Historical use, fishing management and lake characteristics explain the presence of non-native trout in Pyrenean lakes: Implications for conservation

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Abstract

High mountain lakes are naturally fishless, although many have had introductions of non-native fish species, predominantly trout. Predation on native fauna by introduced trout involves profound ecological changes. The objective of this study was to reconstruct the historical process of trout introduction in 520 high mountain lakes >0.5 ha of the southern Pyrenees and quantify which particular factors either environmental or anthropogenic, best explained their present distribution and lake conservation status. The first written evidence of trout introductions dated back to 1371 AD. By 1900, trout had been introduced to 26.5% of lakes during preceding centuries. A subsequent wave of introductions began in 1960 when stocking led to trout becoming established in 52.5% of lakes. From 1900–1950, walking distance from nearby urban centres was the dominant factor explaining 29–60% of the variation in trout distribution, indicating that trout introductions were in lakes closer to human settlements. In contrast, with the onset of modern fish management that took place during the period 1960–2000, the most significant factors were both the management practices and lake characteristics which are likely related with the probability of survival of the fish populations. After 2000, the remaining fishless lakes were those of highest altitudes, and the shallowest and those with the lowest surface area. The recent fishing ban in protected areas of National Parks has resulted in a stabilization of the number of lakes with fish, suggesting that this is the only reliable management policy to avoid new introductions and preserve the conservation status of high mountain lakes.

Keywords: invasive species, trout, high mountain lake, National Park, lake conservation, fish introductions

1. Introduction

Previous studies of the effects of invasive species in freshwater ecosystems have shown negative ecological consequences (Vitule et al. 2009). Freshwater fishes are one of the animal groups with higher number of invasive species, as has been shown in Europe (Hulme et al. 2009). At present, the introduction of freshwater fishes is closely related to human activities (Marchetti et al. 2004), and particularly with angling in the case of salmonid fishes (Cambray 2003). Within high mountain lakes, the introduction of non-native fish species (mainly salmonids), is a global threat with common origins, mainly related with recreational angling and often promoted by different relevant governmental agencies (Pister 2001; Schindler and Parker 2002). These ecosystems are originally fishless due to natural barriers that have prevented the natural colonisation of fish species from lower streams (Knapp et al. 2001a; Pechlaner 1984).

Introduced trout become the top predators of high mountain lake ecosystems leading to profound ecological changes. Predation on native fauna can lead to the elimination of amphibian and reptile populations (Knapp 2005; Knapp et al. 2001b; Martinez-Solano et al. 2003; Orizaola and Brana 2006; Pope et al. 2008), changes in zooplankton and benthic macro-invertebrate species composition and size structure (Brancelj 2000; de Mendoza et al. 2012; Knapp et al. 2001b; Toro et al. 2006), alteration of ecosystem process such as nutrient cycling (Schindler et al. 2001) and indirect effects through resource depletion (Epanchin et al. 2010). Trout introductions are therefore a threat for the conservation of high mountain lake biodiversity in general and in particular for the most conspicuous animal groups.

In order to implement effective conservation measures, it is necessary to have a precise knowledge of trout distribution and the factors that determine their presence. Detailed information on the causes of the spread of salmonid fishes exist for some high mountain areas

such as western North America (Bahls 1992; Schindler 2000; Wiley 2003), where introductions took place between the end of the nineteenth and beginning of the twentieth century. Trout introductions were undertaken initially by individual fishermen and, a few decades later, by governmental agencies responsible for fisheries management (Schindler 2000). In contrast to the American continent, in European high mountain lakes the colonisation process has not been studied in detail (Gliwicz and Rowan 1984; Pechlaner 1984; Sostoa and Lobón-Cerviá 1989). The first introductions in the Alps were carried out at the end of the sixteenth century (Pechlaner 1984) and in the Tatra mountains at the end of the nineteenth century (Brancelj 2000; Gliwicz and Rowan 1984). In the Cantabric mountains (Iberian Peninsula) introductions also occurred at the end of the nineteenth century (Terrero 1951) and even more recently in the Sistema Central and Sistema Iberico, also in the Iberian Peninsula (Martinez-Solano et al. 2003; Toro et al. 2006).

In the Pyrenees it has been suggested that most fish introduction events are relatively recent (Sostoa and Lobón-Cerviá 1989). The different trout species introduced in the Pyrenees include *Salmo trutta*, *Oncorhynchus mykiss* and *Salvelinus fontinalis* in the southern (Spanish) Pyrenees (Sostoa and Lobón-Cerviá 1989) and these species together with *Salvelinus alpinus* and *Salvelinus namaycush* in the northern (French) Pyrenees (Delacoste et al. 1997). At a global scale, *S. trutta* and *O. mykiss* are considered among the 100 most invasive alien species of the world (Lowe et al. 2000). Within Europe, *O. mykiss*, *S. fontinalis* and *S. namaycush* have been introduced from North America, while *S. trutta* and *S. alpinus* which are native European species, have been widely introduced beyond their native range, mostly in high mountain areas. Therefore they are all classified as European alien species (Hulme et al. 2009).

