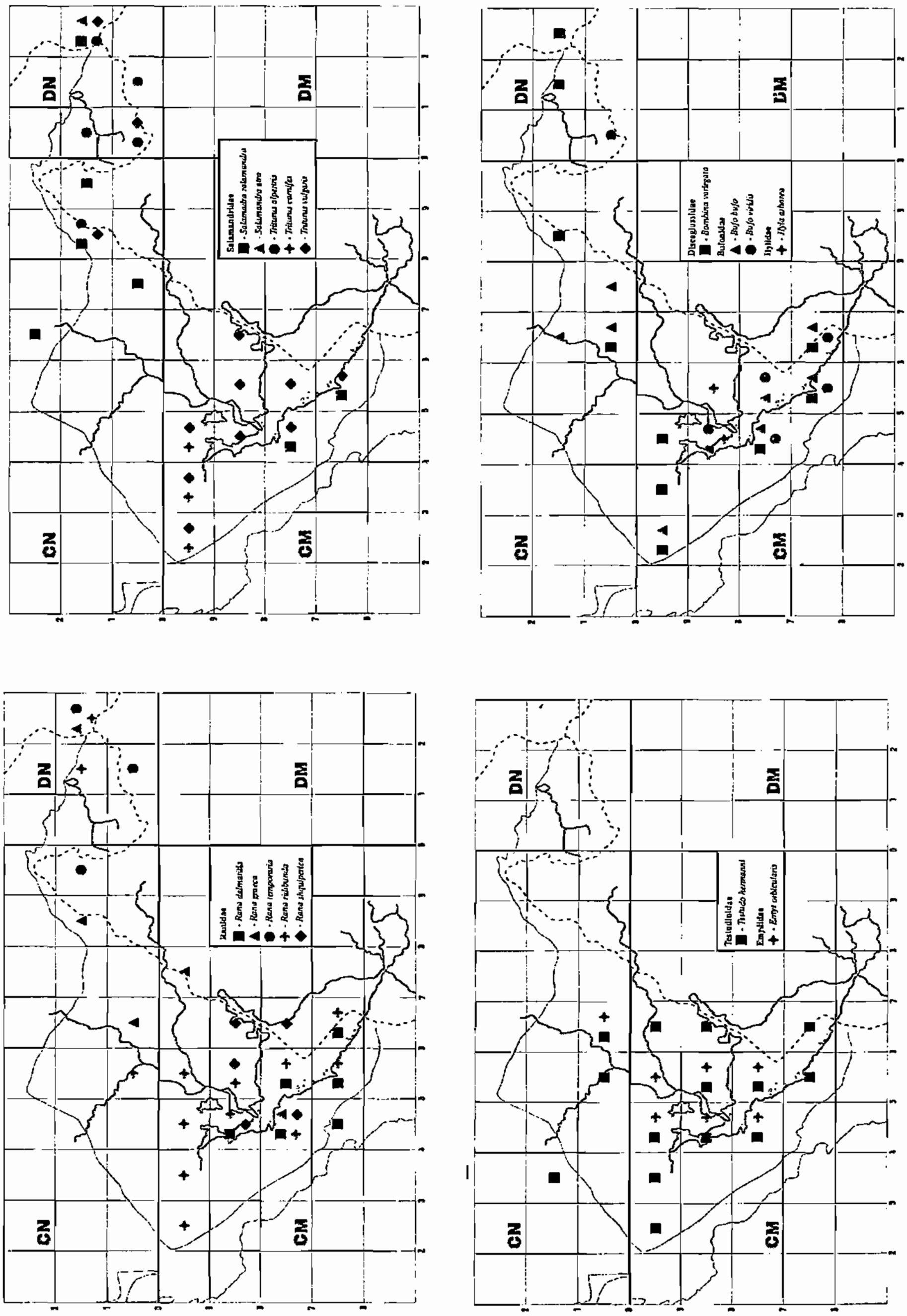
Two reserves are established within Skadar Lake Region (VIDAKOVIC, 1989): National Park Lovcen with total surface area of 200 km^2 and National Park Skadar Lake with surface area of 400 km^2. Part of Prokletije massif, Plav-Gusinje ravine, represent third important area which will be proclaimed a National Park until the end of this century (VUCKOVIC et al, 1985). According to the international standards, all elements of flora and fauna within an national park are protected by the low and any exploitation of nature reserves is strictly forbidden. Formally, all herptiles distributed on the territory of SRY are protected by the low. Also, in "Official Register of SR Montenegro", issue 36/82 by "decision on putting under protection of rare, thinned out, endangered and endemic plant and animal species" permanently have been protected 7 amphibian and 24 reptilian species.

