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ORIGINAL PAPER

Evidence of exotic trout mediated minnow invasion in Pyrenean high mountain lakes

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Abstract Although high mountain lakes are naturally fishless, there have been numerous trout introductions to such ecosystems in many areas of the world with negative ecological consequences. In recent decades other fishes, such as minnows, have been introduced to some mountain areas, including the Pyrenees. These introductions may cause further ecological problems, since minnows also occupy the top of the food chain, and are difficult to manage since such introductions occur without permission from the authorities. In this study we have analyzed the process of minnow introductions in all high mountain lakes of the southern slope of the Pyrenees to find out which particular factors best explained their present distribution and to evaluate which management measures have been most effective for stopping introductions. We found 27 % of the lakes had minnows (*Phoxinus* sp.) present, 52 % had trout and 47 % were fishless. Trout presence was the most significant variable explaining 27 % of deviance of minnow presence data in a generalized additive model. Recreational fishing using minnows as live bait is likely responsible for these introductions. Minnow introductions are therefore mediated by a preceding invasive species and facilitated by human activity. We also compared the number of minnow introductions in non-fishing areas of National Parks with other areas where managed fishing takes place. We found that the number of lakes with minnow introductions was increasing in all areas except those where fishing was prohibited, indicating that prohibiting fishing is an effective management practice for stopping minnow introductions.

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Introduction

Invasions by human-introduced non-indigenous species are one of the main threats to biodiversity. They have been identified as the main cause of extinction of wildlife in many occasions, but also as a potential phenomenon generating evolutionary stress and moreover causing biotic community homogenization (Clavero and García-Berthou 2005; Lockwood et al. 2013; Marr et al. 2013; Simberloff et al. 2013). Freshwater fishes are one of the animal groups with a high number of invasive species (Hulme et al. 2009). Ecological impact of invasive species in aquatic environments has been described as severe when they become top predators (Vitule et al. 2009), since in addition to decreasing the relative abundances or completely eradicating their prey, they also alter the trophic relationships inducing trophic cascades to the base of the food web (e.g. Byström et al. 2007; Wahl et al. 2011). Furthermore, the introduction of multiple predator species can have cumulative effects on the receiving ecosystems (Nyström et al. 2001).

Angling practices with live bait represents a worrying pathway for alien species introductions (Kerr et al. 2005; Webb 2007; DiStefano et al. 2009; Ward et al. 2012). The largest organisms used as live bait are several species of small fishes, but other animals such as amphibians, earthworms, crayfishes, grubs and insects are also used (Lindgren 2006; Keller and Lodge 2007). Improper disposal of live bait has been attributed as the source of introduction of at least 14 species of fishes in Ontario (Kerr et al. 2005). In the English Lake District, individuals of at least 12 native and non-native fish species have been brought to Windermere for the purpose of live-baiting (Winfield et al. 2011). Live-bait use was also responsible for the introduction of 47 known freshwater species in United States Mid-Atlantic slope drainage systems, among which are at least 5 non-native fishes, 4 non-native crayfishes and 9 non-native earthworm species (Kilian et al. 2012). Live baits may arrive in the environment by accidental escape or more often, by being released into the water at the end of the fishing trip (Winfield et al. 2011; Kilian et al. 2012). In some cases, it has been shown that bait-related introductions have resulted in established populations of invasive species (e.g. Callaham et al. 2006; Migge-Kleian et al. 2006).

The use of small fishes as live bait, mainly used to catch salmonids, has previously been reported in boreal and mountain lakes. For example, during the second half of the twentieth century, Scottish lochs have received, at least 7 non-native species used as live-bait, minnows among them (Maitland and Campbell 1992). Also, minnows have expanded outside their native distribution in Scandinavia since the beginning of the twentieth century, mainly in mountain areas and in association with angling practices (Museth et al. 2007). However, invasions linked with released live-bait have not been very well described in high mountain watersheds so far.

High mountain lakes are originally fishless ecosystems due to natural barriers that have prevented the natural colonization of fish species from lower streams or dispersal among interconnected lakes (Knapp et al. 2001a; Pister 2001). However, the Pyrenees like many other high mountain areas of the world nowadays have introduced trout in more than half of the lakes (Miró and Ventura 2013). These introductions first took place in lower altitude lakes (ca. 25 % of the lakes) historically associated with traditional fish exploitation dating back at least to the fourteenth century. More recently, during the second half of the twentieth century, introductions have taken place in another 25 % of the lakes, mainly for recreational fishing purposes and promoted by local fishermen associations, environmental administrative offices and hydroelectrical power companies (as compensatory actions) (Miró and Ventura 2013).

Complementary to the spread of trout, minnows have also been detected in many Pyrenean lakes during the last few decades (Miró 2011). Although minnows apparently are used as live-bait for trout angling, this type of angling is forbidden in the southern part of the Pyrenees since 1966. The illegal introduction of minnows, therefore, occurs without the control of public authorities and generates a challenge for resource managers tasked with the conservation of high mountain lake ecosystems. Within the Pyrenees, there are areas with different degrees of protection (e.g. National Parks where fishing is prohibited and other areas where fish management takes place) that offer an interesting opportunity to explore how different fish management practices (authorized or prohibited trout angling) have affected the spread of minnows.