Among the different approaches used to reconstruct the fish introduction processes in lakes, the review of historical information has been very useful (Le Cren et al. 1972; Pechlaner 1984). Studies with a historical perspective are necessary to understand the impacts of introduced species (Kulhanek et al. 2011), which in turn are the basis for establishing more appropriate management policies. For example, the historical perspective has been used successfully in the North American Great Lakes, where it has had a significant potential for establishing restoration goals (Steedman et al. 1996). The combined analysis of historical information and the factors that might explain the distribution of high mountain lake fishes has not yet been conducted.

The objective of this study was to reconstruct the historical process of trout introduction events in high mountain lakes of the southern slope of the Pyrenees, to quantify which particular factors, either environmental or anthropogenic, best explain their present distribution and to evaluate which conservation measures have been most effective. In particular, we had the following specific hypotheses: (i) we expected that lakes with historical presence of trout populations would be those closer to towns and only hold the autochthonous species *S. trutta*; (ii) that modern introductions would be more likely in areas where active fish management has taken place and would involve the occurrence of other species than *S. trutta*; (iii) the absence of trout would be more likely in lakes with acid pH, higher altitudes and lower lake sizes; and finally (iv) prohibitions on fishing in National Parks would result in stopping fish introductions.

2. Materials and Methods

2.1. Study Area

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 1062 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. A more detailed description of the lake characteristics is provided in Appendix 1.

2.2. Data collection and environmental variables

The occurrence data for the different fish species were obtained from various sources depending on the period. Historical information of fish introductions (before 1900) was obtained from historical documents from local, regional and national archives. For introductions between 1900 and 2000 we collected *ca.* 6000 citations of species presence from interviews with local elderly fishermen or nature reserve guards, from local reports of fishing or walking societies and from our own visual encounter surveys. In a few cases we used gill nets to validate the information from the interviews. On average, we collected 11.6 citations per lake. We chose this method instead of only using our own surveys, since our aim was to provide a historical perspective of fish introductions and to be able to provide an objective account of the main factors responsible for these introductions.

To quantify which factors were responsible for fish introductions we generated a set of environmental variables summarizing both physical lake characteristics that might have affected the distribution of fish species and anthropogenic factors. Physical attributes from each water body included altitude, surface area, temperature and pH. Anthropogenic factors

were divided into two groups, those describing or indicators of the mode of fish introductions (walking distance from the nearest town, the size of the population of the nearest town and their number of hotel beds, records of helicopter stocking and the presence of forestry roads), and those describing the management practice where the lake is situated (Table 1). A detailed description of how the variables were obtained is described in Appendix 1.

2.3. Statistical analysis

We compared the data on the presence / absence of the different fish species in the Pyrenean lakes with the different environmental variables including lake characteristics, site location variables, mode of introduction variables and fish management practice descriptors. We used generalized additive models for the analyses because they are similar to generalized linear models, but relax 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 variables (Hastie and Tibshirani 1991; Knapp 2005). See Appendix 1 for details on the regression procedure. The analysis was run first to obtain a historical perspective every decade from 1900 until present (year 2000) with all the taxa together and secondly with the present day data for each species separately.

Finally, we compared the presence/absence of the different fish species with the categorical predictor variables by 2 x 2 contingency tables. With the different continuous predictor variables, we tested if there were significant differences between the lakes that are fishless, those stocked before the year 1900 and those after 1900 with one-way ANOVA and a Tukey post-hoc test. Data had previously been normalised.

3. Results

3.1. Historical process

More than 98% of the 520 studied lakes had actually natural barriers left after the glaciers retreated that had prevented the natural colonisation of fishes. Therefore, most fish populations found in them at present are presumed to have come from non-natural sources. Research in historical archives confirmed that exploitation of lakes by local citizens was a common traditional activity in the Pyrenees. In this case, lakes were stocked with brown trout from nearby streams where it is native. The first explicit citations dated back to the 14th and 15th centuries in Lake Evol (1371 AD and 1423 AD), in Lakes Naut and Major de Saboredo (1581 AD), in Piedrafita Lake (1624 AD) and Escrita and Peguera catchments (1674 AD) (Miró 2011). These old documents describe both the presence of trout in some lakes, and fishing for commercial exploitation of trout. After these initial historical documents, we found an increase in the number of citations, probably due to the increased amount of preserved written information. Our findings suggest that human exploitation of some of these lakes might have originated further back in time, possibly with the human colonisation of the Pyrenees.