Nevertheless the national lows of nature protection, decline of amphibian and reptilian species within Skadar Lake Region is reality. Causes are various, but they all are connected with development of industry, agriculture and tourism. In the lowland part of the Region, agricultural intensification is one of the most complex factors of threat for amphibians and reptiles. It include afforestation by burning or clearing natural vegetation, drainage of meadows and marshes around Lake Skadar, as well as pollution by use of agricultural fertilizers. The impact of industry is evident through pollution derived from iron plant, brewery, hog raising station, milkproduct, dry cleaning plant,cotton combine, slaughterhouse, tannery and, most important, aluminium plant from Podgorica, whose wastewaters directly influence the Skadar Lake drainage area. In the mountainous part, the main threats could be afforestation, intensive development of fishery by exploitation of glacial lakes, overgrazing and tourism development (especially intensification of winter sports).Intensive tourism development (including new road and piste constructing and following urbanization) could be the most dangerous factor of threat for whole Skadar Lake Region. Under present political and economical conditions, it is attractive way for improvement of an area without significant investment. But, it is always followed by uncontrolled sale and exploitation of natural rarities and destruction of habitats important for diversity maintaining.

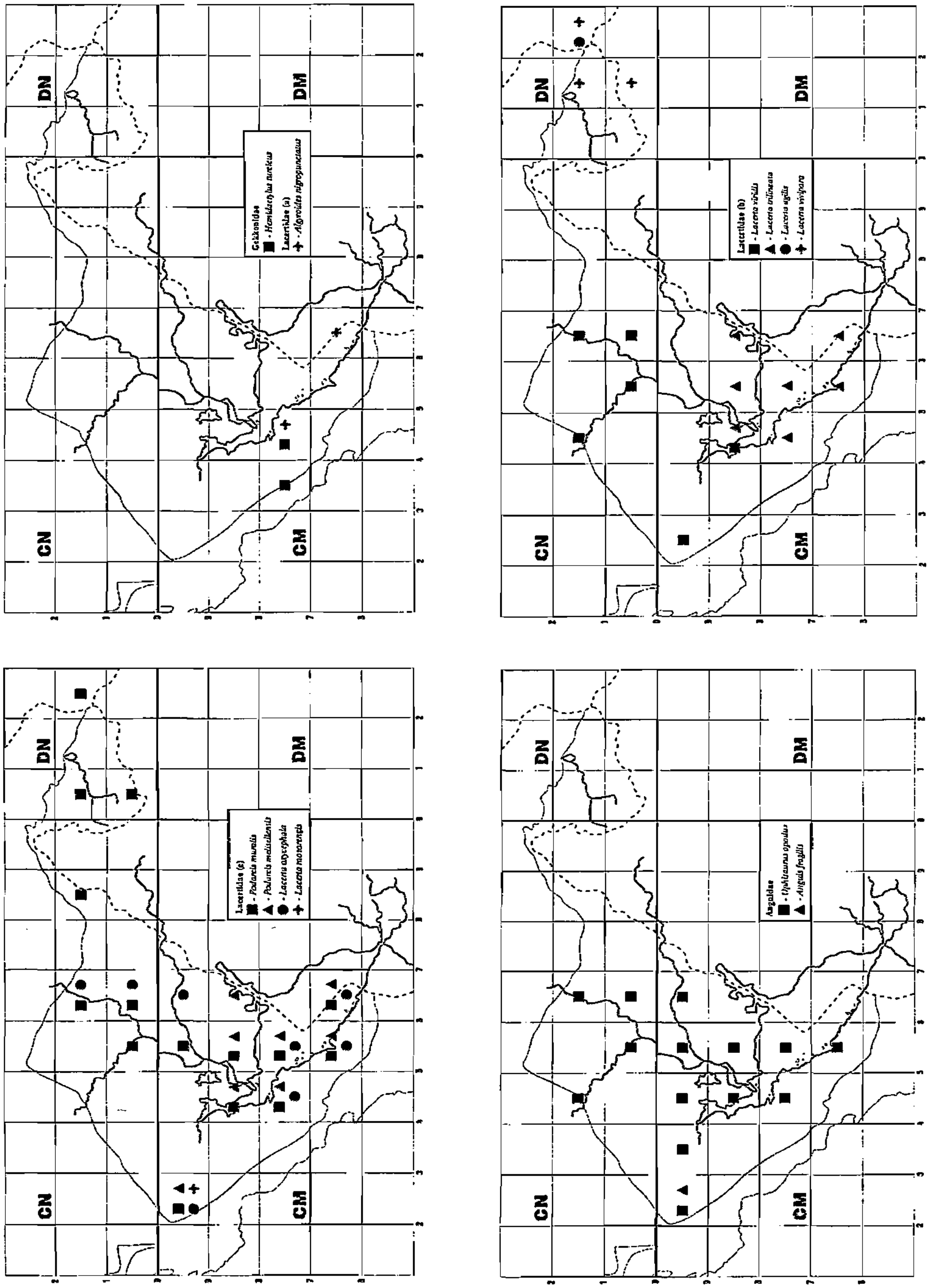
Our opinion is that Skadar Lake Region has its value, by both the number of amphibian and reptilian species present and the diversity of landscape types. Area which maintain great number of mediterranean as well as species common throughout the Europe, deserves appropriate protection. The first step in that process would be the evaluation in international dimensions.

ACKNOWLEDGEMENTS

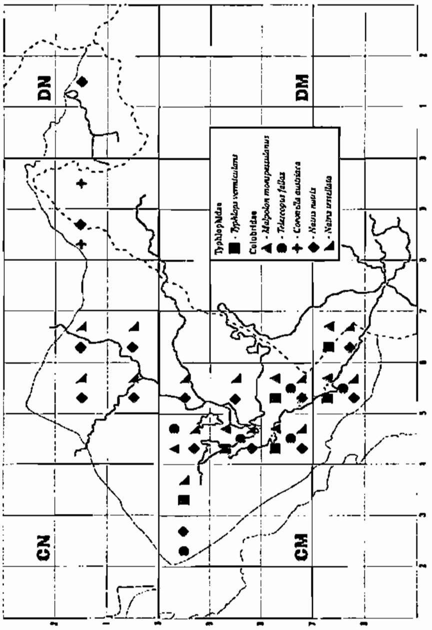
We thank Oliver Isailovic for careful preparing of illustrations.

