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EFFECT OF INTRODUCED FISH ON AMPHIBIAN SPECIES RICHNESS AND DENSITIES AT A MONTANE ASSEMBLAGE IN THE SIERRA DE NEILA, SPAIN

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We examined the effect of fish stocking practices on the populations of seven amphibian species in a montane area in the Sierra de Neila (north-central Spain). We compared values for amphibian species richness and amphibian densities between ponds where fish have been introduced and ponds where fish are absent. Our results show that (1) amphibian species richness was significantly lower in ponds where fish have been introduced; (2) we found contrasting patterns of pond occupancy by the different amphibian species: on the one hand, two out of seven species (*Bufo bufo* and *Rana perezi*) coexist with fish, whereas the other five species breed exclusively in ponds where fish are absent; (3) based on comparisons of presence/ absence data for species present in the area in 1981, 1991 and 2001, we concluded that two amphibian species have suffered severe declines in the last decades. Presently, *Alytes obstetricans* is almost exclusively confined to a few fishless streams, whereas *Salamandra salamandra* appears to have been completely extirpated from the whole area. This local decline of *S. salamandra* seems to be general for the whole region of the Sistema Ibérico (North-central Spain). The possible role of fish stocking practices in these declines is discussed.

Key words: amphibian decline, conservation, exotic fish, Spain

INTRODUCTION

Multiple causes have been proposed to explain documented patterns of amphibian declines throughout the world (reviewed, for example, in Alford & Richards, 1999). Among these factors, much interest has focused on analysing the effect of exotic fish on amphibian populations. Several cases of exclusion – and even extinction (Bradford, 1991) – of amphibian species from ponds after the introduction of non-native fish species have been reported in the literature (Aronsson & Stenson, 1995; Drost & Fellers, 1996; Fisher & Shaffer, 1996; Gamradt & Kats, 1996; Galán, 1997). Allotopic patterns of distribution of introduced fish and amphibians, as well as reduced values of amphibian species diversity in ponds stocked with fish, are both well documented at local (Woodward, 1983; Bradford, 1989; Brönmark & Edenham, 1994; Braña *et al.*, 1996) and regional scales (Hecnar & M'Closkey, 1997).

We have explored the effect of fish stocking practices during recent decades on amphibian species richness and densities in a montane protected area in the Sierra de Neila, in Burgos (north-central Spain). The zone is appropriate for the study of the effect of fish on amphibian populations because of the coexistence of ponds where several species of fish have been introduced and ponds where fish are absent. There is evidence that the populations of some of the amphibian species present at the area (for example, *Salamandra salamandra*) have strongly declined over recent decades, a trend that might also be occurring at a higher (regional) scale (Barbadillo & García-París, 1991; Lizana & Barbadillo, 1997; Barbadillo & Sánchez-Herráiz, 1998; Barbadillo, unpublished data). The availability of amphibian presence/absence data for several water bodies in the study area in the years 1981 and 1991 led us to conduct a more comprehensive field study in 2001. Our objectives were (1) to document possible changes in amphibian species composition in recent years by analysis of data collected over the last two decades; and (2) to explore the effects of introduced fish on the relative abundance of the amphibian species breeding in the study area.

MATERIALS AND METHODS

The study area is located in the Sierra de Neila and included within the limits of the protected area of the "Espacio Natural de la Sierra de la Demanda" (81270 ha), in SE Burgos (Sistema Ibérico, north-central Spain). The area consists mainly of alpine grasslands surrounded by *Pinus sylvestris* forests. The ponds studied in 2001 are of glacial origin and include Laguna Negra, Laguna de la Cascada, Laguna de los Patos, Laguna Brava, Laguna de las Pardillas, Laguna Haedillo and Laguna Oruga (Fig. 1). We also sampled two temporary ponds located in the vicinities of the Laguna Cascada. At Laguna Haedillo we only recorded presence or absence of amphibian species because of problems in carrying out nocturnal transects posed by difficult access in relation to the other ponds. In addition, other sites where historical records of *S. salamandra* were available (Fuente Sanza and Fuente Rialares) were sampled in order to determine their presence or absence in 2001.