The objective of this study was to understand the distribution and spread of minnows in high mountain lakes of the southern slope of the Pyrenees, to find out which particular factors, either environmental or anthropogenic, best explained their present distribution and to evaluate which conservation measures have been most effective. We expected to confirm that successful minnow introductions were closely related with their use as live-bait for trout fishing. In particular, we had the following specific hypotheses: (1) we would only find minnows in lakes where trout had been previously introduced; (2) minnow introductions would be more likely in areas where active fish management has taken place; (3) the probability of finding minnows would be higher in lower altitude lakes and in those with larger sizes; and finally (4) fishing prohibition (for trout) in National Parks should result in stopping minnow introductions.

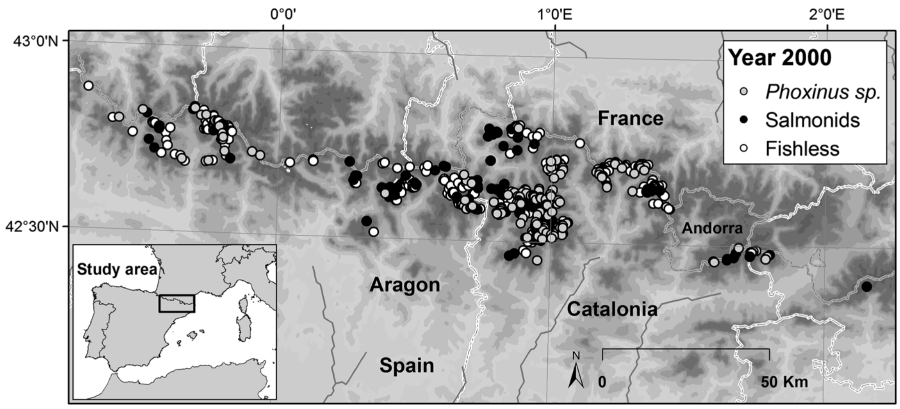

Fig. 1 Distribution of the 520 studied lakes along the southern Pyrenean range and fish presence at year 2000. Minnows (*Phoxinus* sp.) usually share the lake with trout or are alone in some cases; see "Results" section to more details

Materials and methods

Description of the study area and species

The studied lakes are spread along the Pyrenean mountain range (0°42′W–2°09′E, 42°52′–42°23′N; Fig. 1). From among 1,062 lakes >0.5 ha, we surveyed 520 water bodies which comprise all those lying within the Catalan-Aragoneese (Spanish or southern) Pyrenees. Pyrenean lakes originated from glacial processes or were modified by the activity of quaternary glaciations. The surveyed lakes range in altitude between 1,600 and 2,960 m with the highest frequency found at ca. 2,400 m and are relatively small and deep (average surface area of 2 ha and average maximum depth of 17 m, with the largest of 54 ha and deepest 105 m). Due to their common origin, there is a close positive relationship between surface area and maximum depth (Catalan et al. 2009). Most of the lakes are above the tree line with catchments partially covered by meadows, although some of them are within or below the tree line. Approximately half of the lakes have catchments on granodiorite bedrock, the remaining being located in catchments with metamorphic (25 %), detrital (15 %) or carbonate (10 %) bedrock with a minority with Silurian slate (Casals-Carrasco et al. 2009). The latter bedrocks, due to their high sulphate content, give natural acidity to the waters (pH <5.5).

Within the study area there is a National Park, Aigüestortes i Estany de Sant Maurici National Park which has 37 % of the total studied lakes. These lakes are under two different management regimes: a core area with strict regulations where fishing has been prohibited since 1988 (with 75 lakes) and a peripheral area where fishing is allowed (with 116 lakes).

The study species is the European minnow (*Phoxinus phoxinus* (L. 1758)), that was considered a single species until recently, and therefore most citations were referred as *P. phoxinus.* A recent taxonomical revision has distinguished various species: *P. phoxinus* sensu stricto in the northern (French) Pyrenean slope, *Phoxinus bigerri* Kottelat, 2007 native in the north and south-western Pyrenean streams, and *Phoxinus septimaniae* Kottelat, 2007 native in the north-eastern Pyrenean streams (Kottelat 2007). During summer of 2013 we were able to examine the species present in fifteen lakes of both north and south slopes of Pyrenees, and only found *P. phoxinus* sensu stricto. To avoid taxonomical confusion, in this paper we use the term minnow to refer to *Phoxinus* sp. found in the Pyrenean lakes.

Data collection and environmental variables

We collected 435 citations of minnow presence and 379 of minnow absence in the study area from interviews with local elderly fishermen or nature reserve wardens, from local reports of fishing or walking societies and from our own littoral's visual encounter surveys. In fifteen lakes, we also used minnow traps to validate the information from the interviews, confirming all cases. Historical information of minnow absence was also obtained from historical documents from local, regional and national archives. The collected data from the different complementary sources covered the whole twentieth

Table 1 Description of predictor variables used in the generalized additive models

Variable type	Variable name	Description
Physical and chemical	Altitude (ALT)	Elevation of the lake (m)
	Surface area (SURF)	Surface of the lake (ha)
	Water body location (LOCATION)	Latitude and longitude of the lake, UTM reference system
	Connectivity (CONN)	Binary factor that has a value of 1 in each lake that has a direct stream connection without any natural barrier with another lake with an established minnow population
	Accumulated degree days (ADD)	Thermal accumulation in degree days >7.8 °C (°C day)
	pH < 5.5 (pH)	Binary factor indicating lakes with pH < 5.5
Mode of introduction	Walking Effort (WALK)	Walking effort from the nearest town (minutes)
	Population (POP)	Inhabitants of the nearest town to the lake
	Hotel beds (HOT)	Number of hotel beds in the nearest town to the lake
	Helicopter stocking (HEL)	Binary factor determined by the existence of helicopter stocking in the lake
	Forestry road (FROAD)	Binary factor marking the lakes with forestry road access
Management practice	Fishing zone (FIZ)	Binary factor determined by the existence of active fish management, at present or in the past
	National Park (NATP)	Binary factor indicating the lakes which belonging to the part of the National Park where fishing is prohibited at present
	Hydroelectrical power (HEP)	Binary factor marking the lakes with water level regulation
Trout facilitation	Trout presence (TROUT)	Binary factor determined by trout presence in the lake

century and allowed us to reconstruct the decade when minnows arrived to each lake. We chose this method instead of only using our own surveys, since our aim was to provide a historical perspective of minnow introductions and to be able to provide an objective account of the main factors responsible for these introductions.