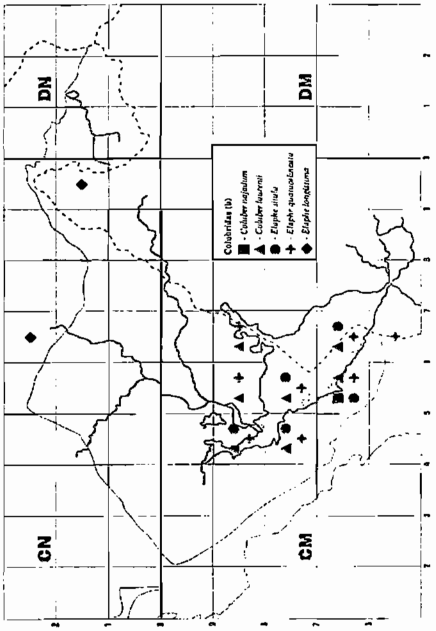


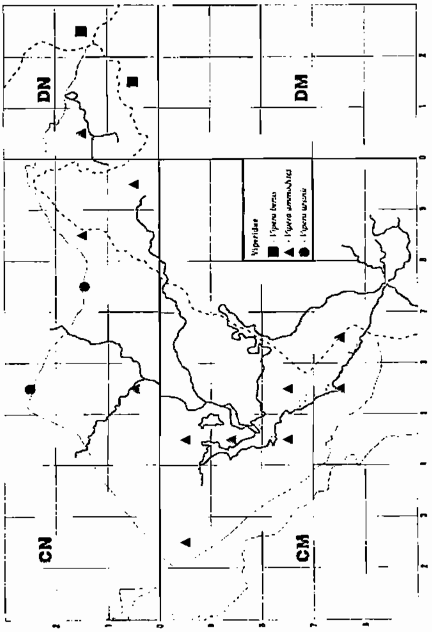
Figures 3-6: Records of amphibian and reptilian species inhabiting Skadar Lake Region.

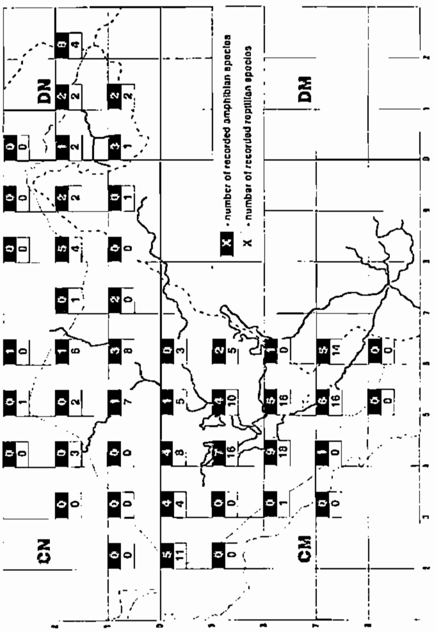


Figures 7-10: Records of amphibian and reptilian species inhabiting Skadar Lake Region.









Figures 11-13: Records of amphibian and reptilian species inhabiting Skadar Lake Region.
Figure 14 (right bottom corner): Summary of recorded amphibian and reptilian species per UTM square within Skadar Lake Region.

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An inventory of amphibian breeding sites of national importance in Switzerland

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Abstract: Since 1988, new legislation has allowed the Swiss federal government to designate habitats of national importance and to oblige the cantons (states) to protect these sites. To help stop the continuing decline of amphibian populations in Switzerland, a group of herpetologists was mandated in 1989 yo prepare an inventory of amphibian breeding sites of national importance. All breeding sites in Switzerland (nearly 8000) were assessed based on known data on species diversity, population sizes, and rarity of the species in Switzerland as a whole as well as on a regional basis. About 10% of the highest ranking sites were intended to be included in the inventory and were studied in 1990 and 1991. Field workers definded three zones requiring varying degrees of protection level. The inventory contains 891 breeding sites, averaging 7.0 ha in size (median 1.8 ha). Before the inventory can be put into force, the cantons will be consulted. The federal government considers the inventory as an important contribution towards implementing the Berne Convention.

Key Words: Amphibia, Switzerland, Inventory, Breeding sites.

INTRODUCTION

Despite being protected by federal law since 1966, amphibian populations in Switzerland have experienced a substantial and continuing decline in the past few decades (GROSSENBACHER, 1988). As a result, most species are considered threatened (Tab. 1, GROSSENBACHER, 1994). Responsible for this decline are mainly the loss or deterioration of breeding sites and habitat fragmentation. In reality there is a considerable gap between the relatively good conservation laws and their effective application.

