# Effects of Introduced Salmonids on a Montane Population of Iberian Frogs

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**Abstract:** *Amphibians are declining worldwide because of multiple factors, including human-mediated introduction of fishes into naturally fishless areas. Although several studies have focused on the effect of exotic fishes on native amphibians breeding in ponds or lakes, little is known about their effects on stream-breeding species. We studied the effects of introductions of native brown trout (*Salmo trutta*) and exotic brook trout (*Salvelinus fontinalis*) on the stream-breeding, endemic Iberian frog (*Rana iberica*) in a protected area in central Spain. We assessed occurrence patterns of tadpoles and salmonids and compared habitat use of the three species. We also determined experimentally whether chemical cues from salmonids elicited antipredator behavior in tadpoles. Finally, we assessed the relative influence of tadpole habitat preferences, differences in salmonid species, and invasion geography on tadpole occurrence. Despite widely overlapping habitat preferences, tadpoles and trout did not coexist, with the former restricted to fishless habitats. Tadpoles detected chemical cues from both trout species and reacted by decreasing their activity, although the response toward the native brown trout was stronger. The residual distribution of Iberian frogs in Peñalara is better explained by the geography of fish invasions than by the fish species involved. Measures such as fish extirpation from certain areas, aimed at recovering lost habitat and improving connectivity among remaining populations of Iberian frogs, seem critical for the species' long-term survival in central Spain.*

**Key Words:** antipredator behavior, introduced fishes, Peñalara Natural Park, *Rana iberica*, stream-dwelling amphibians

Efectos de Salmónidos Introducidos sobre una Población Montana de Rana Ibérica

**Resumen:** *Los anfibios están declinando mundialmente debido a múltiples factores, incluyendo la introducción de peces por humanos en áreas naturalmente carentes de peces. Aunque se han realizado varios estudios sobre el impacto de peces exóticos sobre anfibios nativos que se reproducen en charcas o lagos, se conoce poco de sus efectos sobre especies que se reproducen en arroyos. Estudiamos los efectos de la introducción de la trucha nativa (*Salmo trutta*) y la trucha exótica (*Salvelinus fontinalis*) sobre la rana ibérica (*Rana iberica*) en un área protegida en el centro de España. Evaluamos los patrones de ocurrencia de renacuajos y salmónidos y comparamos la utilización del hábitat de las tres especies. También determinamos, experimentalmente, si las señales químicas de los salmónidos producían comportamiento antidepredador en los renacuajos. Finalmente, evaluamos la influencia relativa de las preferencias de hábitat de renacuajos, las diferencias en especies de salmónidos y la geografía de la invasión sobre la ocurrencia de renacuajos. A pesar de preferencias de hábitat ampliamente traslapadas, los renacuajos y truchas no coexistieron, los primeros restringidos a hábitats sin peces. Los renacuajos detectaron señales químicas de ambas especies de trucha y reaccionaron disminuyendo su actividad, aunque la respuesta hacia la trucha nativa fue mayor. La distribución residual de ranas Ibéricas en Peñalara esta mejor explicada por la geografía de las invasiones de peces que por las especies de peces involucradas. Medidas como la extirpación de peces de ciertas áreas,*

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*enfocada a la recuperación de hábitat perdido y al mejoramiento de de la conectividad entre poblaciones remanentes de ranas Ibéricas, parecen críticas para la supervivencia a largo plazo de la especie en el centro de España.*

**Palabras Clave:** anfibios habitantes de arroyos, comportamiento antidepredador, peces introducidos, Parque Natural Peñalara, *Rana iberica*

## Introduction

Amphibians are declining worldwide because of multiple and complex factors, frequently with synergistic effects (Fisher & Shaffer 1996; Gillespie & Robertson 2001). Prominent among those factors is human-mediated introduction of species to areas where they were historically absent (Moyle & Leidy 1992; Simon & Townsend 2003). In the past century, such introductions occurred worldwide, including protected areas (Cole & Landres 1996; Pister 2001), with subsequent negative effects on native amphibian assemblages (Knapp & Matthews 2000). Most amphibians have evolved in fish-free environments and thus they are poorly suited to coexist with fishes (Kats et al. 1988; Holomuzki 1995).

Salmonid fishes have been extensively stocked for recreational fishing (Fausch 1988). They are typically cold-water fish and thus have been introduced into montane areas, where unique amphibian assemblages have been affected dramatically (Bradford 1989; Drost & Fellers 1996). Most research on the consequences of salmonid introductions has focused on lakes (Bradford 1989; Braña et al. 1996; Knapp et al. 2001*a*; Vredenburg 2004). In comparison, information on the effects on stream-dwelling amphibians is scarce (but see Gillespie & Robertson 2001).

