Effect of salmonid introduction and other environmental characteristics on amphibian distribution and abundance in mountain lakes of northern Spain

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Abstract
Amphibians are currently experiencing a severe worldwide decline. Several factors, such as habitat alteration, climate change, emerging diseases or the introduction of exotic species, have been signalled as being responsible for the reduction of amphibian populations. Among these, the introduction of fish predators has been repeatedly indicated as a factor affecting the distribution of many species. The present study was developed to examine the effect of fish presence and other environmental factors on the distribution and abundance of amphibian species in mountain lakes of the Cantabrian Range in northern Spain. We found no effect of salmonid presence on the distribution and abundance of two widespread anuran species Bufo bufo and Alytes obstetricans, whereas Rana temporaria showed a non-significant tendency to be absent from salmonid-occupied lakes. However, the presence of introduced salmonids was the main negative factor explaining the distribution of the newt species Triturus helveticus, Triturus alpestris and Triturus marmoratus. The effect on these species is likely to be due to increased larval mortality, as adult and egg predation by fish, or oviposition avoidance by female newts has rarely been recorded. Fish removal and the creation of alternative breeding habitats for amphibians are proposed as conservation measures to recover amphibian populations in the vicinity of fish-stocked lakes.

Introduction
In recent years, amphibians have been the focus of important conservation efforts owing to severe population reductions and even extinction in many species (Houlahan et al., 2000; Stuart et al., 2004; Beebee & Griffiths, 2005). The causes of these declines are complex and sometimes difficult to identify as they are reported to occur in very different parts of the world, including pristine habitats (Alford & Richards, 1999). Habitat destruction, water pollution, climate change, emerging diseases or introduced species have been identified as factors most often affecting amphibian populations (Collins & Storfer, 2003; Beebee & Griffiths, 2005). Interactions between these factors may create new environmental pressures that lead to further severe reductions of amphibian populations (Kiesecker, Blaustein & Belden, 2001). The study of the environmental variables that influence the distribution of amphibian species in the wild can contribute to a better understanding of the impact that factors of anthropogenic origin can have on amphibian populations, allowing us to distinguish between natural and non-natural phenomena influencing amphibian distribution.

Amphibian species are exposed to a great variety of breeding habitats, ranging from permanent lakes to temporary ponds, where predation and desiccation are the main mortality factors (Wellborn, Skelly & Werner, 1996; Leips, McManus & Travis, 2000; Babbitt, Baber & Tarr, 2003). Pond desiccation is highly unpredictable and causes near-total breeding failure during periods of drought. Predation pressure is more consistent between years and so has a relatively constant impact on amphibian assemblages (Wilbur, 1980; Alford, 1999). Among the different habitats that amphibians occupy for breeding, mountain lakes represent permanent localities that are often isolated from neighbouring river basins and that have limited predator communities, commonly lacking top predators such as fish (e.g. Bahls, 1992 for western North America). Amphibians that are able to occupy a broad range of habitats (see Van Buskirk, 2003) can use lakes as refuges during dry seasons; the lakes provide a source of individuals for immigration to neighbouring areas after a period of drought. Large permanent water bodies can therefore be considered as hot spots for the conservation of amphibian assemblages in mountain areas.

Mountain lakes present considerable variation in characteristics such as water volume, shore structure, vegetation cover, refuge availability and type of substrate, which can contribute towards explaining the patterns of variation in amphibian abundance and distribution, and the great
variation in species composition among lakes. One of the main factors affecting amphibian assemblages in mountain lakes is the introduction of exotic predators (Collins & Storfer, 2003). In particular, the introduction of predatory fish as a plague control agent or for recreational purposes has been reported to cause severe declines in many natural amphibian populations (Bradford, 1989; Brønmark & Edenhann, 1994; Braña, Frechilla & Orizaola, 1996; Hecnar & M’Closkey, 1997; Knapp & Matthews, 2000; Denoël, Dzukic & Kalezic, 2005). Reduction of amphibian presence in an area occupied by fish predators can be because of either predator avoidance behaviours (mainly selective oviposition avoidance; see Resetarits & Wilbur, 1989; Hopey & Petranka, 1994; Binckley & Resetarits, 2002) or direct consumption (Gamradt & Kats, 1996; Laurila & Aho, 1997; Tyler et al., 1998; Binckley & Resetarits, 2002). The impact of fish predators may be increased by the fact that distances among mountain lakes are frequently outside amphibian dispersal capacities (e.g. Jehle & Arntzen, 2000; Holenweg, 2001; Schabetsberger et al., 2004), which can prevent recolonization displacements (see e.g. Bradford, Tabatabai & Graber, 1993).