New legislation, passed in 1988, allows the federal government to designate habitats of national importance and to oblige the cantons to protect these sites (Switzerland consists of 26 relatively independent cantons or states). Since then, four inventories have been worked out or are presently in work: Inventories of raised bogs, of fenlands, of floodplain woods and of dry prairies. In order to improve the protection of amphibian breeding sites, the federal department of environment, forestry and landscape mandated in 1989 the Coordination Center for the protection of amphibians and reptiles, an independent institution funded by federal and canton governments, to prepare an inventory of amphibian breeding sites of national importance. The goal of the inventory was to determine the most important reproduction sites and to assure their effective protection and restoration, respectively. About 10% of all breeding sites, representing all regions, were intended to be included. This inventory would be the first which was not intended to protect particular vegetational units, but to help preserve the diversity of a particular taxon of animals.

METHODS

Selection of breeding sites

Basis of the inventory were the available data on the approximately 8000 known amphibian spawning sites in Switzerland, gathered from the mid-seventies to the late eighties (GROSSENBACHER, 1988). We first established a formula to assess the value of all breeding sites and to express it as number (BORGULA *et al.*, 1994).

Criteria for the assessment were number of occurring species, rarity of these species and population sizes. Since the distribution of amphibians in Switzerland varies strongly with altitude and on a regional basis, the value for each breeding site was calculated twice, using first an average rarity factor for the whole of Switzerland, and second, a regional rarity factor. For this purpose, Switzerland was divided in 19 geographical regions. If either of the calculated values exceeded a certain threshold, the breeding site was provisionally included in the list of breeding sites of national importance. In order to avoid a lack of breeding sites in the higher regions, where species richness is greatly reduced, thresholds were lowered for alpine regions. Not considered in the inventory for methodological reasons were the two salamanders *Salamandra salamandra* and *S. atra* as well as the introduced Marsh frog *Rana ridibunda*.

Definition of zones of protection

For the delimitation of the breeding sites, three zones requiring varying levels and measures of protection were definded:

Zone A: Core area, which includes the actual breeding ponds and surrounding wetlands, as well as adjacent important structures for amphibians such as uncultivated land or hedges. The core areas should assure successful reproduction of the amphibians. In these core areas nature protection has to dominate over all other potential land uses such as agriculture, forestry or recreation.

Zone B: The surrounding zone, which includes neighbouring agricultural land or woods. By imposing restrictions on current land use, this zone is intended to fulfill various functions. First, it should protect the core areas from detrimental influx of pesticides and fertilizers. Second, it should improve the breeding sites by adding suitable land habitat. Third, it should preserve the most important migration routes and help maintain the connection with other breeding sites.

Zone G: Includes gravel and clay pits in use and similar habitats. That is, artifical breeding sites which experience constant habitat changes. The zone G does not define a particular area to be protected, but demands that the area is managed in an optimal way for the breeding amphibians. This usually requires certain changing parts of the pit to be spared from use for some time.

Field work, description

In 1990, all provisionally listed breeding sites as well as other potentially important sites were visited by experienced field workers. They described the site, and delimited and defined one or a combination of the three zones of protection. Additionally, field workers suggested appropriate measures to improve or maintain the quality of the breeding sites and, if known, they added information on land owners, existing protection and other inventories. The occurrence of amphibians was not systematically checked except in cases where potentially important breeding sites did not reach the threshold value. Most breeding sites which reached either the national or regional threshold value where included in the inventory. Destroyed sites which can not be restored as well as some very small and obviously overestimated sites, on the other side, were excluded. As a substitute for destroyed sites, field workers included, if possible, an other spawning site within a 2-km-radius in the inventory and suggested appropriate measures for improvement.

For each breeding site, we created a double-sided sheet, containing the mentioned text information as well as maps of two different scales: 1:50.000 showing the position of the breeding site and of neighbouring reproduction sites, and 1:10.000 showing the various zones of protection as well as known or supposed migration routes.