Often, it is implicitly assumed that larger range expansions should have more harmful consequences on amphibian assemblages because coexistence mechanisms have less opportunity to evolve (Sih et al. 2000). For example, chemical cues eliciting antipredator behavior in anuran larvae can be specific for a predator species or even a population (Griffiths et al. 1998). The impact of small range expansions, however, should not be dismissed lightly. Numerous amphibian species show naturally allotopic distributions relative to native fishes, suggesting that they lack effective mechanisms for coexistence with them (Sih 1992; Brönmark & Edenhamn 1994; Resetarits 1997; Azevedo-Ramos et al. 1999).

Moreover, there is little information on how landscape and invasion geography modulate the effects of salmonid introductions on amphibians. Spatial features are likely to be important (With 2002), and different stocking practices often result in differences in the geography of salmonid invasions into montane aquatic systems (Adams et al. 2001). For example, introductions into headwater areas facilitate subsequent colonization of a drainage by downstream dispersal because barriers are more effective for upstream than downstream movement (Adams et al. 2000, 2001). Government agencies typically target headwater lakes in their stocking programs because these habitats are not likely to dry or freeze completely (Pister 2001). In contrast, local anglers have often moved fishes from downstream sites into fishless reaches immediately upstream (Thompson & Rahel 1996).

Presumably, the eradication of amphibian species by introduced salmonids occurs largely by predation on larvae (Gillespie & Robertson 2001; Vredenburg 2004). However, nonlethal effects can also be relevant because amphibians may not occupy sites where fish are locally absent but fish chemicals are present (Pilliod & Peterson 2001). Some adult amphibians avoid breeding in water bodies containing chemicals from fish predators (Hopey & Petranka 1994; Binckley & Resetarits 2003). Therefore, introduced salmonids not only reduce amphibian population size but also increase their fragmentation and isolation (Bradford et al. 1993), increasing the likelihood of local extinction.

During the 1970s, brook trout (*Salvelinus fontinalis*), native to North America, were introduced into the Laguna Grande, the only deep, permanent lake in Peñalara Natural Park, to start a recreational fishery. The species was subsequently observed in the Peñalara stream, an outlet of the Laguna Grande. Simultaneously, local anglers moved native brown trout (*Salmo trutta*) from nearby downstream sites to upstream reaches in the Pepe Hernando stream. Those previously fishless reaches were located above a high-gradient section of the stream that had prevented trout colonization. Brown trout dispersed some 300 m upstream (Fig. 1), up to a 1-m-high vertical waterfall with no jump-pool at its bottom, establishing a small, isolated, self-sustaining population (P.A.R., J.B., & L.B., unpublished data).

Following salmonid introductions, Iberian frogs (*Rana iberica*) disappeared from stream reaches where they were formerly present (M. García-París, personal communication). The Iberian frog is an Iberian endemic that is listed nationally and regionally as vulnerable (Esteban & Martínez-Solano 2002). The species typically breeds in running waters, and adults are rarely found far from streams, although juveniles are more terrestrial. In the park, larvae are generally found from June to September, but a few overwinter in the stream (J.B., personal observation). The species is more abundant in northwestern Iberia, where it occupies diverse lotic habitats. In central Spain, however, it is restricted to mountain streams,
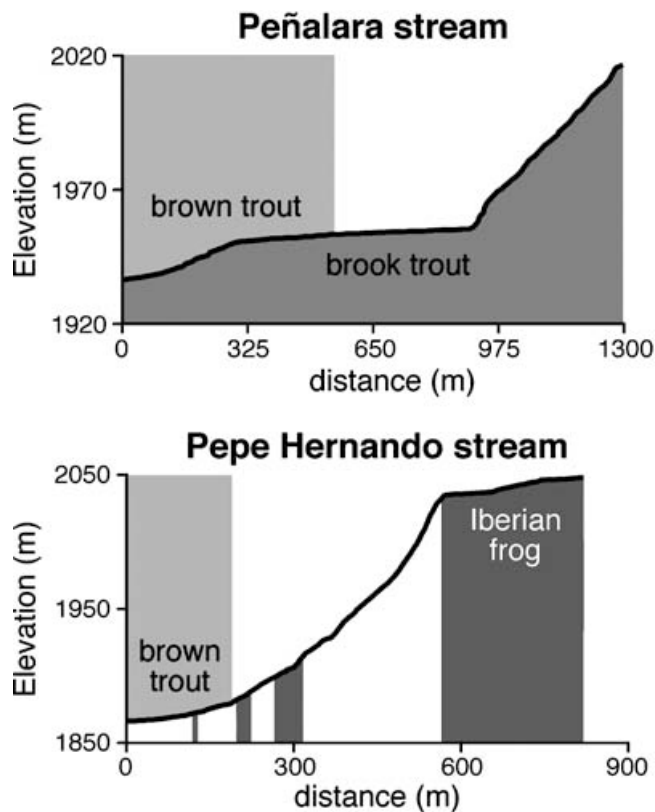
## Peñalara stream