This study evaluates the environmental correlates of amphibian distribution and abundance in lakes of the Cantabrian Mountains (northern Spain), and the effects that introduced salmonid fish have on amphibian species. Some lakes of the area are occupied by several salmonid and cyprinid species (Braña et al., 1996). We have focused on salmonid fish because they are one of the vertebrate groups exerting a stronger predation pressure in permanent lakes (e.g. Bradford, 1989; Resetarits, 1997; Tyler et al., 1998; Gillespie, 2001; Matthews et al., 2001; Piliod & Peterson, 2001; Knapp, 2005), whereas most cyprinids are partially herbivorous or feed on small aquatic invertebrates and seem to have a less important predatory capacity (Hecnar & M’Closkey, 1997). The introduction of salmonids into ‘fishless’ lakes in the study area has previously been recorded to have affected amphibians, as reported by Terrero (1951). He described intense consumption of newts by introduced trout, which was associated with a rapid decline of these amphibians. A preliminary study carried out in a few lakes in the area also reported a reduction of amphibian distribution associated with the presence of introduced predatory fish (Braña et al., 1996). In this study, we considered both anuran and urodele species and used the six most abundant species (common toad Bufo bufo, common frog Rana temporaria, midwife toad Alytes obstetricans, marbled newt Triturus marmoratus, alpine newt Triturus alpestris and palmate newt Triturus helveticus) to further analyse the influence of habitat characteristics on amphibian presence and abundance.

Materials and methods

Habitat characterization

The study area is located in the central sector of the Cantabrian Mountains, which runs along the north coast of Spain for c. 480 km, rising to maximum altitudes of 2600 m. Field studies were conducted in a 5900 km² area, where 30 lentic permanent water bodies were selected to provide a wide sample of lakes and ponds varying in environmental characteristics (Supplementary Material Appendix S1). The study period extended from late May to mid-July in 1998 and covered the larval developmental period of all the amphibian species inhabiting these mountain areas (G. Orizaola & F. Braña, pers. data). The water bodies considered in this study were naturally unoccupied by fish (except for two lakes, Lillo and Embalse del Valle, for which a natural origin of Salmo trutta is possible). However, some of the lakes have been historically stocked with fish to provide recreational angling. Several field and laboratory measures were recorded to describe the physical, chemical and biological characteristics of each lake. Lake elevation (ALT) was obtained from 1:25 000 topographic maps (Instituto Geográfico Nacional), and perimeter (PER) and maximum depth (DEP) were obtained from Fernández & García (1987). Shore slope (SLO), in degrees, was estimated considering the distance from the shore to the point at 0.50 m in depth. Water temperature (TEM) was measured at 0.50 m depth using a digital thermometer. Acidity (pH) was measured by reflectometric methods (Reflectoquant®), Merck, Darmstadt, Germany, and RQflex 2 scanner. The concentration of suspended matter (SMA) was determined in 11 samples, filtered using 47-mm-diameter Whatman (Maidstone, England, UK) GF/A filters, which were subsequently dried at 50 °C for 24 h to obtain dry weight to the nearest 0.0001 g. Per cent surface occupied by aquatic vegetation growing in the lakeshore (VEG) was visually estimated by two observers examining a strip of 5 m of the lake shore; the mean value of both estimates was used for analyses. Plankton abundance (PLA), expressed as individuals/litre, was obtained by filtering 11 samples through a 53-µm mesh. Samples were preserved in formaldehyde (10%) until analysis in the laboratory. All the measures and samples were taken in five evenly distributed points of each lake and averaged for analysis. Lake isolation (ISO) was estimated by the straight-line distance to the nearest permanent lake on 1:25 000 maps.

Fish and amphibian surveys

Data on fish presence/absence and species composition were obtained by means of visual surveys, communication with local fisheries authorities and from previous surveys involving electrofishing and gillnet catching (Braña et al., 1996). Amphibian presence was determined by visual detection of eggs, adults or larvae, by removal of rocks on the shore and adjacent terrestrial habitats and by dip-netting surveys on the entire shoreline. As amphibian breeding activity depends on temperature and lake freezing and starts early in lower, warmer areas (e.g. Laurila, Kartunen & Merilä, 2002; Laugen, Laurila & Merilä, 2003), the surveys were scheduled to follow the elevation gradient, from lowest to highest places. This procedure should be considered reliable, as surveys conducted in the following years did not detect any...
new amphibian species in the lakes. Besides, larvae of the studied species remain in the water for several weeks or even months, so the risk of having false-negative records should be minimum. Amphibian abundance was estimated as the average number of individuals (adult and larvae) hand netted by two people during 5-min exhaustive sampling in five points of the lake (the same points where habitat parameters were recorded). The same two people surveyed all the lakes. All the surveys were conducted in a short period of time (6 weeks), at the same time of the day and always following the elevation gradient described previously. Adult anurans were not included in abundance analyses as they were captured only in small numbers.