To detect which factors were associated for minnow introductions, we generated a set of environmental variables summarizing both physical lake characteristics and anthropogenic factors that might have affected the distribution of minnows (Table 1). Altitude and surface area were obtained from a GIS generated from 1:25,000 maps from the Spanish, Aragonese an Catalan geographical agencies (Casals-Carrasco et al. 2009). A binary factor of connectivity (CONN) was included to assess the possible natural dispersion of minnows through streams or their secondary spread after introduction in one lake, coding the presence/absence for a given lake of a stream connection with another lake with an established minnow population. Water temperature was the accumulated degree days (ADD) during the ice-free period calculated from daily max–min temperature data using the sine-wave method, assuming the trigonometric sine curve as an approximation of the diurnal temperature curve and adding the area under the curve and above the lower threshold for each day (Baskerville and Emin 1969). As a lower threshold we used 7.8 °C, the lower developmental temperature below which *Salmo trutta* reproduction is not feasible (Elliott et al. 1995). Daily water temperatures were obtained from 27 automatic thermometers (Vemco Minilog-T) deployed at a depth of ca. 1 m in lakes covering a wide range of altitudes, surface areas >0.5 ha and catchment sizes from 2008 to 2009. The calculated ADDs from those lakes with automatic thermometers were extrapolated to all other lakes using a multiple linear regression model between ADD and various morphometric parameters and choosing the minimum combination of morphometric variables that described most of the variance with stepwise forward selection procedure. The following model obtained was:

$$\begin{aligned}&\text{Degree days}(>7.8\,^{\circ}\text{C}) = 4410.4874 - 1.39\,\text{ALT}\\&- 21.73\,\text{SURF} - 117.72\ln(\text{CS}/\text{SURF})\\&- 0.08\,\text{CS} - 53.71\ln(\text{DC}/\text{CS})\\&R^2 = 0.833;\ F_{5,20} = 19.9, P < 0.0001\end{aligned} \tag{1}$$

where ALT and SURF are lake altitude and surface area (Table 1), CS is the lake total catchment size and DC is the lake direct catchment size (i.e. the proportion of catchment not shared with other lakes that are upstream of the lake). This latter parameter equals CS when there are no lakes upstream and the ratio DC:CS is relevant since the presence of other lakes in the catchment changes the temperature of the streams flowing out of them. The ratio CS:SURF is indicative of the rate of water renewal.

When using pH as an environmental variable, we used a binary variable which separated out lakes with pH <5.5, as below this pH fish are known to be unable to survive (Rosseland et al. 2000).

Among the factors indicating the mode of fish introductions, we estimated the walking effort from the nearest town (WALK; in minutes) which could be a good predictor of the likelihood of a lake having fish introduced. To estimate it, we chose a representative subset of 72 lakes from different areas, for which we quantified the walking time from the nearest town by ourselves climbing to each of these lakes. Then we regressed the walking time with two predictor variables: the altitudinal difference between the lake and the town (ALTDIFF; in meters) and the linear distance between them (DIST; in meters), both obtained from a GIS and 1:25,000 cartographic maps. Since climbing uphill takes more time than downhill, we used two regressions, one for uphill (UPWALK) and the other for downhill (DOWNWALK):

$$\mathrm{UPWALK} = -4.279 + 0.157\ \mathrm{ALTDIFF} + 0.008\ \mathrm{DIST} \quad (2)$$
$$R^2 = 0.942;\ F_{2,72} = 563.6;\ p < 0.001$$

$$\mathrm{DOWNWALK} = -5.732 + 0.078\ \mathrm{ALTDIFF} + 0.013\ \mathrm{DIST} \quad (3)$$
$$R^2 = 0.885;\ F_{2,69} = 258.9;\ p < 0.001$$

The total walking effort was then obtained by adding UPWALK with DOWNWALK. We also used the size of the nearest town (POP) and the number of hotel beds (HOT) at the time of the minnow introduction, both obtained from national twentieth century historical inventory surveys. We also recoded if there was helicopter stocking of trout (HEL) in the area nearby to the lake during a certain period (1970s–2000s) or if the lake had a forestry road giving car access to the lake (FROAD). The management practice variables considered were whether the location was within an area with active fish management (FIZ), whether lakes belonged to fishing prohibited areas of National Park of Aigüestortes i Estany de Sant Maurici (NATP) or if the lake had water fluctuations for hydroelectric production (HEP). Hydroelectric production (HEP) is not only relevant for the potential direct effect of changing water levels on minnow breeding, but also because HEP companies performed trout introductions in order to compensate local towns from the potential negative effects of the impounding. Finally we also used the presence of trout species (TROUT) as a categorical variable to explore potential interferences and relationships between minnow and trout species.