According to data from the "Consejería de Medio Ambiente" of the "Junta de Castilla y León", between 1976 and 1995 three of the studied ponds (Negra, Patos

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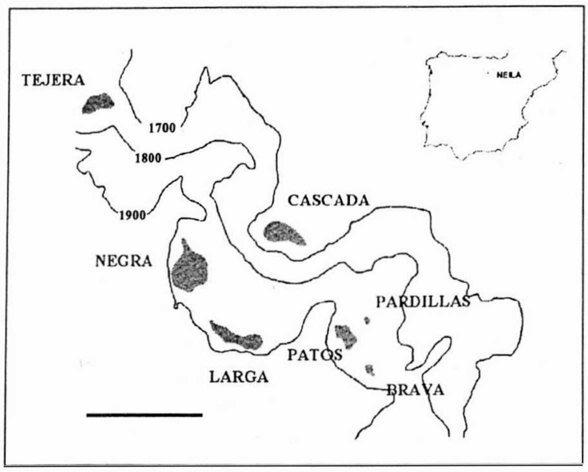

FIG 1. Study area. Location of Neila in the Iberian Peninsula (upper-right corner) and ponds that were sampled in 2001 (except for Laguna Oruga, which is located 8 km east of Laguna Patos; and Laguna Haedillo, located 4 km north-west of Laguna Negra). Scale bar equals 1 km.

and Cascada) were used as sport fishing reserves (no data were available for Laguna Brava). These ponds were stocked with brown trout (*Salmo trutta*), rainbow trout (*Oncorhynchus mykiss*) or both species. During those years, 69 000 kg (4000 kg/yr on average) of these fish species were released into the ponds. Apart from these official stocking practices, uncontrolled introductions of cyprinid species have also taken place: *Cyprinus carpio, Gobio gobio*, *Phoxinus phoxinus*, *Carassius auratus* and *Chondrostoma arcasii* have been identified in one or several of the ponds (C. Temiño, Junta de Castilla y León, personal communication; authors, personal observations).

Eight amphibian species have been reported to breed in the area: *Salamandra salamandra, Triturus helveticus*, *T. marmoratus*, *Alytes obstetricans*, *Bufo bufo*, *B. calamita*, *Hyla arborea*, and *Rana perezi* (Barbadillo & Sánchez-Herráiz, 1998; Barbadillo, unpublished data, Table 1).

We visited the area weekly during the months of May to July 2001, coinciding with the period of reproductive activity of amphibians at the area (Barbadillo, 1987), in order to obtain estimates of adult amphibian densities at each pond. Densities for each species were obtained from standardized transects that included visual encounters – both during the day and at night – and nocturnal acoustic transects (see Heyer *et al.*, 1994). We dip-netted some of the ponds in order to detect amphibian larvae and thus confirm the presence of breeding populations at each pond. We also carried out standardized transects of fixed length in which we visually recorded fish densities. Electrofishing or fishing nets were discarded because of difficulties imposed by the large extent, depth, and rocky bottom of most of the ponds. We scored separately presence/absence and densities of salmonids and cyprinids. Densities of amphibians and fish were scored as number of specimens per metre of transect. Maximum, minimum and average density values across visits were calculated. The small, peripheral ponds located in the vicinities of Laguna Cascada dried very early in the season and data about amphibian densities were only available for one of the visits.

In order to document possible changes in species richness at each pond, we compared presence/absence data for each amphibian species at each pond between previous, non-standardized surveys carried out in 1981 and 1991 and the results of 2001 samplings. In the samplings of 1981 and 1991 we obtained data on the amphibian species breeding at each pond. These samplings (1981 and 1991) also included multiple visits during the breeding season which combined nocturnal transects in order to detect adults and diurnal transects and dip-netting in the ponds selected in order to detect larvae of the different species. In all cases, presence was scored for the analyses only when evidence of reproductive activity was detected (presence of calling males, clutches or larvae).

We also compared present values of amphibian species richness and densities between fish-stocked ponds and fishless ponds. Again, only data for species that breed at each of the ponds were considered. We tested for correlation of fish and amphibian densities, considering separately each of the seven amphibian species breeding at the area. For the statistical analyses, only maximum densities were used as they were assumed to reflect more precisely the potential of a pond to hold amphibian populations while being at the same time less sensitive to sampling bias.

To explore the relationships between the presence/absence and the densities of each of the amphibian species and the characteristics of the ponds, we recorded the following variables: pond area, pond depth, percentage of aquatic vegetation on the surface, and type of substrate at the bottom of each pond. This latter variable was calculated as the ratio between the percentage of the bottom of the pond composed by rocks and the percentage of the bottom composed by mud. Because most ponds were quite large and the visibility of the bottom was restricted to a few meters form the shore, the percentages refer to the characteristics of the bottom of the pond in a two-meter wide ring immediately adjacent to the shore all along the perimeter of the pond. All comparisons were made using non-parametric statistical analyses (Spearman correlations).