We found sufficiently detailed information to be able to perform a detailed historical reconstruction of introductions in the 20th Century. As of 1900, there were 138 lakes (26.5% of the total) that had been stocked with trout during preceding centuries for traditional exploitation (Fig. 1). The first modern introductions using large stocks of juvenile trout grown in local fisheries occurred occasionally at the beginning of the 20th century, but it was not until 1960 that most fish stockings were recorded. During this period the number of lakes with introduced trout increased to 272 (52.5% of the sample). Prior to 1900, the trout species introduced was exclusively brown trout, which was also the species with the highest increase

during the last decades of the 20th Century, increasing from 153 lakes in 1950 (29.4%) to 262 (50.4%) in 2000 (Fig. 2). The other species found, *Oncorhynchus mykiss* and *Salvelinus fontinalis*, originally from North America, were first recorded in 1950 being present in 32 (6.2%) and 33 (6.3%) lakes respectively (Fig. 2). These latter introductions were carried out mostly by governmental agencies but also by hydroelectric exploitation companies and local fishermen's societies.

Those lakes that had been stocked before 1900 had significantly higher surface area, shorter walking distance from the nearest town and higher catchment area compared to the lakes stocked after 1900. In contrast, fishless lakes had the highest altitudes, greatest walking distances and lowest temperatures (Fig. A1). Lakes stocked after 1900 had intermediate values for these variables.

Among the different factors explaining trout introductions during the 20th century, there was a clear difference between the first and second half of the 20th century. From 1900-1950, walking distance from the nearest settlement was clearly the dominant factor explaining from 29 to 60 % of deviance: the lakes where fish were introduced were those closer to human settlements. This factor was followed in significance by lake surface area (13 – 30% of deviance increase) and lake location and altitude (Fig. A2). In contrast, with the onset of modern fish management, walking distance ceased to be the most explicative factor during the period 1960-2000. Management factors, such as fishing zone or helicopter stocking area (only during the last decade, 1990-2000), and lake characteristics such as lake surface area, altitude and temperature were the most relevant factors. These results indicated that both the management practices and lake characteristics were the most relevant factors for explaining the presence of the fish population in modern fish introductions.

3.2. Factors explaining the present distribution of the three introduced salmonids

Among the three trout species found in south Pyrenean lakes, *Salmo trutta* is clearly the most widely distributed. It was found in 258 (49.6%) lakes and had a significantly higher chance of being found in the lakes within active fish management areas ($\chi^2 = 138.7$, $p < 0.0001$), in lakes with HEP ($\chi^2 = 44.7$, $p < 0.0001$), with nearby forestry roads ($\chi^2 = 44.7$, $p < 0.0001$) or in helicopter stocking areas ($\chi^2 = 77.65$, $p < 0.0001$). In contrast, the probability of occurrence in lakes within National Parks was not statistically different than outside them ($\chi^2 = 0.55$, $p = 0.457$).

The generalized additive model selected nine of the thirteen predictor variables (helicopter stocking, active fish management, surface area, altitude, location, pH < 5.5, walking distance from nearest settlement, forestry road and HEP) which were significantly associated with the probability of *S. trutta* occurrence (Table A1). The relationship between the probability of *S. trout* occurrence (on a logit scale) and the important continuously distributed predictor variables were all significantly nonlinear (Fig. 3). The response curve describing the estimated effect of lake surface area on the probability of *S. trutta* occurrence (p_i) indicated that p_i was low at the smallest lakes, but increased steadily until ca. 3 ha when it was constant. *S. trutta* had an increasing function of altitude until 2300m, when it decreased progressively, and it had a constant, decreasing function with walking distance from the nearest town. Categorical variables representing different management practices contributed to approximately half of the deviance explained by the model (28.1%), with morphometric variables representing lake characteristics the other half (23.9%). The response surface for water body location is not provided in this or subsequent figures as it was often complex and offered no additional insights into the effects of the different predictor variables on species occurrence.

O. mykiss was found in 32 (6.2%) of the lakes and had a significantly higher chance of being found in lakes within active fish management areas ($\chi^2 = 196, p < 0.0001$), in lakes with HEP ($\chi^2 = 5.2, p = 0.023$), with nearby forestry roads ($\chi^2 = 43.2, p < 0.0001$), and within National Parks ($\chi^2 = 17.9, p < 0.0001$), but its occurrence was independent of the lake being within helicopter stocking areas ($\chi^2 = 0.05, p = 0.817$). The generalized additive model selected two of the thirteen predictor variables (active fish management area and forestry roads) which were significantly correlated with the probability of *O. mykiss* occurrence (Fig. A3a). Both variables were equally important within the model.