Statistical analyses

First of all we calculated the introduction rate of minnow and trout for the twentieth century by dividing the number of lakes where they have been introduced by the period in years that the introductions lasted. In the case of trout we only included the modern trout introductions that took place during the second half of the twentieth century to compare the rate with those of minnows. Then, we used generalized additive models (GAMs) as statistical analyses to compare the data on the presence/absence of minnows in the Pyrenean lakes with the different environmental variables described above. We used GAMs for the analyses because they are similar to generalized linear models, but distend the assumption that the relationships between the dependent variable (when transformed to a logit scale) and predictor variables are linear by estimating a nonparametric loess smooth function for each continuous predictor variable (Hastie and Tibshirani 1990; Knapp 2005). Prior to analyses with the GAMs, we tested for collinearity among the predictor variables by Pearson correlation coefficients (r) for all pair-wise combinations of continuous predictor variables. The strongest correlation coefficient was $|0.72|$, below the suggested cut-off of $|r| \geq 0.85$ that would indicate collinearity for the sample size used in these analyses (Berry and Felman 1985). Therefore, all predictor variables were included initially in the regression models. In the regression models, p_i is the probability of finding the species at location i, and is defined as:

$$p_i = \frac{e^{\theta_i}}{1 + e^{\theta_i}},$$

where the linear predictor (i.e., logit line) θi is a function of the independent variables. For minnow, the specific relationship we used for θ was the following function of covariates:

$$\begin{aligned}\theta = {} & \mathrm{lo(ALT)} + \mathrm{lo(SURF)} + \mathrm{lo(LOCATION)} \\ & + \mathrm{CONN} + \mathrm{lo(ADD)} + \mathrm{pH} + \mathrm{lo(WALK)} \\ & + \mathrm{lo(POP)} + \mathrm{lo(HOT)} + \mathrm{HEL} + \mathrm{FROAD} + \mathrm{FIZ} \\ & + \mathrm{NATP} + \mathrm{HEP} + \mathrm{SALM}\end{aligned} \tag{4}$$

where lo(·) represents a nonparametric loess smoothing function that characterizes the effect of each continuous independent variable on p_i. The location covariate lo(LOCATION) was a smooth surface of UTM easting and northing (see Table 1 for variable abbreviations).

From Eq. (4) we selected a subset of significant variables explaining the greatest proportion of deviance. The best combination of independent variables was selected by stepwise forward selection using AIC criteria. The use of this procedure ensured that at each selection step only those variables explaining a significant proportion of previously non-explained variance would be selected. In other words, while altitude, temperature (ADD) and surface area share a significant amount of variance, their inclusion in the model would only take place if each variable would explain a fraction of variance not explained by the others. The proportion of variance explained by each variable was determined by evaluating the change in deviance resulting from dropping each variable from the model in the presence of all other variables. Analysis of deviance and likelihood ratio tests (based on the binomial distribution) were used to test the significance of the effect of each predictor variable on the probability of occurrence by minnow. Because the large sample sizes used in the regression models could result in predictor variables being statistically significant despite very weak associations with species presence/absence, predictor variables were considered to have significant effects only when $p \leq 0.01$.

The relationship between the significant predictor variables and the probability of minnow occurrence is shown graphically in separate plots separating the unshared fraction of variance that each factor explains (Fig. 3). Each plot depicts a response curve that describes the contribution of the predictor variable to the logit line. More generally, the response curve shows the relative influence of the predictor variable on the probability of minnow occurrence. This response curve is based on partial residuals, is plotted on a log-scale, and is standardized to have an average value of 0. For example, a hump-shaped response curve for the predictor variable "elevation" indicates that minnow was, in a relative sense, most likely to be detected at sites at low elevations and less likely to be detected at sites at high elevations (Knapp et al. 2003).

We used the estimated effect of previous trout presence in the binomial equation to approximate the change in the likelihood (i.e. odds ratio) of finding minnow in the presence versus absence of trout after having controlled for the effects of habitat and spatial variables (Hastie and Tibshirani 1990; Welsh et al. 2006).

All regression-related calculations were conducted using R statistical software (R Development Core Team 2013) with the function library *gam* (Hastie 2013). The analyses were run with the data up to the year 2000.

To analyse in more detail the factor variables, we compared the presence/absence of minnows with the categorical predictor variables by 2 × 2 contingency tables. To test if the morphometric characteristics of the lakes where minnows were introduced were different from the other lakes, we compared the values of the continuous predictor variables with one-way ANOVA and a Tukey post hoc test, for distinguishing among three groups: fishless lakes, lakes stocked with trout only and lakes stocked with trout and minnows. Data had previously been normalised.

Finally, in order to assess the effectiveness of banning fishing to prevent new introductions of minnows, we compared the changes in the number of lakes with minnows in Aigüestortes i Estany de Sant Maurici National Park during the twentieth century. Drawing line charts, we compared the number of lakes with new minnow introductions since the fishing ban (1988) in the 75 lakes where fishing was banned and the 116 lakes where fishing continued. We grouped the rest of the lakes, outside of the National Park, in a third group (with 329 lakes) and plotted the changes in each category during the twentieth century.