RESULTS

Salmonids and cyprinids were both present in Lagunas Brava, Negra, and Patos (Table 1). The maximum densities of cyprinids were observed at Lagunas Negra and Patos (2.250 and 2.010 individuals per metre of transect, respectively). Only Laguna Cascada contained cyprinids (in low densities, only 0.005 individuals/metre) but not salmonids. As commented above, this pond was stocked with salmonids in recent decades, but in our surveys in 2001 we only found cyprinids. The present lack of salmonids in this pond was probably related to

TABLE 1. Presence (+) or absence (-) of the eight amphibian species at Neila in ponds sampled in 1981, 1991 and 2001. For amphibians, presence was scored only when evidence of reproduction was recorded. For *A. obstetricans*, +(-) indicates that, although calling males were detected in the surroundings of the ponds, no larvae were detected during the surveys at those sites.

	S. salamandra			*T. helveticus*			*T. marmoratus*			*A. obstetricans*			*B. bufo*			*B. calamita*			*H. arborea*			*R. perezi*		
	1981	1991	2001	1981	1991	2001	1981	1991	2001	1981	1991	2001	1981	1991	2001	1981	1991	2001	1981	1991	2001	1981	1991	2001
WITH FISH																								
Brava			-			-			-			+(-)			+			-			-			+
Negra	+	-	-	+	-	-	-	-	-	+	+	+(-)	+	+	+	-	-	-	-	-	-	+	+	+
Patos			-			-			-			+(-)			+			-			-			+
Cascada			-			+			+			+			+			-			+			+
FISHLESS																								
Casc. (per. 1)			-			+			+			-			-			+			+			+
Casc. (per. 2)			-			+			+			-			-			+			+			+
Haedillo	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Oruga	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+
Pardillas	+	-	-	+	+	+	+	+	+	+	+	+(-)	+	+	+	+	+	-	+	+	+	+	+	+
Fuente Sanza	+	+	-																					
Fuente Ria.	+	+	-																					

TABLE 2. Maximum value (D_{max}), average (Av) and standard deviation (SD) of the densities recorded at each pond for the amphibian species present at the study area. Average densities were calculated from the values obtained at each visit to a certain pond; for ponds with fish and those peripheral ponds only one record was available.

	Salm.	Cypr.	*T. helveticus*			*T. marmoratus*			*A. obstetricans*			*B. bufo*			*B. calamita*			*H. arborea*			*R. perezi*		
			D_{max}	Av	SD	D_{max}	Av	SD	D_{max}	Av	SD	D_{max}	Av	SD	D_{max}	Av	SD	D_{max}	Av	SD	D_{max}	Av	SD
FISH																							
Brava	0.020	0.180										1.292	0.435	0.554							0.041	0.017	0.018
Negra	0.024	2.250										1.418	0.449	0.582							0.110	0.044	0.044
Patos	0.015	2.010										0.340	0.121	0.126							0.140	0.056	0.060
Cascada		0.005	0.076	0.043	0.029	0.005	0.002	0.002	0.014	0.006	0.006	0.426	0.169	0.185				0.014	0.005	0.007	0.143	0.053	0.064
FISHLESS																							
Casc. (per. 1)				0.513	0		0.162	0								0.135	0		0.081	0		0.027	0
Casc. (per. 2)				0.411	0		0.109	0								0.109	0 .		0.027	0		0.027	0
Pardillas			0.037	0.012	0.011	0.061	0.027	0.024				0.035	0.013	0.011				0.207	0.038	0.065	0.700	0.232	0.235

TABLE 3. Relationships between pond characteristics, density of predatory fish, and maximum densities of amphibian species at Neila (results of Spearman non-parametric tests). For densities of *R. perezi*, only data on their relationship with fish densities was included because this species breeds in all the ponds studied. *A. obstetricans* was not included because we found evidence of reproduction of this species in only one of the ponds studied. N.S., P>0.05.