Statistical analyses

Stepwise logistic regression analyses were used to evaluate the effect of habitat characteristics on lake occupation by the six most frequent amphibian species. In order to fit the standard procedures of logistic regression analyses (see Harrell, 2001), we first performed a principal component analysis (PCA) to reduce the number of variables to be included in the analysis. By means of PCA, we transformed nine lake descriptors (ALT, PER, DEP, SLO, pH, TEM, SMA, VEG and PLA) into compound variables, using the scores from the significant PC axes for each lake in logistic regression analyses. Salmonid fish presence/absence and lake isolation were also included in the logistic regression analyses as independent variables. The effect of environmental characteristics on salmonid fish occurrence was analysed using the same procedures, using lake characteristics (PC axes) and ISO as factors. The effect of salmonid predators on amphibian abundance was investigated by means of Mann–Whitney tests. The level of significance was set at $\alpha = 0.05$ for all tests.

Results

Five anuran and five urodele species were detected in the surveys, the common toad *B. bufo*, common frog *R. temporaria* and midwife frog *A. obstetricans* being the most frequent anurans (Fig. 1a), and palmate newt *T. helveticus*, alpine newt *T. alpestris* and marbled newt *T. marmoratus* being the most frequent urodèles (Fig. 1b). All these species are commonly found in northern Spain, occupying a broad range of habitats from temporary ponds to larger lakes (Barbadillo et al., 1999; Van Buskirk, 2003). Seven fish species were detected in the surveyed lakes (salmonids: brown trout *S. trutta*, rainbow trout *Oncorhynchus mykiss* and brook trout *Salvelinus fontinalis*; non-salmonids: minnow *Phoxinus phoxinus*, tench *Tinca tinca*, carp *Cyprinus carpio* and Iberian roach *Chondrostoma arcessii*; Supplementary Material Appendix S1). The results of the PCA conducted on group lake descriptors showed a first axis (PC1) that explained 28% of the total variance of lake characteristics and a second one (PC2) that explained 19% (Table 1). Four variables loaded significantly in the first axis: PER, DEP and SLO with a negative sign and PLA with a positive sign. In the second axis, only pH loaded significantly, with a negative sign (Table 1).

Eight out of 30 lakes, mainly comparatively deep, with a large perimeter, steep shore slope and low plankton abundance, contained predatory fish (Table 2, Supplementary Material Appendix S1). Analyses on habitat variables showed that *B. bufo* distribution was influenced by lake characteristics (PC1, Table 2), being more frequently found in deep lakes with a large perimeter and steep slope, as well
as in areas with low plankton density (Supplementary Material Appendix S1). In the case of *A. obstetricans*, the distribution was mainly influenced by lake isolation (Table 2), being preferentially found in lakes far apart from other permanent lakes (Supplementary Material Appendix S1). The distribution of *R. temporaria* was not significantly affected by any of the considered factors (Table 2), despite showing a tendency to be absent from salmonid-occupied lakes (only two salmonid lakes had *R. temporaria*, Fig. 1a).

The presence of salmonid fish did not affect the distribution (Table 2) and abundance of *B. bufo*, *A. obstetricans* and *R. temporaria* ($U_{12,8} = 69.5, P = 0.365$; $U_{22,8} = 79.5, P = 0.665$; and $U_{22,8} = 59.5, P = 0.146$, respectively; Fig. 2). The presence of salmonid fish in mountain lakes was strongly associated with the absence of urodeles (Table 2, Fig. 1b). Considering altogether the three most frequent species (*T. helveticus*, *T. alpestris* and *T. marmoratus*), both larvae and adult abundance were negatively affected by salmonid presence ($U_{22,8} = 32, P = 0.004$ for larvae and $U_{22,8} = 39.5, P = 0.019$ for adults, Fig. 2). The distribution of *T. helveticus* and *T. marmoratus*, and to a lesser extent *T. alpestris*, was also correlated with lake characteristics (PC1, Table 2), these species being preferentially found in lakes with low perimeter, depth and slope and high plankton abundance (Supplementary Material Appendix S1).