Species	Status
Salamandra atra	3
Salamandra salamandra	3
Triturus alpestris	3
Triturus carnifex	3
Triturus cristatus	2
Triturus helveticus	3
Triturus vulgaris	2
Alytes obstetricans	3
Bombina variegata	3
Pelobates fuscus	0
Bufo bufo	3
Bufo calamita	3
Bufo viridis	0
Hyla arborea	2
Rana arvalis	0
Rana dalmatina	3
Rana kl. esculenta	3
Rana lessonae	3
Rana latastei	1
Rana temporaria	n

0 = "ausgestorben" (extinct)
1 = "vom Aussterben bedroht" (endangered)
2= "stark gefdhrdet" (vulnerable)
3= "gefährdet" (vulnerable, to a lesser degree)
n= not threatened

Table 1: Amphibian species of Switzerland and their status according to the Swiss Red List (Grossenbacher, 1994). The introduced *Rana ridibunda* is not included.

RESULTS

The inventory contains 891 breeding sites, 11.4% of all known spawning sites in Switzerland. Data on size, altitudinal distribution and species richness are summarized in Table 2. With on average 5.45 reproducing species, the sites exceed average species richness of breeding sites in Switzerland (2.67) more than twofold. Most reproduction sites of endangered or rare species are included in the inventory: At least 68% of *Triturus cristatus*, 69% of *T. vulgaris*, 58% of *Hyla arborea*, 84% of *Rana dalmatina* and 100% of *R. latastei* reproduction sites. 72% of the breeding sites of national importance are at altitudes under 600 m, 89% under 1000 m.

The breeding sites encompass a great variety of habitat types. An important portion, about 20%, is represented by gravel pits and similar habitats. The artificial dynamics of these sites mimics the lost natural dynamics of floodplains and provides often excellent conditions for amphibians, particularly for the Natterjack toad *Bufo calamita* and the fire-bellied toad *Bombina variegata*, but also for the treefrog *Hyla arborea* and the midwife toad *Alytes obstetricans*.

Field workers found that a considerable number of breeding sites, about 30%, have been deteriorated since they have first been described in the mid-seventies to late eighties. About 15% have been completely destroyed. The most important cause was the filling up of ponds or parts of it. Only a minority of the spawning sites has already a sufficient legal status of protection.

	N	mean	Med.	Total	Range
A	773	7.0	1.8	5424	0.004-374
B	616	11.2	6.2	733	0.05-234
G	176	8.6	4.2	1511	0.35-192
Tot	876	15.8	7.6	13667	0.004-607
Alt.	891	716	485		95-2240
sp.	891	5.45	5		1-11

sp. (number of species): not including *S. atra*, *S. salamandra* and *R. ridibunda*.

Table 2: Size (ha), altitude (m) and species richness of amphibian breeding sites of national importance.

DISCUSSION, FURTHER PROCEDURE

Switzerland has lost over 90% of its wetlands in the past 150 years. The remains are mostly small and isolated. Not surprisingly therefore, the inventory, although representing the best amphibian reproduction sites, largely contains rather small wetlands or areas with few water bodies. As an additional negative aspect, a large number of them is surrounded by intensively used agricultural land.

Nevertheless, the inventory is a chance to secure a grid of the best still existing breeding sites. This may be crucial for maintaining regional amphibian populations by enabling large population sizes which can be the starting point for colonisation or reçolonisation of surrounding ponds, and by helping to preserve endangered and rare species by offering special habitats. The federal government considers the inventory as an important contribution towards implementing the Berne Convention.

On the other hand, the inventory can for various reasons only be a part of the conservation strategy for the amphibian fauna of Switzerland. First, since it contains only about 10% of all sites, the need to preserve or restore the additional breeding sites persists. Second, the inventory only covers the reproduction sites. Summer and winter habitats could only partly be included since they are mostly unknown. Third, amphibian breeding sites are often characterized by great dynamics. This often requires flexible measures which go beyond defining fixed protected areas.

The inventory and the final report are published in 1994 (BORGULA et al., 1994). They will be mailed to the canton governments and to interested organizations for consultation. The cantons are obliged by the law to implement the inventory and to prepare appropriate measures to protect or restore the breeding sites or to assure a management that helps preserve the quality of the site. The federal government provides financial help as well as advice.