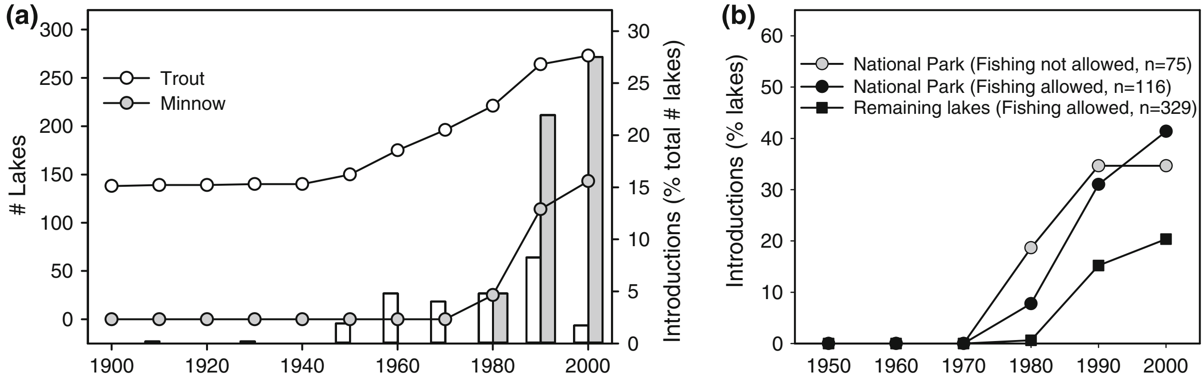

Fig. 2 **a** Introduction process of the minnow compared to trout in the southern Pyrenees during the twentieth century. *Vertical gray bars* are the decadal total number of lakes that were stocked with trout in percentage of the total number of lakes >0.5 ha of the study area (n = 520). **b** Effect of Aigüestortes i Estany de Sant Maurici National Park on the introduction of minnows in high mountain lakes of the Pyrenees. *Circles* are the lakes within the National Park, and *squares* are lakes outside the National Park. *Grey circles* are the area of the National Park where fishing is not allowed and *black circles* and *squares* are the lakes in fishing allowed areas

Results

We found minnows in 141 of 520 (27 %) of the surveyed high mountain lakes (Fig. 1). In 133 of these lakes there were also at least one trout species. The remaining 8 lakes had trout in the past which had gone extinct but still had minnows present. In addition, we found 132 lakes (25.4 %) with only trout (mainly *S. trutta*, but also in some lakes *Salvelinus fontinalis* and *Onchorynchus mykiss*) and 247 lakes (47.5 %) that were fishless. All the minnow introductions registered up to the year 2000 took place during the last three decades of the twentieth century (Fig. 2a). Before 1970 there was no Pyrenean high mountain lake with minnow. Since then, the number of minnow introductions has been much higher than those of trout. The introduction rate for minnow was 4.7 pa (141 lakes introduced between 1970 and 2000) and 2.2 pa for trout (133 lakes introduced between 1940 and 2000). The documented causes of introduction of minnows were transport to the lake in water containers by fishermen to be used as live bait. According to our interviews, minnows were then introduced to the lakes by throwing the remaining individuals into the lake in the belief that this species would be food for trout.

The forward selection procedure for the generalized additive model selected six of the fourteen predictor variables by order of importance which were significantly correlated with the probability of minnow occurrence: trout presence, surface area, location, altitude, helicopter stocking and forestry road (Table 2). The relationship between the probability of minnow occurrence (on a logit scale) and the important

Table 2 Results of generalized additive models developed for minnows (*Phoxinus* sp.) introduced in the Pyrenean lakes

Parameter	*Phoxinus* sp.	
Null deviance	608	
Degrees of freedom (null model)	519	
Model deviance[a]	288	
Degrees of freedom (full model)	502	
Explained deviance (% of total)	53	
Deviance increase[b]		
Trout presence	86.8	(27.2)***
Surface area	38.8	(12.1)***
Location	37.1	(11.6)***
Altitude	34.3	(10.8)***
Helicopter stocking	15.5	(4.8)***
Forestry road	2.9	(0.9)***

Only variables that were significant at the stepwise procedure are included in the table

[a] Sometimes referred to as "residual" deviance

[b] Deviance increase means the increase in deviance resulting from dropping the selected variable from the model. The percentage increase is given in parentheses, and was calculated as [deviance increase/(null deviance-model deviance)] × 100 (Knapp 2005)

Asterisks indicate the level of statistical significance associated with each variable: * $p \leq 0.01$ and $p > 0.001$; ** $p \leq 0.001$; $p > 0.0001$; *** $p \leq 0.0001$ and *NS* not significant ($p > 0.01$)