## Pepe Hernando stream



*Figure 1. Stream profiles of the sampled sections of the studied streams of Peñalara Natural Park, indicating where tadpoles and salmonids are present. The gray blocks indicate the stream sections where tadpoles and salmonids are present*

peat bogs, and ponds of glacial origin (García-París et al. 1989; J.B., personal observation). Populations in central Spain appear to be declining in recent times, allegedly because of habitat loss and fish introductions (Esteban & Martínez-Solano 2002).

There have been recent attempts to extirpate brook trout from the Laguna Grande (Granados & Toro 2000), but the effects of introduced salmonids in the streams of Peñalara Natural Park remain unexplored. For example, habitat preferences of Iberian frog larvae and introduced salmonids have not been examined. Those preferences may determine the degree of sympatry between native and invasive species (Scoppettone 1993; Rincón et al. 2003) and, hence, the latter's impact on stream-dwelling amphibians. Furthermore, such information should provide valuable insight on whether subtle differences in the ecology of invasive species, a priori assumed to be ecologically similar, and in invasion geography mediate their effects. Finally, such knowledge should provide the basis for future recovery measures for Iberian frog populations in the area.

Our objectives were to (1) assess and analyze the patterns of occurrence of Iberian frog tadpoles, brown trout, and brook trout in streams of Peñalara Natural Park, (2)

determine whether habitat use is similar between Iberian frog tadpoles and salmonids, and (3) experimentally establish whether chemical cues from native and non-native salmonids elicit antipredator behavior in Iberian frog tadpoles.

## Methods

### Study Area

Peñalara Natural Park (Sierra de Guadarrama, central Spain, 40°50′N, 3°57′W) is an alpine area composed of several small glacial valleys (*hoyas*) between 1800 and 2430 m asl. It contains hundreds of interconnected ponds and small rivulets, most of which drain into two larger streams, the Peñalara and Pepe Hernando, which eventually join and flow into the Lozoya River (Tajo drainage). The Hoya de Peñalara (HP) begins in a glacial cirque containing the Laguna Grande (surface area 5452 m²; 2017 m asl). The Pepe Hernando stream originates in a system of ponds at about 2050 m asl and flows through the adjacent Hoya de Pepe Hernando (HPH). In preliminary surveys, Iberian frogs were found only in the headwaters of this stream within the park (Fig. 1). The past distribution of the species is not accurately documented, but it was present in HP in the early 1980s (M. García-París, personal communication).

The HP and HPH streams are physiographically similar. Both are small, clear mountain streams with cobble substrate and pools, riffles, and runs constituting most of the habitat. Whereas runs are the most abundant habitat type (by area) and cascades are rare in the HP stream, pools are dominant in the HPH stream and cascades are relatively common. The HP is wider and more open than the HPH, practically lacking trees. The HPH has steeper sides and some conifer thickets. Ponds at varying distances and degrees of connection from the main channel are present in both valleys. Most ponds in the HP dry in the summer, however, whereas the HPH also contains permanent springs in which water forms relatively cold and well-oxygenated permanent ponds and then flows into the main channel. Brown trout have not colonized those habitats, and Iberian frogs still use them to breed.

### Habitat Unit Preferences and Spatial Distributions

We sampled trout and Iberian frog tadpoles and assessed stream habitat in the HP and HPH streams in early August 2002, after the Iberian frog breeding period and before froglet emergence. In a study reach (usually 50 m long), we first performed visual counts of tadpoles, then electrofished for trout, and finally mapped the reach to estimate the relative abundance (proportion of total surface area) of backwaters, pools, runs, riffles, and cascades (following Bisson et al. 1988; Hawkins et al. 1993).

In the HPH stream, we started the surveys at the downstream limit of the introduced population of brown trout

at 1865 m asl and proceeded up to the stream sources, sampling 17 study reaches and 815 m of stream. In the Peñalara stream, we began sampling at a gauging station at 1936 m asl and fully surveyed the subsequent upstream 437 m (9 reaches). From this point to the Laguna Grande (848 m) we performed only tadpole counts and electrofishing to confirm the absence of Iberian frog tadpoles and to assess the distribution and remove brook trout from the Peñalara stream, but these data were not used in subsequent statistical analyses.