S. fontinalis was found in 33 (6.3%) of the lakes and had a significantly higher chance to be found in lakes within active fish management areas ($\chi^2 = 9.62, p = 0.002$), in lakes with HEP ($\chi^2 = 22.4, p < 0.0001$), with nearby forestry roads ($\chi^2 = 51.3, p < 0.0001$), and within National Parks ($\chi^2 = 4.66, p = 0.031$), but its occurrence was independent of the lake being within helicopter stocking areas ($\chi^2 = 0.43, p = 0.518$). The generalized additive model selected two of the thirteen predictor variables (to have a forestry road access and to be within an active fish management area) which were significantly correlated with the probability of *S. fontinalis* occurrence (Fig. A3b). Access to a forestry road had a stronger weight in the model explaining 71.3 % of deviance compared to the other significant variable (20.1%).

Finally, for the three trout species, none of the biological variables (e.g. the occurrence of other trout species) were significant, suggesting that the probability of finding one particular trout species is not directly linked with the previous presence of another species.

3.3. The role of National Parks in the conservation of lakes

In the previous statistical models, National Parks did not appear as a significant variable in explaining the historical changes or the present trout distribution. Therefore, in order to evaluate the particular role of National Parks in lake conservation, we compared the changes

in the number of lakes with fish during the twentieth century in Aigüestortes i Estany de Sant Maurici National Park with the other areas, distinguishing the two management areas of the National Park (Fig. 4a). We found that among the three areas, the number of lakes with trout only stopped increasing at the time of the ban (1988) in the area where fishing was prohibited, while at the other areas it continued to increase.

The increase in the number introductions could partially be masked if, at the same time, there were some lakes in which trout populations had gone extinct. Therefore we counted the number of lakes that had been stocked during the twentieth century and the number that at present are fishless in the three areas. The percentage of lakes that lost salmonid populations was lowest in the fishing area of the National Park, while it was higher in the area of the National Park where fishing is not allowed (Fig. 4b), although, these differences were not significant in the chi square tests ($P > 0.1$ in all cases). In contrast, the lakes where fish disappeared had a significantly higher altitude and only half of the surface area (Fig. 4c and d respectively), suggesting that the disappearances basically occurred in lakes where populations cannot persist and that had not been re-introduced, independently of the type of protection area. Within the National Park, the slightly higher rate of population disappearances in the fishing-prohibited area was likely due to re-stocking in the fishing-allowed area.

4. Discussion

4.1. First introductions: traditional exploitation of lakes

The first written documents describing the existence of fishing rights in high mountain lakes of the Pyrenees dated back to the 14th and 15th centuries. These initial introductions for traditional exploitation resulted in 26.5% of the lakes having introduced trout by 1900. Similarly, in the northern side of the Pyrenees, it has been described that ca. 25% of the lakes had fish prior to the onset of widespread introductions after 1936 (Delacoste et al. 1997), which might also be attributed to traditional fishing activities by local fishermen. Our findings suggest that human exploitation of some of these lakes might have originated further back in time, possibly back to Neolithic times, when primitive residents already used the high altitude pastures (Miró 2011). However, written evidence found suggest that the first major historical introductions occurred within the medieval warm period (1000–1300 AD) when the human population in the Pyrenees was highest (Miró 2011). Our results contrast with what has been described from other alpine areas of the world where trout introductions are a relatively recent process. In North America, introductions started in the 19th century with the arrival of European colonists (Schindler 2000; Wiley 2003) similar to most areas of Europe where it was not until the end of the 19th and 20th centuries that most alpine areas were populated with trout (Gliwicz and Rowan 1984; Martinez-Solano et al. 2003; Sostoa and Lobón-Cerviá 1989; Toro et al. 2006). Similarly, in northern European regions with many alpine lakes, such as Scotland or Scandinavia, trout introductions have occurred since the Middle Ages but were mainly at low altitudes, and it was not until the late nineteenth century that introductions to remote high mountain lakes began, mainly related with angling activities (Maitland and Campbell 1992; Nilsson 1972). The only exception is in Tyrol, in the Alps, where there are records of introduction of Arctic char (*Salvelinus alpinus*) at the end of the 15th and beginning

of the 16th centuries (Pechlaner 1984). The main difference between the introductions in the Alps and the Pyrenees was that in the Alps the introductions and fishing were performed by the nobility (e.g. King Maximilian I; Pechlaner 1984), while in the Pyrenees the rights of exploiting the lakes were given to local towns in order to keep the population in the area. As a result of this historical process those lakes that had been traditionally exploited for fish were typically those closer to towns, and were also those of higher surface area, lower altitude and bigger catchment size (Fig. 1 and A2).