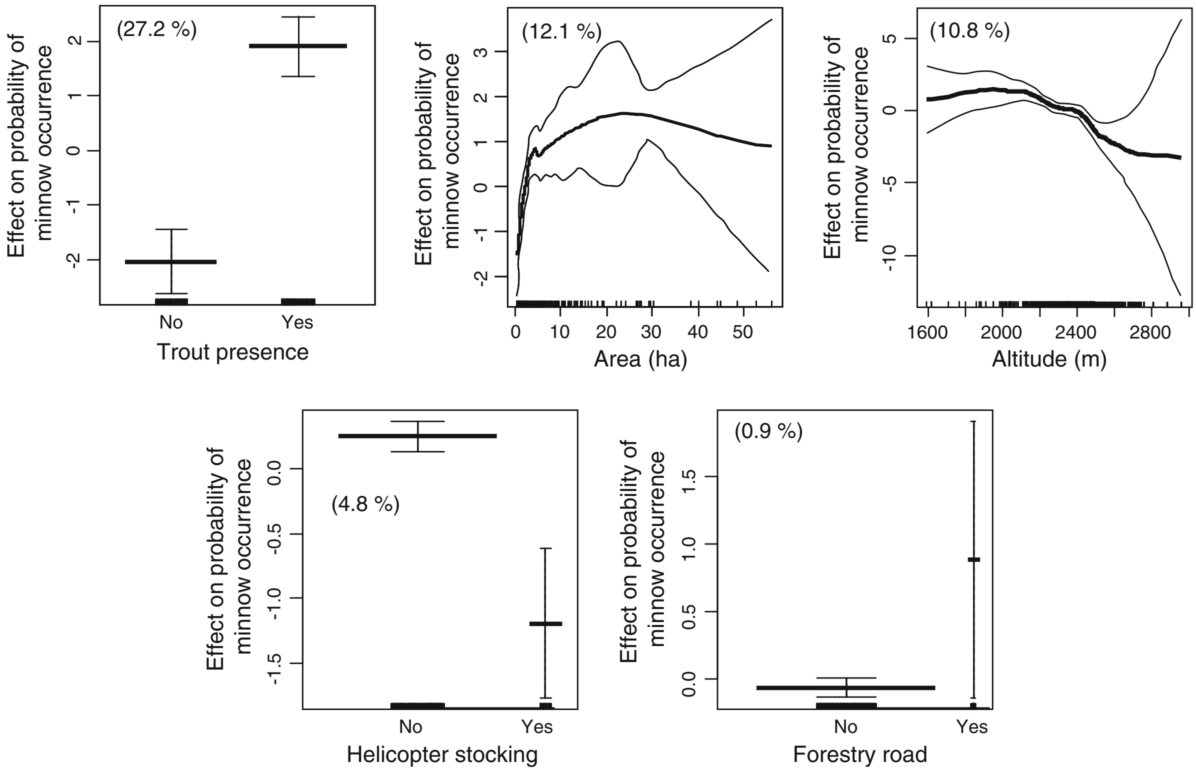

Fig. 3 Estimated effect of each of the highly significant ($p \leq 0.01$) predictor variables on the probability of occurrence by minnow, as determined from the generalized additive model (span = 0.5). Response curves are based on partial residuals and are standardized to have an average probability of zero. Thin *lines* are approximate 95 % confidence intervals and hatch marks at the bottom are a descriptor of the frequency of data points along the gradient in continuous variables or within each category for categorical variables. The width of *horizontal lines* in categorical variables is proportional to the frequency of the data within each category. *Numbers* in *parenthesis* are the percentage of explained deviance of each variable. See Table 2 for model details

continuously distributed predictor variables were all significantly nonlinear (Fig. 3). The presence of trout explained 27.2 % of deviance of minnow occurrence (higher probability when trout were present either now or previously in the lake), more than twice than the second most important variable. The response curve describing the estimated effect of lake surface area on the probability of minnow occurrence (p_i) indicated that p_i was low at the smallest lakes, but increased steadily until ca. 3 ha when it increased slowly until ca. 25 ha and then decreased gradually. Minnow presence was a constant function of altitude until 2,300 m, when it decreased progressively. Categorical variables representing different management practices (helicopter stocking and forestry road) contributed only a little part on the deviance explained by the model. The response surface for water body location is not provided in this figure as it was complex and offered no additional insights into the effects of the different predictor variables on species occurrence.

Minnow appears to be particularly linked to the previous trout presence in the lake. After controlling for the effects of habitat and spatial variables, minnows were 54 times more likely to be found in trout-containing water bodies than lakes without trout (odds ratio, with approximate 95 % confidence limits 17–169).

The lakes with introduced minnows had significantly lower altitude, greater surface area, higher accumulated temperature, shorter walking effort from the nearest town and greater catchment area compared to the lakes stocked only with trout. In contrast, fishless lakes had the highest altitudes and walking effort and lowest temperatures and catchment areas (Fig. 4). Lakes stocked only with trout had intermediate values for these variables.