Variable		*Triturus marmoratus*	*Triturus helveticus*	*Bufo bufo*	*Bufo calamita*	*Hyla arborea*	*Rana perezi*	Salmonids	Cyprinids
Area									
	r_s	-0.78	-	0.88	-		-	-	0.86
	t	-2.77	-	4.20	-	-		-	4.17
	P	0.039	N.S.	0.008	N.S.	N.S.		N.S.	0.006
Submerged veg.									
	r_s	0.96	0.92	-0.93	0.79	0.90		-0.87	-0.88
	t	7.09	5.11	-5.54	2.85	4.55		-4.25	-4.63
	P	<0.001	0.004	0.003	0.036	0.006		0.005	0.003
Substrate									
	r_s	-0.92	-0.85	0.79	-	-0.92		0.84	0.89
	t	-5.37	-3.55	2.84	-	-5.37		3.84	4.83
	P	0.003	0.016	0.036	N.S.	0.003		0.009	0.003
Depth									
	r_s	-0.99	-0.95	0.887	-0.82	-0.93		0.84	0.92
	t	-15.97	-6.92	4.30	-3.17	-5.75		3.85	5.57
	P	<0.001	<0.001	0.008	0.02	0.002		0.008	0.001
Salmonids									
	r_s	-0.86	-0.86	0.82	-	-0.86	-0.39	1.00	-
	t	-3.75	-3.75	3.15	-	-3.75	-0.09	-	-
	P	0.013	0.013	0.025	N.S.	0.013	N.S.	-	-
Cyprinids									
	r_s	-0.92	-0.85	0.86	-	-0.92	0.04	0.90	1.00
	t	-5.37	-3.55	3.77	-	-5.37	0.08	5.08	-
	P	0.003	0.016	0.013	N.S.	0.003	N.S.	0.002	-

recent restoration practices that involved removal of artificial dams, which affected water level and probably made it an unsuitable habitat for these fish species. In fact, according to data from the "Consejería de Medio Ambiente", when this pond was emptied in 1999 very few salmonids (fewer than 10) remained (C. Temiño, pers. comm.).

Present values for amphibian species richness in the ponds studied in 2001 ranged from two to seven species (Table 1). The lowest values (two species of amphibian) were found at ponds that presently have fish (Lagunas Negra, Brava and Patos), whereas the highest values were found in fishless ponds (seven species of amphibian in Haedillo and Oruga, and five species in Pardillas and the peripheral ponds near Laguna Cascada). Six species of amphibian were breeding in 2001 at Laguna Cascada, where only cyprinids (but not salmonids) are present.

According to the comparisons of amphibian presence/absence data from the sampling carried out in 1981, 1991 and 2001, two out of eight species have experienced severe declines at the area: *S. salamandra* and *A. obstetricans*. In the case of *Salamandra salamandra*, we did not find adults or larvae in any of the sampled sites during the surveys in 2001, which indicates the extinction of all of the six populations that were known at the study area in 1981 (Table 1).

With respect to *Alytes obstetricans*, we detected calling males at very low densities (~10 calling males per night) in all of the ponds where salmonids were present (Negra, Brava and Patos). However, in 2001 surveys, no larvae were detected in any of these ponds. At Laguna Cascada, where cyprinids but not salmonids are present, we have detected larvae of *Alytes obstetricans*, but at very low densities compared with the density values obtained in the fishless pond Laguna Oruga (0.01 *vs.* 0.93 larvae per metre of transect, respectively).

There is a significant negative relationship between amphibian species numbers and presence of fish (r=-0.84, df=7, P=0.005) and also between amphibian species richness and density of fish (for salmonids: Spearman tests: r_s=-0.82; n=9, P=0.007; for cyprinids: r_s=-0.78, n=9, P=0.013). The relationship between amphibian and fish densities varied among species. In fact, in ponds where salmonids are present only *B. bufo* and *R. perezi* are abundant and breed successfully.

The highest values of densities of both species of *Triturus* were recorded at ponds where salmonids were absent (Table 2). The newts were, however, also present at Laguna Cascada (Table 1). The same pattern (a significant negative correlation between maximum or average densities of amphibians and densities of salmonids) was evidenced for *H. arborea*. *B. calamita* was only detected in fishless, temporary ponds, although density values were always very low (Table 2).

On the other hand, *B. bufo* is relatively abundant in ponds that have been stocked with fish. The highest densities of *B. bufo* correspond to the ponds with higher densities of salmonids (1.29 and 1.41 adults of *B. bufo* per metre of transect at Lagunas Brava and Negra, Table 2). Consequently, the relationship between *B. bufo* and fish densities is positive and significant (Table 3).