To estimate the abundance of Iberian frog tadpoles, three observers moved carefully upstream along the banks recording the number of tadpoles visible in each channel habitat unit. Each observer counted tadpoles at least three times, and the grand average was retained for further analyses. Differences among observers were almost nonexistent. Visual censuses have been successfully used for other stream-breeding anurans (Griffiths et al. 1998; Schley et al. 1998), and Iberian frog tadpoles were easily detected because of their dark color, their restriction to nonturbulent areas, and the crystal-clear water and scarcity of aquatic vegetation. Subsequent electrofishing further confirmed the absence of tadpoles from fast, turbulent water areas and the accuracy of visual counts in slow water.

We assessed the abundance of both brook and brown trout through electrofishing with a backpack custom-made electroshocker (DC 200–400 V, <1 amp). We performed three consecutive passes in an upstream direction in each reach. In more than 95% of the cases, no fish was captured in the third pass, which agrees with the documented effectiveness of the method in small streams (Thompson & Rahel 1996; Kruse et al. 1998). Each captured individual was measured (fork length in millimeters) and the type of habitat unit where it was found was recorded. Scale samples for age determination were taken from representative specimens. Individuals were assigned to age classes (0+, 1+, 2+, etc.). The 0+ age class comprised fishes in their first growing season whose scales, consequently, did not show an annual checkmark. These individuals emerged from the redds in mid- to late May and hence were about 3.5–4 months old at the time of our sampling. Fishes in their second growing season, and, therefore, with an annual checkmark visible on their scales, were assigned to the 1+ class and so forth.

Once tadpole counts and electrofishing were finished, we measured stream width (nearest 5 cm) every 2 m and recorded the length of each channel habitat unit to estimate the total surface area of the reach and of each habitat unit type.

## Statistical Analyses

To identify habitat preferences, we tested for differences between the proportions of stream area corresponding to each habitat category and the proportions of individuals captured (both trout species) or observed (tadpoles) with a goodness-of-fit chi-square test. For brown trout in the HPH stream, habitat availability was only obtained from those reaches inhabited by the species. Similarly, we did not use data from the HP stream to estimate habitat availability for Iberian frog larvae because it contained none (see Results).

To assess the influence of trout species, invasion geography, and habitat unit type on the occurrence (presence or absence) of Iberian frog tadpoles in habitat units, we used log-linear analysis of contingency tables (Quinn & Keough 2002). We built multidimensional tables by cross-tabulating our whole survey data according to (1) stream in which the unit was located, (2) tadpole presence or absence, (3) habitat type, and (4) presence or absence of trout (regardless of species). Although we found no Iberian frog tadpoles in the HP stream, this was not an a priori condition, and we attributed this fact to the more widespread occurrence of salmonids there. Therefore, we believe we were justified in using the data from the HP stream and including a "stream" term in our analyses.

Log-linear analysis tested whether tadpole presence was significantly affected by other factors (both main factors and their interactions) by using a contingency table when independence (i.e., no influence of those factors) was assumed. The multidimensional table of expected values (often referred to as a "fitted table") was compared against the table of observed values with a maximum-likelihood chi-square test. With four variables, the complete model is complex (15 terms, including all interactions), so we used a backward stepwise procedure to find the simplest model that would still produce a fitted table not significantly different from the observed one. We considered that factors left out of this minimum model had no significant influence on tadpole occurrence. We carried out log-linear analyses with the Statistica package version 6 (Statsoft, Inc., Algés, Portugal). Unless otherwise indicated by the user, Statistica treats zeros in contingency tables as nonstructural. However, because we excluded those areas where we judged tadpole presence to be structurally impossible (i.e., tadpole absence not attributable to the presence of salmonids or to active habitat choice, rather than structural limitations on habitat use), structural zeros were absent from our tables.

The HP and HPH streams differ in both introduced fish species identity and invasion geography. Hence retention of the "stream" term in the minimum model would not indicate whether differences in tadpole occurrence between streams are due to different impacts of each trout species or to their different spatial distribution. To ameliorate this problem, we recorded "salmonid presence" in three different ways that capture these between-stream differences in salmonid distribution to varying extents. Thus, we recorded whether the habitat unit (1) contained salmonids (salmonid presence 1); (2) was located within a reach that contained salmonids (salmonid presence 2);

or (3) was linked in the upstream direction to at least one totally fishless area (no salmonids from the water source to the habitat unit) that could potentially act as a source of tadpoles (salmonid presence 3). Salmonid presence 1 reflects differences in the overall proportion of habitat units containing salmonids between streams; salmonid presence 2 accounts for between-stream differences in the abundance of relatively large fish-free stream sections (the position of fish-free areas [potential tadpole sources] can be relevant [Bradford et al. 1993]); and salmonid presence 3 focuses on differences in the frequency of fish-free areas upstream of habitat units.