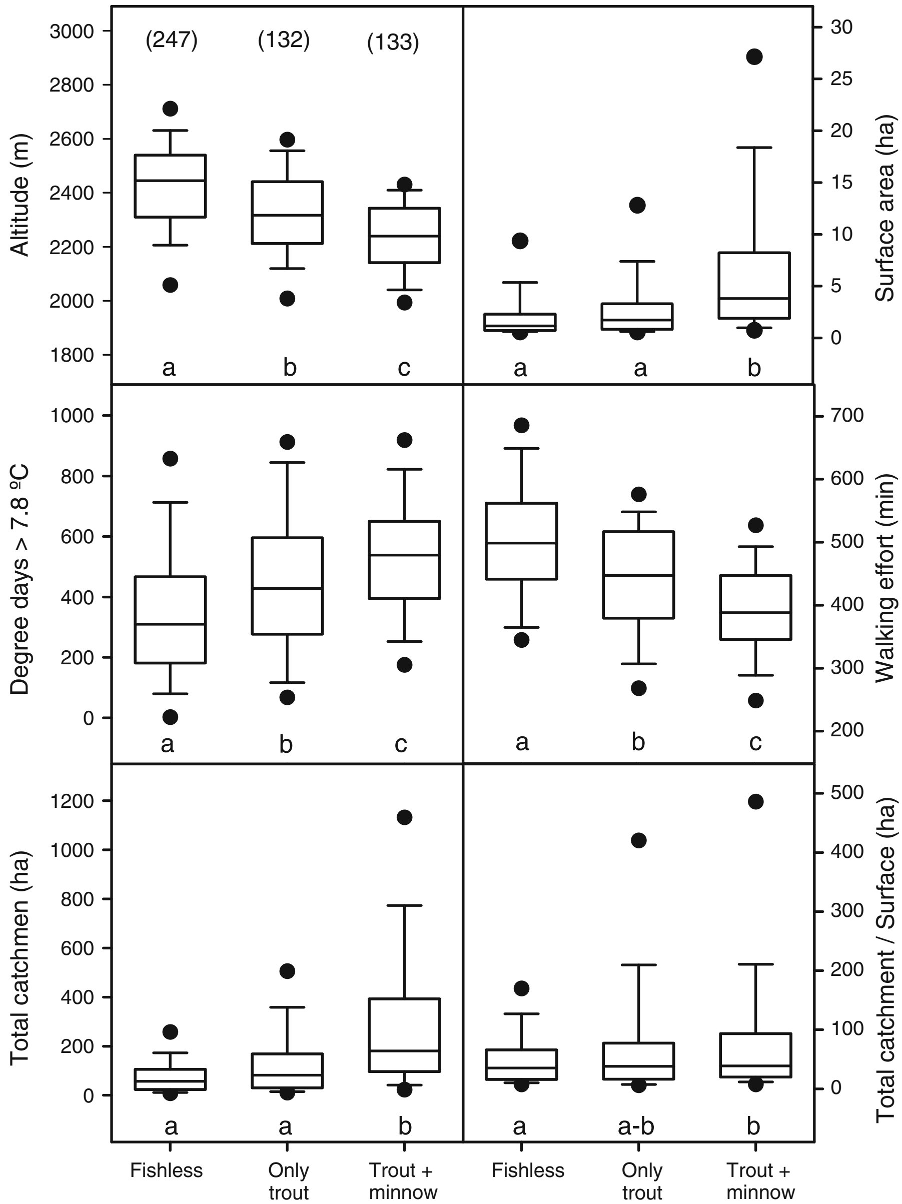

Fig. 4 Box plots showing elevation, surface area, accumulated degree days above 7.8 °C, total catchment, the ratio of total catchment to lake surface area, and the walking effort from the nearest town to each lake, of fishless lakes and stocked lakes with trout and with trout + minnow. The *line* within each box marks the median, the bottom and *top* of each box indicate the 25th and 75th percentiles, the *whiskers* below and above each box indicate the 10th and 90th percentiles, and the points above and below the *whiskers* indicate the 5th and 95th percentiles. Sample sizes for each category are given between brackets in the first panel. Categories with *different letters* are significantly different at the $p < 0.05$ level (one way ANOVA, Tukey post hoc)

Minnows were significantly more likely to be found in the lakes with prior trout presence ($\chi^2 = 159.9$, $p < 0.0001$), within active fish management areas ($\chi^2 = 49.8, p < 0.0001$), in lakes with hydroelectrical power exploitation ($\chi^2 = 44.1$, $p < 0.0001$), or with nearby forestry roads ($\chi^2 = 44.8$, $p < 0.0001$). In contrast, the probability of occurrence in lakes within National Parks was not statistically different from the lakes outside them ($\chi^2 = 1.36, p = 0.244$), as well as in lakes with helicopter-based trout introductions compared to lakes where trout were introduced with other methods ($\chi^2 = 2.36$, $p = 0.124$).

In the previous statistical models, National Park does not appear as a significant variable in explaining minnow distribution. However, if we examine the accumulated percentage of lakes with minnow at the final part of the twentieth century (Fig. 2b), we can see that only in the non-fishing area, the number of lakes with minnows stopped increasing from the time of the prohibition, while in the other areas it continued to increase.

Discussion

Introduction mediated by invasive trout

The results of this study strongly suggest that the invasion of minnows in the high mountain lakes of the Pyrenees is mediated by a preceding invasive species and facilitated by human activity. We found that the introduction of minnow is a more recent and faster process than those of salmonids (Fig. 2a). Since 1970, when the first introduction took place, it has now spread to 27 % of the lakes of the southern Pyrenees with an introduction rate of 4.7 pa, compared to those of trout, at 2.2 pa for the period between 1940 and 2000. The results of GAM indicate that the presence of trout before minnow introduction is by far the most important variable explaining minnow's distribution. In fact, we have not found any case of minnow introduction in naturally fishless lakes, indicating that recreational fishing with live-bait is likely to be responsible for these introductions (ca. half of the lakes with salmonids; Miró and Ventura 2013). The higher probability of finding minnows at lower altitude lakes and closer to forestry roads also support this idea, since both are elements facilitating the access of fishermen to lakes.

In high mountain lakes, trout are the only species group authorised for introduction by governmental agencies worldwide (e.g. Sostoa and Lobón-Cerviá 1989; Wiley 2003), mainly associated with recreational fishing (Cambray 2003). Unlike trout, minnow introductions are in general not authorised by governmental authorities and their introduction is often an illegal angling practice. The end result in many cases is that fish unused as live-bait are released at the end of the fishing expedition (Maitland and Campbell 1992; Kerr et al. 2005; Winfield et al. 2011; Kilian et al. 2012). This has been quantified to be done by 36 % of the fishermen in Michigan and Wisconsin, 41 % in Ontario and 65 % in Maryland (Litvak and Mandrak 1993; Kerr et al. 2005; Kilian et al. 2012). To prevent the widespread release of non-native species used as live-bait, many US states and Canadian territories have restricted the use, sale, or transport of bait (Kerr et al. 2005; Peters and Lodge 2009). A similar situation exists on the southern slope of Pyrenees, where the release of any organism to the environment without government authorization is also strictly prohibited (Miró 2011). Nevertheless, similar to our findings from the southern valleys of the Pyrenees, in some regions of North America a large proportion of anglers appear to be unaware of, or choose to ignore, the current regulation prohibiting the release of live organisms because they believe their actions are compassionate and that the released unused bait is suitable food for angling fishes (Kerr et al. 2005; Kilian et al. 2012). Our results strongly suggest the need to intensify preventive actions by giving accurate information of the potential negative effects for the local environment of the release of non-native organisms to fisheries boards and local communities. These actions are one of the best guiding principles to prevent the spread of invasive species together with regulation and legislation (Simberloff et al. 2013).