### Discussion

As permanent habitats, mountain lakes provide a crucial environment for amphibian reproduction, especially during dry summers when reproduction can fail in adjacent temporary habitats because of desiccation (Pilliod, 2001). Lakes stocked with salmonids may act as sink habitats for amphibians, as predation can reduce the number of individuals that reach metamorphosis and can consequently produce recruitment failure (Pilliod & Peterson, 2001). The effect of fish introductions could be increased as a consequence of the high fidelity in oviposition site selection shown by some amphibian species (Berven & Grudzien, 1990; Laurila & Aho, 1997). The results of the present study indicate that the presence of salmonid fish is the main factor explaining the species richness and the composition of amphibian communities in mountain lakes of northern Spain. Salmonids were found in comparatively deep lakes with large perimeters and high shore slopes, characteristics associated with the large water volume necessary to maintain physiological and feeding requirements of large fish. Amphibian diversity and abundance were considerably reduced in this type of lake. The results obtained in this work are similar to those of previous studies, reaffirming the crucial role that fish introductions could exert at the local level on native amphibian fauna (Bradford, 1989; Bradford *et al*., 1993; Brönmark & Edénhamn, 1994; Hecnar & M'Closkey, 1997; Tyler *et al*., 1998; Knapp & Matthews, 2000; Gillespie, 2001; Pilliod & Peterson, 2001; Hamer, Lane & Mahony, 2002).

The impact of salmonid fish was, however, notably different depending on the amphibian species. *Bufo bufo* inhabits most lakes stocked with salmonids, where high larval densities are present. This could be related to the presence of highly toxic compounds (bufotoxins) in the skin of adults and larvae of this species, effective against vertebrate predators (Denton & Beebee, 1991; Crossland

### Table 2 Results of logistic regression analyses for the presence of salmonids and each amphibian species

<table>
<thead>
<tr>
<th></th>
<th>Salmonids</th>
<th>PC1</th>
<th>PC2</th>
<th>ISO</th>
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<tr>
<td></td>
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<td>$P$</td>
<td>Coefficient</td>
<td>$P$</td>
</tr>
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<td>–</td>
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</tr>
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<td>0.099</td>
<td>NE</td>
<td>1.714</td>
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<tr>
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<td>7.130</td>
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<tr>
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<td>0.024</td>
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<td>2.294</td>
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<tr>
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<td>0.046</td>
<td>–</td>
<td>6.879</td>
</tr>
</tbody>
</table>

Abundance of amphibians (mean number ± 1 se) in lakes with or without fish predators. Anuran abundance represents only larval abundance.

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**Figure 2** Abundance of amphibians (mean number ± 1 se) in lakes with or without fish predators. Anuran abundance represents only larval abundance.
& Alford, 1998; Benard & Fordyce, 2003). However, the effects of salmonid presence on *A. obstetricans* and *R. temporaria* seem far more complex. *Alytes obstetricans* did not appear to be strongly affected by the presence of salmonids (it was detected in about half of the lakes occupied by salmonids), although larvae density was lower in lakes occupied by these predators. The mechanisms that allow *A. obstetricans* to coexist with fish predators are unclear; nevertheless, this species shows several life-history characteristics that could contribute to a tolerance to salmonid fish. For example, larvae of this species hatch at an advanced developmental stage and with a large size (Máñquez, 1996), characteristics associated with high escape capabilities and low larvae mortality because of predation (Cronin & Travis, 1986; Richards & Bull, 1990). *Alytes obstetricans* larvae were also located hiding in the vegetation more frequently than other amphibian species, which could increase their survival. The difference in our results from those reported for Central Spain, where *A. obstetricans* apparently fails to reproduce in favourable areas when these are occupied by fish predators (*S. fontinalis*; Martínez-Solano, Barbadillo & Lapeña, 2003), suggests that differences in habitat structure could be responsible for a differential impact of fish predators. In the case of *R. temporaria*, a lower survival was reported for larvae exposed to predatory fish (Laurila & Aho, 1997; Laurila, 1998), which could be associated with the disappearance of frogs from lakes stocked with predatory fish. This agrees with the findings of other studies reporting a negative spatial correlation between predatory fish and different *Rana* species (Bradford, 1989; Bradford et al., 1993; Knapp & Matthews, 2000; Knapp, Matthews & Sarnelle, 2001; Pilliod & Peterson, 2001). In our study, *R. temporaria* showed a nonsignificant tendency to be affected by the presence of salmonid fish in the surveyed lakes, being absent from the majority of lakes stocked with predatory salmonid fish.