4.2. Modern introductions and the role of management practices

With the onset of modern fish management that took place during the period 1960–2000, the most significant factors explaining trout distribution were related with management practices. However, different factors were important for the different species. For *Salmo trutta*, the probability of occurrence was highest in helicopter stocking areas, and in active fish management areas. The repeated use of helicopter stocking in recent decades has resulted in extensive occurrence of trout in these areas and, as a result, in a high percentage of lakes with fish (52.5%). In these areas the inverse relationship between trout presence and altitude is lost. This result is similar to that found in other areas of the world where helicopters and aeroplanes have been used for stocking trout, such as several mountain areas of West USA, where trout is present in ca. 60% of lakes (Bahls 1992).

In contrast to *S. trutta*, *S. fontinalis* was preferentially found in lakes with forestry road access, while for *O. mykiss* it was preferentially found in lakes that had a forestry road access but in areas that active fish management has been carried out. These two last species are present in a much lower number of lakes, and also were introduced mainly between 1960 and 1980 (Fig. 2). Their presence is closely linked with the majority of construction of hydroelectric schemes at high altitudes in the Pyrenees (Catalan et al. 1997). Hydroelectric

companies compensated local citizens by developing local fisheries that were used to introduce these two species to the lakes around the area where hydroelectric schemes were developed. The same hydroelectric development was responsible for the construction of forestry roads that were in turn, the main routes serving for introducing these two species. Amongst the species, *S. fontinalis* was mainly introduced in lakes with vehicle access. In the 1980s administrative changes shifted stocking responsibilities to local fishermen's societies together with governmental agencies. This favoured the closure of *S. fontinalis* and *O. mykiss* hatcheries and the consolidation of North and Central European strains of *S. trutta* in official fish hatcheries. As a result, there has been a shift from using local stocks to using races from different parts of central and northern Europe (Araguas et al. 2009).

Modern management practices have therefore resulted in high fish introductions during the last few decades with a maximum during 1990s (Fig. 2). As a result, trout have been introduced in most lakes with higher fishing interest (e.g. lower altitude or bigger surface area). This also explains that fish introductions dropped considerably during 2000.

4.3. Limits to trout survival: the role of lake characteristics

Lake characteristics were the second most important group of variables (altogether explaining 23.9% of deviance; Table A1) after those related with management. Amongst them lake surface area was the most important variable. Similarly, in the Sierra Nevada (USA) Knapp (1996) also found that those lakes with fish were significantly bigger than those without. The possible role of lake surface area on trout occurrence is likely related with the probability of population persistence. We found that those lakes that lost their fish populations had significantly smaller surface area and higher altitude. Lake surface area and lake depth are highly correlated in alpine lakes due to their common glacial origin (Catalan et al. 2009). Smaller, and therefore shallower, lakes are those where ice-cover thickness (e.g. 2–4 m,

typical of these lakes; Ventura et al. 2000) can reach lake bottom or close to it, and therefore kill all fish either directly due to disappearance of water or indirectly through disappearance of oxygenated layers below the ice-cover. The second most important factor is lake altitude, strongly correlated with lake temperature in alpine areas (Thompson et al. 2009). However, temperature itself does not seem to be the main factor under altitude, since we explicitly incorporated the accumulated summer temperature (Table 1) in the model and this variable was not selected in the analysis. It is possible that the fraction of deviance explained by altitude is more related with lake characteristics, such as benthic substrate composition, or the probability of finding inlet streams, both needed for trout reproduction and therefore population persistence (Wiley 2003).

The presence of lakes with $\text{pH} < 5.5$ also explained a significant proportion of deviance of *S. trutta*, being the probability of occurrence positively associated with pH. This species cannot survive below this pH (Rosseland et al. 1999). The Pyrenees has some lakes with such a naturally low pH occurring due to substrate geology (Casals-Carrasco et al. 2009), where although they have been stocked in the past, we do not find the presence of trout in any of them. Other trout species such as *S. fontinalis* potentially would be able to survive in some of these lower pH lakes (Korsu et al. 2007; Westley and Fleming 2011). However, the history of *S. fontinalis* introduction was closely linked with hydroelectric exploitations (see section 4.2.), and therefore the species has never been introduced in these lakes.