The success of the inventory will now mostly depend on the efforts of the cantons to implement its proposed measures. Unfortunately, the will to do so probably does not exist in all cantons. The way the inventory is implemented, however, may be crucial in deciding the future of severely threatened species such as the treefrog, *Hyla arborea*, the Italian agile frog, *Rana latastei*, the agile frog *Rana dalmatina*, the crested newt, *Triturus cristatus*, and the smooth newt, *T. vulgaris*.

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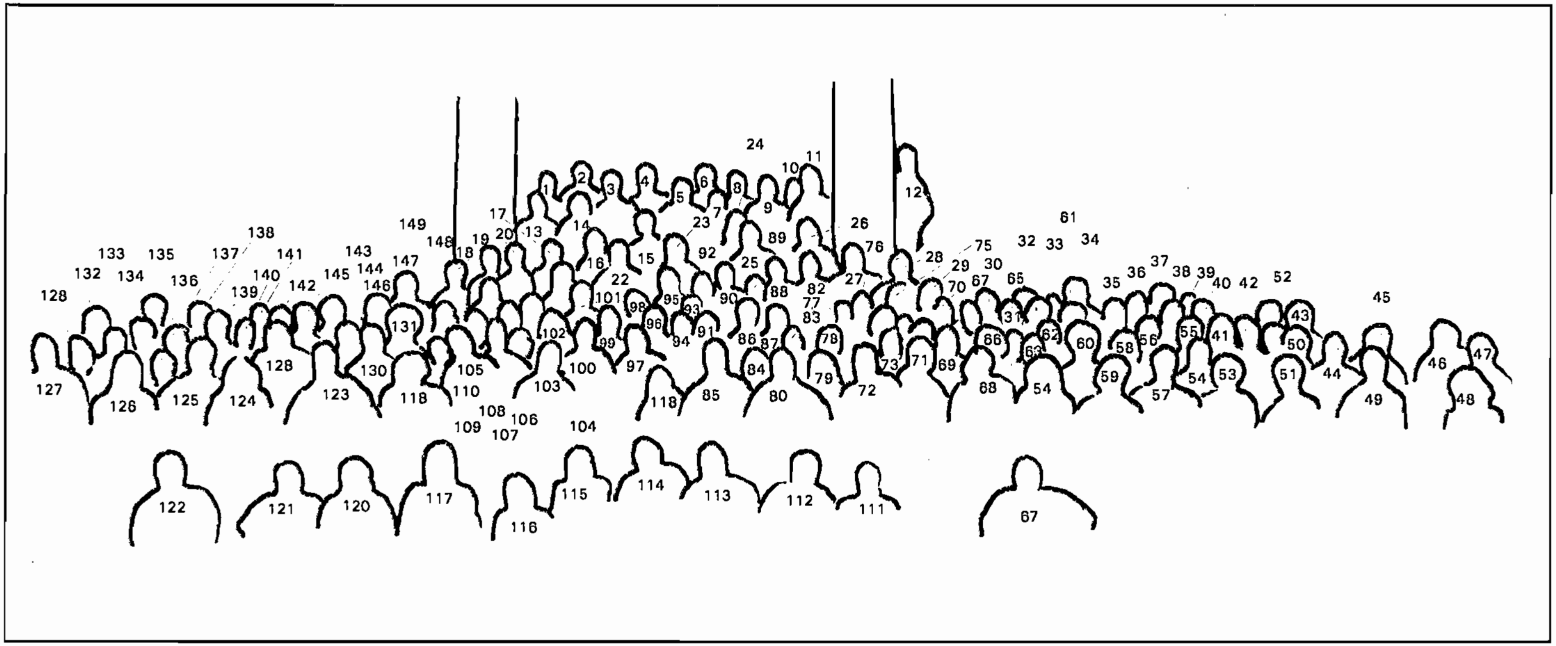

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