We ran the log-linear analysis three times successively with salmonid presence 1–3 for the "trout presence or absence" term. By comparing the terms retained in the three corresponding minimum models, we expected to be able to infer the relative importance of salmonid spatial distribution and species identity. For example, if species was a relevant factor (due to, for example, differences in predatory ability or habitat use), the "stream" term was retained in all three minimum models. On the other hand, if differences in distribution patterns as captured by either salmonid presence 1, 2, or 3 were the most relevant factor, the "stream" term would be redundant and, hence, was dropped from at least some of the three minimum models.

Perfectly untangling the roles of spatial features and species identity would ideally require additional data from other stream networks inhabited by Iberian frogs where brown trout had been introduced into headwaters and brook trout into downstream reaches. Unfortunately, we do not know of any stream in Spain where the Iberian frog occurs and brook trout have invaded in an upstream direction. In Spain brook trout seem to have been invariably stocked into headwater mountain lakes (Doadrio 2002) and, to our knowledge, have never subsequently invaded an inlet stream containing Iberian frogs.

We surveyed 292 habitat units in the HPH stream and 118 in the HP stream. Backwaters and cascades, however, were excluded from analyses because they never contained tadpoles. Also, we excluded data from a high-gradient section (average 58.2%) of the HPH stream (reaches 8–12) that lacked tadpoles. Thus, 186 habitat units from the HPH stream and 101 from the HP stream were included in the log-linear analyses.

## Antipredator Behavior of Iberian Frog Tadpoles

To test whether Iberian frog tadpoles respond to chemical cues from brown and brook trout and whether the response to the two species differs, we carried out the following experiment. Two plastic containers (34.5 × 23.5 × 20 cm), furnished with a small refuge (a thin, 10 × 6.5 cm piece of dark plastic held 1.5 cm from the bottom by three plastic legs), were placed close to the bank

of the HPH stream at the downstream end of its fishless section. Each container was randomly assigned to either the experimental or control treatment for each replicate. Water from two opaque 20-L holding tanks flowed constantly into the containers via clear plastic tubing fitted with closing valves. Plastic tubing also drained the containers. Flow rate was about 275 cc/minute and water volume was about 4.8 L. The holding tanks constantly received stream water from the upstream fishless area. One of the tanks received one big (20 cm) and two small brown trout, and the other remained empty.

In each trial, five Iberian frog and one common fire salamander (*Salamandra salamandra*) larvae (a proportion similar to that found in the stream) captured in nearby areas of the HPH stream were placed in each container. After 10 minutes of acclimation, the experiment (two 10-minute stages) started. In the first stage of each trial, the two containers received water from the tank with no fish. In the second stage, inflow in the experimental container was switched to the fish tank, whereas in the control container flow from the fishless tank was briefly interrupted and then resumed. Tests with a dye indicated that incoming water dispersed completely throughout the container in <25 seconds. This design mimicked natural conditions in relevant aspects such as no water recirculation and water with the full array of diverse chemical cues naturally encountered by the tadpoles (Irving & Magurran 1997). Also, Iberian frog tadpoles are largely restricted to pools (see Results), where, similar to the experimental arenas, both current velocity (typically < 10 cm/s) and turbulence are low (P.A.R., personal observation). Salmonid density in our experiments was in the upper part of the range of density values at the habitat unit scale.

After each trial, we returned larvae to their capture locations, emptied containers, and thoroughly rinsed them with stream water from the fishless section. We repeated the procedure with brook trout (of a size similar to the brook trout) from the HP stream. We obtained 12 replicates for each fish species.

During the experiment, we videotaped tadpole movement for further analysis in the laboratory. We digitized the recordings and counted swimming tadpoles every 30 seconds. We then calculated the difference in the average proportion of swimming tadpoles between the two stages. Finally, we tested for differences in this variable between treatments (fish presence or absence) and fish species (brown trout or brook trout) with analysis of variance (ANOVA) with post hoc Fisher's exact tests.