Live-bait related introductions of minnows have occurred in lower, boreal and arctic lakes where trout are present, but they have not been previously described in high mountain lakes. The distribution of minnow expanded considerably throughout the 1900s in the north European lakes of Scotland and Norway, especially in mountain areas, due mainly to the use of minnows as live bait for angling (Maitland and Campbell 1992; Museth et al. 2007). When minnow is introduced in lakes with autochthonous trout, it reduces recruitment and annual growth rates of trout, causing a decrease of the trout abundance by 35 % on average; however, the effect on other native fauna takes place primarily in the shallow littoral areas (Museth et al. 2007).

In the particular case of extreme habitats such as high mountain lakes that are naturally fishless, such as those of the Pyrenees, the introduction of trout results in a substantial impact for native fauna, especially for the more conspicuous organisms such as amphibians and macroinvertebrates which may be extirpated (e.g. Knapp et al. 2001b). However, minnows, like other small cyprinids, have an omnivorous and opportunistic diet similar to trout (Oscoz et al. 2008; Museth et al. 2010). They predate on zooplankton, benthic macroinvertebrates (Vinebrooke et al. 2001; Naestad and Brittain 2010) and also feed on fish eggs and hatchlings (Kottelat 2007). Their small size allows them to access shallow areas which trout cannot reach, thus eliminating the possibility that other taxa such as amphibians can shelter in littoral areas of the lake. Consequently, the introduction of minnows as a second top predator, will likely result in a stronger negative effect on the native fauna as has been found in other aquatic ecosystems (Nyström et al. 2001). In fact, preliminary results obtained in Pyrenean lakes seem to confirm the negative effects of this small fish (A. Miró, Pers. Comm.).

Minnow invasive potential

Differences in minnow life history characteristics compared to those of trout give them a higher acclimation success and therefore an extraordinary invasive potential. The minnow, like other small widely-distributed freshwater fish, displays a remarkable variability in its life history depending on the site temperature. For example, minnows have maximum age ranges between 3 and 13 years reaching maturity between 1–2 and 5–7 years in hot and cold places respectively, while show significantly lower growth increments in cold summers (Mills 1988). This plasticity in their life history is what has allowed the species to easily adapt to high mountain lakes, showing higher resistance to harsh conditions than trout. In our dataset, we have not found any lake where minnows have disappeared once established. In contrast, in lakes with trout, between 10 and 44 % of the populations go extinct after 20–30 years due to a lack of favourable conditions (Knapp et al. 2005; Miró and Ventura 2013). Moreover, in Pyrenees, we have found that in eight lakes, preexisting trout populations have disappeared after minnow introductions. Some of these lakes had brown trout introduced centuries ago while others were stocked recently.

Also, minnow presence was more likely at lakes with lower elevations, greater surface areas and higher temperatures. This could be a result of the pattern of introductions (e.g. fishermen using live bait do not fish so often at the upper altitude lakes) or to acceptable habitat conditions for released minnows to become established. Taking into account the high adaptability of minnows, it seems more likely that our finding is a result of the pattern of introduction.

As well as minnows, other species might be used as live bait, increasing the number of invasive species to lakes. This is the case for gudgeon (*Gobio* sp.), which is now found in some lakes of the northern slope of the Pyrenees (Miró 2011). This fish has similar size and flexible life-history features to those of minnows (Tang et al. 2011). Thus the ecological effects of fish introductions in high mountain lakes can result in stronger unpredicted consequences.

Management practices

In this study we have found that the only area where minnow introductions have stopped increasing is at that area of the Aigüestortes i estany de Sant Maurici National Park where fishing has been prohibited since 1988 (Fig. 2b). Similar results have also been found for trout (Miró and Ventura 2013) and this indicates that to date the only management practice that is effective in stopping minnow introductions in high mountain lakes is to ban fishing. This is especially the case for the southern Pyrenean lakes, where the use of live-bait is prohibited, and therefore minnow introductions occur out of the control of governmental agencies. It is necessary to improve the regulation of the activity, and in the cases where maximum protection are required, authorities might even consider the prohibition of fishing.

Conclusions

Minnows have been introduced in 27 % of high mountain lakes of the Southern slope of the Pyrenees as a result of releases by fishermen using it as live-bait. This invasion took place in only the last three decades of the twentieth century. Although this fishing technique is forbidden in the Southern slope of the Pyrenees since 1966, this has not prevented their introduction in high mountain lakes and the spread of the species. In contrast, trout fishing prohibition since 1988 in the core area of Aigüestortes i Estany de Sant Maurici National Park has been an effective measure to stop minnow introductions. Our results show that there is almost no natural minnow spread beyond the lake of introduction. Therefore, if new introductions are prevented, spread may be contained to only those lakes where introductions already occurred. In order to prevent further minnow introductions we suggest, apart from to studying fishing prohibition in high protected areas, conducting intensive information campaigns to fishermen, local communities and administrative offices in the areas where trout fishing is allowed.

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