4.4. The role of National Parks

In this study we have found no significant effect of National Parks in the occurrence of *S. trutta*, and surprisingly the probability of finding *O. mykiss* and *S. fontinalis* was higher inside than outside National Parks. This finding contrasts with previous studies where the probability of finding trout is lower in National Parks where fishing is forbidden (Knapp

1996; Wiley 2003). For the two species with higher occurrences inside the National Park, this apparent contradiction is likely due to the higher abundance of hydroelectric exploitation inside the Park. For these two species the presence of forestry roads (build almost exclusively during lake impoundments) is one of the two most important factors explaining their distribution. In addition, before prohibition of fishing in the National Park, these areas were included within normal fish management areas, and therefore National Parks before the 1980s, had the coincidence of the two most relevant variables for explaining the occurrence of these two species.

A closer examination of the number of lakes with fish in Aigüesteortes i Estany de Sant Maurici National Park compared to the rest of the area (Fig. 4a) showed that in the area of the National Park where fishing is forbidden, the number of lakes with trout stopped increasing just at the time of the prohibition, while at the other areas, it continued to increase. This finding illustrates that the only management practice that had an effect in the protection of lakes is the prohibition of fishing. Although the most obvious route to avoid introductions would be for governmental agencies to stop stocking trout, this is complicated by the fact that through the history of trout introductions in the Pyrenees there have been several other agents involved in these introductions in addition to governmental agencies (e.g. local citizens in the past or local fishermen's societies in the last decades). As a result we have found some lakes that have been stocked recently without the collaboration of the administration.

The social implications of prohibiting fishing would not be relevant if the prohibition is implemented in those lakes that are at present fishless (at the moment there is the paradox that some lakes have fishing rights while never being stocked with fish). It could also be implemented in some particular lakes or groups of lakes that are especially important for the conservation of some animal groups (e.g. Pyrenean newt *Calotriton asper*). Finally,

prohibition on fishing has already been performed in the Aigüestortes i estany de Sant Maurici National Park, with no documented adverse social impact: basically local citizens realised that the social benefits of nature preservation (through higher tourism) have been greater than those that were obtained via fishing.

Although the purpose of this paper was not to explicitly quantify the relative proportion of established (self-sustainable) populations, our results suggest that there is a high proportion of the populations that are established. This is obvious with the historical introductions prior to 1900 (the lakes still have trout at present) but less clear with the introductions that occurred along the twentieth century. By comparing the data of the recorded introductions with the persistence of populations at the year 2000, we were able to quantify the number of lakes where trout populations have not been able to survive. We found a small percentage of stocked lakes where trout disappeared (Fig. 4b). As we discussed in section 4.3. the disappearance was not related with any of the protection figures, but with physical lake characteristics, such as higher altitudes and smaller surface areas (Fig. 4c, d). Moreover, previous studies have suggested that fish naturalisation is relatively infrequent, due to the requirement of stream inlets connected with the lakes (Lek et al. 1996). It is also possible that we did not find a higher reduction of trout disappearances in the non-fishing area of the National Park due to the relatively recent date of fishing prohibition. Previous studies have described that it takes several decades (between 20 and 30 years, but in some circumstances even 40) before populations disappear from the lakes where they have been introduced and that have unsuitable characteristics for trout populations to persist (Knapp et al. 2005). We would expect, therefore, that in the next few decades there will be a higher increase of fish disappearances in the protected area of the National Park.

5. Conclusions

The results found in this study, together with those of other areas (Knapp 1996; Wiley 2003), show that when fishing or fish stocking is forbidden, the number of lakes with trout stop increasing and tend to decrease. In contrast, in National Parks where fishing and stocking are allowed, the number of lakes with trout increase until nearly all have fish (fishing allowed area of Aiguestortes i estany de Sant Maurici National Park, Fig. 4; and National Park of Pyrénées; Delacoste et al. 1997). In this latter case the conservation status of those animal groups affected by fish introductions will be affected.

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Figure 1. Fish species occurrence and distribution of studied lakes along the Pyrenean range at the year 1900 (upper panel) and at the year 2000 (lower panel). Note that in 43 lakes there are more than one trout species per lake that are not visible in the map. In particular we have found that 32 lakes have two trout species, mainly *S. trutta* with *S. fontinalis* or *O. mykiss*. In addition, 11 lakes have the three trout species. Asterisks in the upper panel are the local towns.

Figure 2. Introduction process of non-native trout species in the southern Pyrenees during the twentieth century. Vertical gray bars are the decadal total number of lakes that have suffered fish introductions in percentage of the total number of lakes > 0.5 ha of the study area (n=520).

Figure 3. Estimated effect of each of the highly significant ($P \leq 0.01$) predictor variables on the probability of occurrence by *Salmo trutta*, 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 A1 for model details.