## Results

### Habitat Selection

Iberian frog tadpoles and brown and brook trout preferred deeper habitats with slower water current and used

pools. Secondarily, they inhabited runs more often than expected but avoided riffles and cascades ($\chi^2 = 560.5$, df $= 4$, $p < 0.0001$; $\chi^2 = 49.7$, df $= 3$, $p < 0.0001$ and $\chi^2 = 9.7$, df $= 4$, $p < 0.05$; respectively). The very slow and often debris- or vegetation-clogged backwaters were also completely avoided. Both salmonids occupied riffles to some extent, whereas only one tadpole was observed in a riffle (Fig. 2). Therefore, there seemed to be substantial opportunity for spatial overlap and hence direct interaction between tadpoles and salmonids.
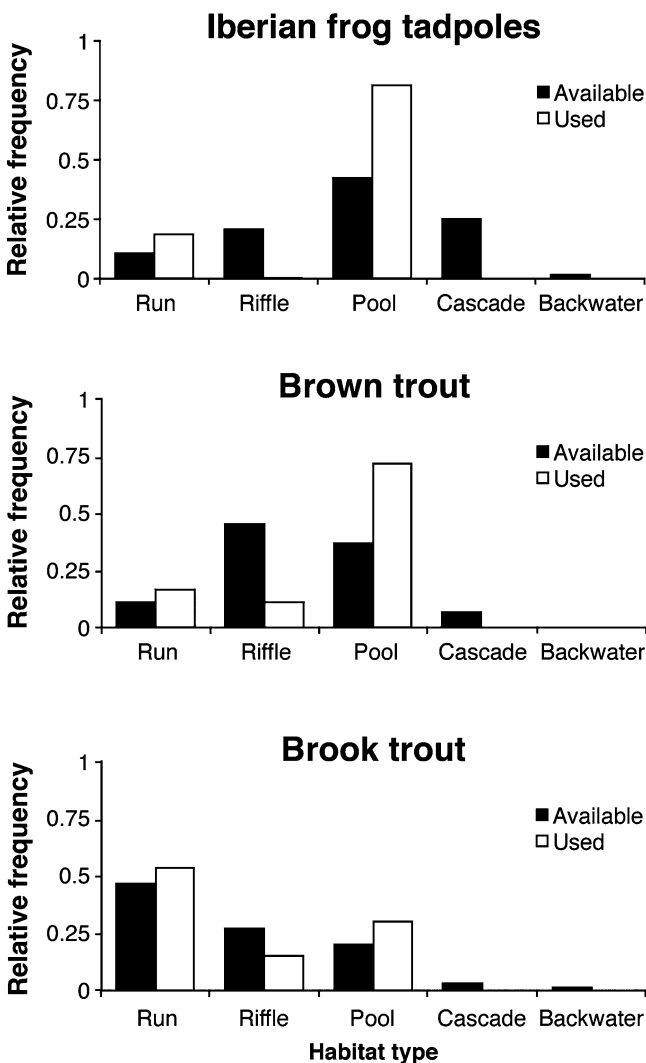


*Figure 2. Habitat selection (expressed as proportion of availability and use of every type of channel habitat unit) by brook trout (n = 72) in the Peñalara stream and Iberian frog tadpoles (n = 631) and brown trout (n = 38) in the Pepe Hernando stream. Habitat availability data for brown trout in the Pepe Hernando are from only those reaches accessible to the species.*

## Spatial Distributions

Tadpoles, adults, and juveniles of Iberian frog were present in the HPH stream, whereas visual censuses and electrofishing failed to detect them in the HP stream. In the HPH stream, tadpoles were common in pools and, to a lesser extent, in slow runs above the waterfall that marked the upstream limit of brown trout presence save for a central section (reaches 8–12) of very high gradient. In that area, slow-water habitats were scarce and small. Above reach 12 and up to the stream sources, tadpoles were again relatively abundant. Within the stream section inhabited by brown trout, some tadpoles were observed in a few pools that did not contain trout and were connected to fish-free areas.

We captured 113 brook trout in the HP stream. The species occurred from the gauging station to the outlet of the Laguna Grande and maintained an average density of 0.1 fish/m² in the nine study reaches. At least three age classes were present (0+ to 2+), and the relative abundance of age 0 individuals (32% of the total) and the frequent presence of newly emerged brook trout in May 2003 suggested that the species reproduces successfully in the stream. We also captured 23 brown trout in the Peñalara stream from several age classes, including 0+, but the species was restricted to the lower part of the study area, below 1953 m asl.

In the HPH stream, brown trout were relatively abundant (average density 0.61 fish/m²) from the point where the surveys began to a waterfall at 1881 m asl. We found individuals of ages 0+ (24% of the total) to 3+, indicative of continued successful reproduction.

## Determinants of Iberian Frog Tadpole Occurrence

The presence of introduced trout and habitat type were the major determinants of tadpole occurrence in a certain habitat unit. The terms for habitat type and salmonid presence (1–3) were always retained in the minimum models, and their removal produced a significant lack of fit between the predicted and observed tables (Table 1). No interaction terms were retained in the minimum models (Table 1); thus, the effects of both habitat type and salmonid presence were not different between streams and the effects of trout presence did not vary among habitat types.