Urodeles seemed to be more severely affected by the presence of salmonid fish than anurans, as introduced salmonids significantly affected the distribution and abundance of all the *Triturus* species considered in the analyses. Newts were restricted almost exclusively to salmonid-free lakes and newt larva was not detected in salmonid-occupied lakes. Especially remarkable is the case of *T. marmoratus*, which was not found in lakes with salmonids, supporting the results found in Switzerland for *T. cristatus*, a closely related species (Van Buskirk, 2005). In this sense, a report at the time of first salmonid introductions signalled a high consumption of adult newts by introduced trout on several of the studied lakes (Terrero, 1951). This report confirms the previous suitability of these lakes for newt reproduction, as well as the impact that salmonid introductions have had on this group, which is currently almost absent from lakes containing introduced salmonids. Similar negative effects of fish presence have been reported for *Triturus* and other urodele species worldwide (Petranka, 1983; Beebee, 1985; Semlitsch, 1988; Ildos & Ancona, 1994; Aronsson & Stenson, 1995; Gamradt & Kats, 1996; Heenar & M’Closkey, 1997; Tyler et al., 1998; Smith et al., 1999; Barr & Babbitt, 2002). In addition to direct predation on any life-history stage, the current distribution of amphibian species could also be explained by the avoidance of unsuitable (because of high predation risk) oviposition places. Avoidance of fish-occupied habitats for oviposition has been reported for several urodele species (Kats & Sih, 1992; Gamradt & Kats, 1996). However, recent studies on newt oviposition behaviour have shown that *Triturus* species do not alter their oviposition site selection in response to fish presence (Orizaola & Braña, 2003, except for *T. marmoratus*), and neither does *R. temporaria* (Laurila & Aho, 1997). Otherwise, larvae of many amphibian species are usual prey of fish (e.g. Kats, Petranka & Sih, 1988; Denton & Beebee, 1991), whereas predation on egg or adult stages of amphibians has been reported less frequently (Wilbur, 1980; Kats & Ferrer, 2003), which could indicate that the main impact of fish predation should operate at the larval stage.

Fish introductions, and more widely the introduction of exotic competitors and predators, have been reported as one of the main causes responsible for the observed global amphibian decline (Bradford, 1989; Wake, 1991; Brönmark & Edénhamn, 1994; Braña et al., 1996; Knapp & Matthews, 2000; Gillespie, 2001; Hamer et al., 2002; Kats & Ferrer, 2003). The results of the present study show that salmonid fish presence differentially affects various amphibian species, altering the composition of the amphibian community and reducing its richness in mountain lakes of Northern Spain. In this area, lakes without salmonids hold comparatively diverse amphibian communities with up to six species frequently coexisting, whereas in lakes stocked with predatory salmonids *B. bufo* and, to a lesser extent, *A. obstetricans* are almost the only species present, *Triturus* sp. and *Rana* sp. being virtually absent. In the light of these results, conservation measures are necessary for the maintenance of amphibian populations in these lakes. Fish eradication would obviously be the most effective, but would probably be only feasible in small lakes isolated from any fish population source (Knapp & Matthews, 1998). For this purpose, application of piscicides (e.g. rotenone, antimycin) is unsuitable because of their great impact on local native fauna (Aronsson & Stenson, 1995; Knapp & Matthews, 1998). Considering the high fidelity of amphibian species to their breeding localities, another way to recover natural amphibian populations could be the creation of alternative permanent habitats for amphibian reproduction in the proximity of fish-occupied lakes, which could be used by a great variety of species without the risk of being preyed by fish. These methods would likely be effective given the apparent great resistance and resilience of amphibian fauna to fish introductions, as proven by the recoveries of *R. muscosa*, *Ambystoma macrodactylum* and *Ambystoma gracile* populations soon after trout eradication in mountain lakes (Funk & Dunlap, 1999; Knapp et al., 2001; Hoffman, Larson & Samora, 2004; Vredenburg, 2004). Preventing new introductions and immediate eradication of recently introduced fish should be the most effective management measures to assure the preservation of amphibian populations in lakes naturally unoccupied by predatory fish.
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References


**Supplementary material**

The following material is available for this article online:

Appendix S1 Physical and biological characteristics of the surveyed lakes.

This material is available as part of the online article from http://www.blackwell-synergy.com