Figure 4. (a) Effect of Aigüestortes i estany de Sant Maurici National Park on the introduction of nonnative trout species in high mountain lakes of the Pyrenees. Circles are the lakes within

538 the National Park, and squares are lakes outside the National Park. White circles are the area
539 of the National Park where fishing is not allowed and black circles are the lakes in fishing
540 allowed areas. (b) Percentage of lakes (respect to the total number of each area, in brackets) in
541 the two management areas of the National Park compared to the rest of the study area. (c and
542 d) Box plots of the altitude and surface area of the lakes that have been stoked with trout after
543 1900 distinguishing those that are still with fish from those that are now fishless. The lines
544 within each box follow Figure 3. Sample sizes are given in parentheses in (c). Categories with
545 different letters are significantly different at the $P = 0.05$ level (Mann Whitney U test).
546

Table 1. Description of predictor variables used in the generalised 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
	Accumulated degree days (ADD)	Thermal accumulation in degree days > 7.8°C (°C Day)
	pH<5.5 (pH)	Lakes with pH < 5.5
Mode of introduction	Walking distance (WALK)	Walking distance from the nearest town (minutes)
	Population (POP)	Inhabitants of the nearest town where the lake is situated.
	Hotel beds (HOT)	Number of hotel beds in the nearest town where the lake is situated.
	Helicopter stocking (HEL)	Helicopter stocking in the lake
	Forestry road (FROAD)	Lakes with forestry road access
Management practice	Fishing zone (FIZ)	Active fish management, at present or in the past
	National Park (NATP)	Part of the National Park where no fishing is allowed at present
	Hydroelectrical power (HEP)	Lakes with water level regulation
Biological parameters	<i>Salmo trutta</i> presence (STRU)	Presence of <i>S. trutta</i> . Only for <i>S. fontinalis</i> and <i>O. mykiss</i> models
	<i>Salvelinus fontinalis</i> presence (SFON)	Presence of <i>S. fontinalis</i> . Only for <i>S. trutta</i> and <i>O. mykiss</i> models
	<i>Oncorhynchus mykiss</i> presence (OMYK)	Presence of <i>O. mykiss</i> . Only for <i>S. fontinalis</i> and <i>S. trutta</i> models

Figure 1

Figure 2

Figure 3

Figure 4

Supplementary information Miro & Ventura Appendix 1

Supplementary methods

1. Materials and Methods

1.1. Additional description of the study area

The studied lakes range in altitude between 1600 and 2960 m with the highest frequency found at ca. 2400 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, they have a close positive relationship between surface area and maximum depth (Catalan et al. 2009b). 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 bedrocks, 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 training waters ($\text{pH} < 5.5$). The ionic content is generally low, being the chemical variability mostly related with bedrock composition (Catalan et al. 1993). The lakes have low phosphorous and chlorophyll content being of oligotrophic nature (Buchaca and Catalan 2007). A general description of the climate, physic-chemical and biological characteristics of Pyrenean lakes and their comparison with other European high mountain lakes can be found elsewhere (Camarero et al. 2009; Catalan et al. 2009a; Kernan et al. 2009; Thompson et al. 2009).

1.2. Detailed description of the data collection and environmental variables

Altitude and surface area were obtained from a GIS generated from 1:25000 maps from the various national agencies (Casals-Carrasco et al. 2009). 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 lower threshold of each day (Baskerville and Emin 1969). We used the lower developmental temperature of 7.8°C as the lower threshold below which salmonid reproduction is not feasible (Elliott et al. 1995). Daily water temperatures were obtained from 45 automatic thermometers (Vemco Minilog-T) deployed at a depth of ca. 1m in lakes covering a wide range of altitudes, surface areas and catchment sizes. This ice-free accumulated temperature was assumed to be representative of the epilimnion (fish mainly feed and spend their time in this upper layer of lakes during the ice-free period) following previous limnological knowledge of these lakes and calibration measurements performed seasonally at different depths. The calculated ADDs from the lakes with automatic thermometers were extrapolated to all other lakes using a regression model between ADD and various morphometric parameters. This was possible since the temperature variability in alpine areas follows a close relationship with altitude and also with lake size and water renewal time among other variables (Thompson et al. 2009). We chose this approach rather than assuming that altitude was the parameter mostly explaining differences in water temperature for getting a closer estimation of the differences in water temperature among this big subset of lakes covering a wide range of altitudes and morphometric variables. We introduced the measured ADD from the 45 lakes as the dependent variable in a multiple linear regression model and chose the minimum combination of morphometric variables that

described most of the variance with stepwise forward selection procedure obtaining the following model:

$$\begin{aligned} \text{DegreeDays}(>7.8^{\circ}\text{C}) = & -1.39 \text{ ALT} -21.73 \text{ SURF} -117.72 \ln(\text{CS}/\text{SURF}) \\ & -0.08 \text{ CS} - 53.71 \ln(\text{DC}/\text{CS}) \end{aligned} \quad (1)$$

$$R^2 = 0.833; F_{5, 20} = 19.9, P < 0.0001$$

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 lake renewal time.