All types of salmonid presence had a strong negative effect on the likelihood of tadpoles occurring in a certain habitat unit. Tadpoles were never present in a unit occupied by trout or not connected to an upstream fish-free area, and only in a few cases did tadpoles occur within a reach containing salmonids. The influence of habitat type highlighted by the analyses agreed well with the strong habitat preferences of Iberian frog tadpoles presented above.

**Table 1. Determinants of the presence of Iberian frog tadpoles in channel habitat units according to the results of log-linear analyses of tadpole and introduced salmonid occurrence in the Peñalara and Pepe Hernando streams in Peñalara Natural Park.**

| Model[a] | Factor | M.L.$\chi^{2b}$ | df | p |
|---|---|---|---|---|
| Same unit | whole model | 12.47 | 12 | 0.409018 |
| | salmonid × tadpole | 43.85 | 13 | 0.000033 |
| | habitat × tadpole | 56.06 | 14 | 0.000001 |
| | stream × tadpole | 79.34 | 14 | <0.000001 |
| | habitat × salmonid | 29.42 | 14 | 0.009157 |
| Same reach | whole model | 15.88 | 13 | 0.255779 |
| | salmonid × tadpole | 33.56 | 14 | 0.002389 |
| | habitat × tadpole | 64.58 | 17 | <0.000001 |
| | stream × tadpole | 27.16 | 14 | 0.018326 |
| | stream × salmonid | 102.41 | 14 | <0.000001 |
| Upstream | whole model | 12.69 | 14 | 0.550670 |
| | salmonid × tadpole | 85.44 | 15 | <0.000001 |
| | habitat × tadpole | 61.39 | 18 | 0.000001 |
| | stream × salmonid | 169.66 | 16 | <0.000001 |

[a]*Models differ in the way in which salmonid presence was measured.*
[b]*The value of the maximum likelihood chi-square statistic obtained when we compared the observed and predicted tables for the whole model and the observed and predicted table obtained by excluding the corresponding factor from the minimum model for individual terms.*

The stream in which the habitat unit was located also influenced tadpole occurrence (Table 1). This effect stemmed from differences in the spatial distribution of salmonids rather than from salmonid species. The stream factor became nonsignificant and disappeared from the final model when differences between streams in the abundance of upstream fish-free areas were accounted for (Table 1). The stream term still appeared in the minimum models when salmonid presence was entered as occurrence within the same reach or within the same unit (Table 1).

Because most tadpoles (81%) were found in pools, a relatively high proportion of empty cells in the multiway contingency tables may have influenced the results presented above. Therefore, we repeated log-linear analyses for tadpole presence in pools only, excluding data from riffles and runs. As before, salmonid presence was always retained in the minimum models, and the effect of stream on tadpole occurrence was excluded when salmonid presence was entered as presence upstream.

### Antipredator Behavior of Iberian Frog Tadpoles

Iberian frog tadpoles markedly decreased their activity when exposed to chemicals from both trout species ($F_{3,44} = 10.5$, $p < 0.0001$; Fisher's test, brown trout vs. control: $p < 0.0001$; brook trout vs. control: $p = 0.0174$; Fig. 3), and this reduction was greater for brown than brook trout ($p = 0.0183$). In contrast, activity in the two groups that did not receive fish water in the second phase changed

little between stages and showed no significant difference in this variation ($p = 0.7830$, Fig. 3).

## Discussion

Our results suggest that the impact of introduced fishes on local stream amphibian populations may be highly dependent on landscape features and invasion geography. They also support previous suggestions of a negative interaction between Iberian frogs and introduced fishes and help explain the absence of frogs from apparently favorable sites (Bosch & Martínez-Solano 2003).

Landscape configuration seemed a major cause of the different impact of fish invasion between streams. In the HPH stream, residual frog populations have persisted in suboptimal breeding sites inaccessible to fish, whereas the HP stream lacked frogs completely. Also, physical barriers to upstream movement have limited salmonid spread in the HPH stream but not in the HP stream. This differential effect of barriers appeared dependent on invasion geography. In the HP stream, brook trout dispersed downstream from a headwater lake. In contrast, in the HPH stream, brown trout spread upstream.