The occupation by *R. perezi* of the ponds studied is apparently unaffected by the presence or absence of fish (Table 3). This species is present in all the ponds sampled (Table 1), although the highest densities were recorded at a fishless pond (0.70 individuals per metre at Laguna Pardillas, Table 2).

According to the results of the analyses of the relationship between pond characteristics and amphibian densities, the most important variables affecting the latter are the type of substrate, pond depth, and density of submerged vegetation (Table 3). Except for pond area, which was positively correlated with the density of *B. bufo* and negatively correlated with *T. marmoratus*, the remaining variables studied clustered amphibians and fish in two groups. *B. bufo* and salmonids are present in deep ponds with little submerged vegetation and rocky bottoms. On the other hand, *T. marmoratus*, *T. helveticus*, *B. calamita* and *H. arborea* breed only in shallow ponds with abundant submerged vegetation and muddy bottoms.

DISCUSSION

The magnitude of the effect of exotic fish on amphibian populations is usually different among species. Not all amphibian larvae are equally vulnerable to predatory fish. For example, Hecnar & M'Closkey (1997) found that, among the species they studied, ranids and bufonids coexisted successfully more frequently with predatory fish than hylids or ambystomatids. We also found this pattern at Neila: only *B. bufo* and *R. perezi* breed successfully in ponds occupied by salmonids. Coexistence of amphibians and predatory fish is generally due to the existence of species-specific predator-avoidance mechanisms (Petranka *et al.*, 1987; Manteifel, 1995). Kats *et al.* (1988) stated that the existence of these mechanisms is best predicted by the frequency of encounter with fish rather than by phylogeny. According to this, amphibian species affected more severely by fish introductions would be those that have not been historically exposed to predatory fish: that is, species that breed in ephemeral ponds. This pattern is evident in our study area. *T. helveticus*, *T. marmoratus* and *H. arborea* are species that usually breed either in temporary ponds or in permanent, fishless ponds (Barbadillo *et al.*, 1999). In Neila they appear to be unable to coexist successfully with fish in permanent ponds, where marginal favourable breeding sites are present. In fact, in our study area, in accordance with other studies on breeding habitat selection, *T. marmoratus* and *H. arborea* selected ponds with abundant aquatic vegetation that provides oviposition sites for newts and support in the water for calling males (Miaud, 1995; Moravec, 1989). On the other hand, *B. bufo* was present in large, permanent ponds with scarce vegetation, a pattern that is general in other high altitude areas in Spain (personal observations). This pattern suggests that habitat requirements of *B. bufo* and fish overlap to a great extent in Neila.

Only two of the seven amphibian species breeding at Neila presently coexist with fish. In other mountainous areas in Central Spain, *B. bufo* usually shares reproduction sites with native salmonid species, such as the trout *Salmo trutta* (personal observations). The situation of long historical coexistence with *S. trutta* suggests that the impact of predation on *B. bufo* populations is probably low.

The case of *R. perezi* is different because they are present in almost every body of water in the study area, independently of the water body characteristics, including presence or absence of fish. *R. perezi* is a ubiquitous species that rapidly colonizes almost every body of water available, including large, permanent ponds (Llorente & Arano, 1997). Galán (1997) found that out of eight amphibian species, only *R. perezi* was able to breed successfully after the introduction of fish (*Gambusia affinis* and *Carassius auratus*) and crayfish (*Procambarus clarki*), although their densities were much lower than before introduction. To our knowledge, avoidance mechanisms or unpalatability in *R. perezi* larvae have not been described.

The remaining five species of amphibian are absent from ponds where fish have been introduced. Of these, three of them (*T. marmoratus*, *T. helveticus* and *H. arborea*) are locally abundant in fishless ponds. The remaining two species (*S. salamandra* and *A. obstetricans*) are respectively, absent or very scarce in the study area. *S. salamandra* has not been seen in the area since 1991. Although this species was not abundant at the Sierra de Neila (only six localities were known in the area in 1981, Table 1), the sampling effort in 2001 was higher than that of previous studies, and thus it is unlikely that they have been overlooked. Populations of this species located at lower altitudes in the same region of the study have also disappeared. This is the case in the two localities in the Sierra de Neila that still held salamanders in 1991, which were sampled in 2001 with negative results. The lack of evidence of reproduction of the species in the last decade suggests that *S. salamandra* populations at Neila might be very close to regional extinction. *S. salamandra* is likely to have been strongly affected by repeated fish introductions, although other causes (i.e. epidemic disease, such as chytridiomycosis) might be also involved. In other high altitude areas in Spain it has been shown to be extirpated from ponds and streams where fish were introduced (Martínez-Solano *et al.*, pers. obs.). An indirect effect of fish as vectors of amphibian pathogens cannot be discarded as some cases of this interaction have already been reported (Kiesecker *et al.*, 2001). The species has also disappeared from other sites in the Sistema Ibérico in the province of La Rioja, where historical records were also available (Barbadillo & García-París, 1991; I. Esteban, pers. comm.).