The influence of geography on the outcome and impact of invasions should be relevant in lotic systems (With 2002). First, the rates and range of upstream and downstream fish dispersal usually differ (Adams et al. 2001). Hence, the location of the introduction can substantially influence its subsequent impact. Second, because
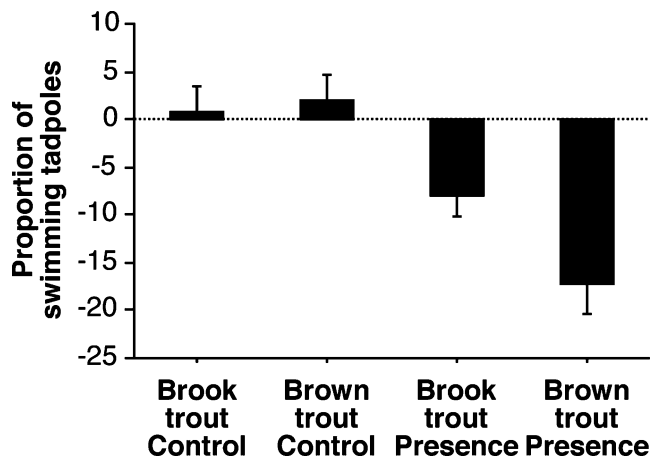


*Figure 3. Difference in the average proportion of swimming tadpoles of Iberian frog (mean ± SE) between the two stages of the experiment on antipredator behavior in tadpoles in response to chemical cues. During the first stage the four groups received water with no fish, whereas in the second stage the two control groups still received water with no fish and the other two groups received water with fish.*

of downstream transmission of chemical cues, fish may elicit avoidance responses in amphibians in areas they do not physically occupy (Binckley & Resetarits 2003). This might result in extensive loss of breeding habitat and dispersal pathways (Bradford et al. 1993). Such nonlethal effects of introduced predators are well documented for other aquatic taxa (McIntosh & Townsend 1996).

Third, stream habitat structure varies geographically and may determine the outcome of fish invasion through its influence on the spatial overlap between introduced fishes and native amphibians (Scopettone 1993; Rincón et al. 2003). Iberian frog tadpoles and salmonids displayed a completely allotopic spatial distribution at the habitat-unit scale in the park, whereas Iberian frog tadpoles have been observed in runs occupied by fishes in low-gradient rivers (J.B., personal observation).

This contrasting pattern may be a consequence of the habitat requirements of fishes and anuran larvae. Tadpoles require slow water because of their limited sustained swimming ability (Liu et al. 1997; Huey 1980). Pilot experiments indicate that about 50% of Iberian frog tadpoles are washed out by currents about 14–15 cm/second (P.A.R., unpublished data). Salmonids, in turn, need deeper water (Heggenes 1988). In high-gradient streams organized in pool-riffle sequences (such as ours), slower water and greater depth are usually linked. In contrast, in the runs that dominate low-gradient streams, slow water is typically found in shallower marginal areas, whereas central, deeper zones exhibit faster current (e.g., Rincón & Lobón-Cerviá 1993). By using these shallow, slow zones within runs, anuran larvae achieve a degree of segregation relative to predatory fishes that in high-gradient streams seems possible only between habitat units (Olson 1989; Holomuzki 1995).

Presumably, under such conditions of partial spatial segregation, the antipredator behavior highlighted by experimental results may enhance the survival of Iberian frog tadpoles and contribute to their coexistence with predatory fishes. Our results, however, suggest that antipredator behavioral responses are inefficient (sensu Sih et al. 2000) in our study area and, possibly, other high-gradient streams.

Our findings support the view that the persistence of Iberian frogs in Central Spain is seriously compromised. These populations are genetically impoverished and show very low genetic variability across six microsatellite loci and high inbreeding coefficients indicative of reduced effective population sizes and high fragmentation (I.M.-S., unpublished data). Habitat loss due to urbanization affects populations at lower elevations (600–1000 m). At higher elevations, the situation is somehow better and some populations still inhabit well-preserved habitats that in some cases are included in protected areas such as Gredos Regional Park or Peñalara Natural Park. Recent surveys, however, show that other high-elevation populations have disappeared from apparently suitable areas (SCV 2001; Martínez-Solano & González-Fernández 2003). Our results indicate that uncontrolled introduction of fishes can have a profound impact on Iberian frog populations in high-elevation streams and may cause some of those local extinctions.

The urgent adoption of measures favoring increases in size of breeding populations and in gene flow among them should be a priority to guarantee the survival of this Iberian endemism at a regional level. These measures should incorporate adequate management of introduced fishes including their eradication from key areas. Amphibian populations have responded to such restoration efforts and recovered pre-fish stocking levels relatively quickly, provided that nearby sources of colonists for the restored sites remain (Knapp et al. 2001b; Vredenburg 2004). Additionally, such actions should also benefit other amphibians that have suffered recent declines in our study area, such as the common fire salamander (Bosch & Martínez-Solano 2005) and the common midwife toad (*Alytes obstetricans*) (Bosch et al. 2001).

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