The decline of *A. obstetricans* in the study area seems to be directly related to fish introductions. The lack of evidence of normal reproductive activity in fish-stocked ponds that were formerly occupied by the species, together with the fact that they are locally abundant in fishless streams both suggest a negative interaction between fish and *A. obstetricans*. The absence of larvae of *A. obstetricans* in ponds occupied by fish might be better explained by direct avoidance of reproduction sites with high densities of predatory fish by adult toads than by direct predation of fish on larvae. High densities of larval *A. obstetricans* were observed in some fishless ponds and streams in the study area. Declines of *A. obstetricans* related to other causes (red-leg disease and chytrid fungi) have been reported in other high altitude populations (Márquez *et al.*, 1995; Bosch *et al.*, 2001). In any case, there is no evidence that these pathogens might be involved in the decline of the species at Neila. In-depth analyses regarding other possible factors affecting *A. obstetricans* populations are needed in order to clarify the situation of this species at the area.

In general, introduced fish species (with 25 species reported in Spain: Elvira, 2001) are a problem for native amphibian populations. Introductions were initially carried out by the authorities in the first half of the last century for sport fishing. Although the negative effect of introduced fish has long been identified and stressed (Elvira, 2001), few efforts have been made to control or eradicate any of the species involved. In areas where massive introductions of fish have taken place, amphibian populations are strongly dependent on ephemeral ponds, and these are subject to greater risks of recruitment failure as they are more unpredictable environments than permanent ponds. Moreover, some species that are linked to permanent sites might be rapidly declining due to their inability to respond to new predation pressures, resulting in a pessimistic future. However, adequate management decisions might avert this situation, because it has been shown that amphibian populations affected by fish introductions may recover to pre-stocking levels within a reasonable period of time (>10 years) (Knapp *et al.*, 2001). As these authors conclude, a key point in achieving this recovery is ensuring that measures are put in practice before connectivity to nearby populations falls below a critical threshold, so that recolonization is still possible.

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REFERENCES

Alford, R. A. & Richards, S. J. (1999). Global amphibian declines: a problem in applied ecology. *Annual Review of Ecology and Systematics* **30**, 133-165.

Aronsson, S. & Stenson, J. A. E. (1995). Newt-fish interactions in a small forest lake. *Amphibia-Reptilia* **16**, 177-184.

Barbadillo, L. J. (1987). *La Guía de Incafo de los anfibios y reptiles de la Península Ibérica, islas Baleares y Canarias*. Incafo, Madrid, Spain.

Barbadillo, L. J. & García-París, M. (1991). Problemas de conservación de los anfibios en España. *Quercus* **62,** 20-25.

Barbadillo, L. J. & Sánchez-Herráiz, M. J. (1998). Reserva de caza de las Sierras de la Demanda y Neila (sector burgalés). In: *Inventario de las áreas importantes para los anfibios y reptiles de España*, 109-114. Santos, X.; Carretero, M. A.; Llorente, G. A. & Montori, A. (Eds.). Ministerio de Medio Ambiente, Madrid, Spain.

Barbadillo, L. J., Lacomba, I., Pérez-Mellado, V., Sancho, V. & López-Jurado, L. F. (1999). *Anfibios y Reptiles de la Península Ibérica, Baleares y Canarias*. Geoplaneta, Barcelona, Spain.

Bosch, J., Martínez-Solano, I. & García-París, M. (2001). Evidence of a chytrid fungus infection involved in the decline of the common midwife toad (*Alytes obstetricans*) in protected areas of central Spain. *Biological Conservation* **97,** 331-337.

Bradford, D. F. (1989). Allotopic distributions of native frogs and introduced fishes in high Sierra Nevada lakes of California: implication of the negative effect of fish introductions. *Copeia* **1989**, 775-778.

Bradford, D. F. (1991). Mass mortality and extinction in a high-elevation population of *Rana muscosa*. *Journal of Herpetology* **25,** 174-177.

Braña, F., Frechilla, L. & Orizaola, G. (1996). Effect of introduced fish on amphibian assemblages in mountain lakes of northern Spain. *Herpetological Journal* **6,** 145-148.

Brönmark, C. & Edenham, P. (1994). Does the presence of fish affect the distribution of tree frogs (*Hyla arborea*)? *Conservation Biology* **8**, 841-845.

Drost, C. A. & Fellers, G. M. (1996). Collapse of a regional frog fauna in the Yosemite area of the California Sierra Nevada, USA. *Conservation Biology* **10**, 414-425.

Elvira, B. (2001). Peces exóticos introducidos en España. In *Atlas y Libro Rojo de los peces continentales de España*, 267-272. Doadrio, I. (Ed.). Dirección General de Conservación de la Naturaleza – Museo Nacional de Ciencias Naturales, Madrid, Spain.

Fisher, R. N. & Shaffer, H. B. (1996). The decline of amphibians in California's Great Central Valley. *Conservation Biology* **10**, 1387-1397.

Galán, P. (1997). Declive de poblaciones de anfibios en dos embalses de La Coruña (noroeste de España) por introducción de especies exóticas. *Boletín de la Asociación Herpetológica Española* **8**, 38-40.

Gamradt, S. C. & Kats, L. B. (1996). Effect of introduced crayfish and mosquitofish on California newts. *Conservation Biology* **10**, 1155-1162.

Hecnar, S. J. & M'Closkey, R. T. (1997). The effects of predatory fish on amphibian species richness and distribution. *Biological Conservation* **79**, 123-131.

Heyer, W. R., Donnelly, M. A., McDiarmid, R. W., Hayek, L. C. & Foster, M. S. (1994). *Measuring and Monitoring Biological Diversity. Standard Methods for Amphibians.* Smithsonian Institution Press. London, UK.

Kats, L. B., Petranka, J. W. & Sih, A. (1988). Antipredator defenses and the persistence of amphibian larvae with fishes. *Ecology* **69**, 1865-1870.

Kiesecker, J. M., Blaustein, A. R. & Miller, C. L. (2001). Transfer of a pathogen from fish to amphibians. *Conservation Biology* **15**, 1064-1070.

Knapp, R. A., Matthews, K. R. & Sarnelle, O. (2001). Resistance and resilience of alpine lake fauna to fish introductions. *Ecological Monographs* **71**, 401-421.

Lizana, M. & Barbadillo, L. J. (1997). Legislación, protección y estado de conservación de los anfibios y reptiles españoles. In: *Distribución y biogeografía de los anfibios y reptiles en España y Portugal*, 477-516. Pleguezuelos, J. M. (Ed.). Granada: AHE-Universidad de Granada, Spain.

Llorente, G. A. & Arano, B. (1997). *Rana perezi* (Seoane 1895). In *Distribución y Biogeografía de los Anfibios y Reptiles en España y Portugal*, 164-166. Pleguezuelos, J. M. (Ed.). Granada: AHE-Universidad de Granada, Spain.

Manteifel, Y. (1995). Chemically-mediated avoidance of predators by *Rana temporaria* tadpoles. *Journal of Herpetology* **29**, 461-463.

Márquez, R., Olmo, J. L. & Bosch, J. (1995). Recurrent mass mortality of larval midwife toads *Alytes obstetricans* in a lake in the Pyrenean mountains. *Herpetological Journal* **5** 287-289.

Miaud, C. (1995). Oviposition site selection in three species of European newts (Salamandridae) genus *Triturus*. *Amphibia-Reptilia* **16**, 265-272.

Moravec, J. (1989). Observation on fertilization and oviposition in the European tree frog (*Hyla arborea*). *Acta Universitatis Carolinae Biologica* **32**, 515-530.

Petranka, J. W., Kats, L. B. & Sih, A. (1987). Predator-prey interactions among fish and larval amphibians: use of chemical cues to detect predatory fish. *Animal Behaviour* **35**, 420-425.

Woodward, B. D. (1983). Predator-prey interactions and breeding-pond use of temporary-pond species in a desert anuran community. *Ecology* **64**, 1549-1555.

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