When using pH as an environmental variable, we used a binary variable which grouped lakes with $\text{pH} < 5.5$, as below this pH fish are known not to be able to survive (Rosseland et al. 1999). In the Pyrenees, lakes with such low pH are naturally found in the areas underlain with Silurian slate. These rocks contain a high proportion of sulphates that give this natural acidity. The occurrence of these lakes was found by combining a lake lithological classification (Casals-Carrasco et al. 2009) with the validation of field measurements of pH.

Among the factors indicating the mode of fish introductions we estimated the walking distance from the nearest town (WALK; in minutes) which could be a good predictor of the likelihood of a lake to have fish introduced, especially for the period prior to 1900, when all introductions are believed to have been undertaken by local citizens on foot. The walking distance is largely a function of two variables, the

difference in height from nearest town and the lake, and the distance. To estimate it, we chose a representative subset of lakes (ca. 70 lakes from different areas) for which we quantified the walking time from the nearest town by climbing ourselves 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):

$$\text{UPWALK} = 0.157\text{ALTDIFF} + 0.008\text{DIST} - 4.279 \quad (2)$$

$$R^2=0.942; F_{2,72}=563.6; P<0.001$$

$$\text{DOWNWALK} = 0.078\text{ALTDIFF} + 0.013 \text{ DIST} - 5.732 \quad (3)$$

$$R^2=0.885; F_{2,69}=258.9; P<0.001$$

The total walking distance was then obtained by adding UPWALK with DOWNWALK. We also used the size of the nearest town and the number of hotel beds at the time of the fish introduction obtained from national historical inventory surveys with the hypotheses that the bigger the town, the higher the probability of having people interested in introducing fish or that those towns with higher number of beds would have more tourists and that this therefore might increase the pressure for introducing fish. We also recoded if there was helicopter fish stocking in the area nearby to the lake during a certain period or if the lake had a forestry road giving car access to the lake. The management practice variables considered were whether lakes belonged to National Parks, whether the location was within an area with active fish management or if the

lake had water fluctuations for hydroelectric production (HEP). Within the study area there were two National Parks: Valle de Ordesa y Monte Perdido National Park, that due to its carbonate lithology has very few lakes (2% of the total) where fishing is allowed. In addition, Aigüestortes i Estany de Sant Maurici National Park has 37% of the total lakes under two different management regimes: a core area with strict regulations where fishing has been banned since 1988 and a peripheral area where fishing is allowed. For evaluating the effectiveness of the two management strategies within National Parks, we only used the data from Aigüestortes i Estany de Sant Maurici National Park (NATP) and did not take into account the other National Park. Hydroelectric production (HEP) is not only relevant for the potential direct effect of changing water levels on fish breeding, but also because HEP companies performed fish introductions in order to compensate local towns (owners of the historical rights of exploitation of lakes) from the potential negative effects of the water level fluctuations. Finally we also used the presence of other trout species as a categorical variable to explore potential interferences and relationships among fish species.

1.3. Statistical analyses

Prior to analyses with the generalised additive models, we tested for collinearity among predictor variables by Pearson correlation coefficients (r) for all pair-wise combinations of continuous predictor variables. Correlation coefficients ranged between -0.71 and 0.60 (Table A2) and were well 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 in Knapp 2005). 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) η_i is a function of the independent variables.

For all fish species, the specific relationship we used for η was the following function of covariates:

$$\eta = \text{lo}(\text{ALT}) + \text{lo}(\text{SURF}) + \text{lo}(\text{LOCATION}) + \text{lo}(\text{ADD}) + \text{pH} + \text{lo}(\text{WALK}) + \text{lo}(\text{POP}) + \text{lo}(\text{HOT}) + \text{HEL} + \text{FROAD} + \text{FIZ} + \text{NATP} + \text{HEP} + \text{STRU} + \text{SFON} + \text{OMYK}. \quad (4)$$

Where $\text{lo}(\cdot)$ represents a nonparametric loess smoothing function that characterizes the effect of each continuous independent variable on p_i . The location covariate $\text{lo}(\text{LOCATION})$ 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 and 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 each fish species. 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 $P \leq 0.01$.

For all regression models, the relationship between the significant predictor variables and the probability of species occurrence is shown graphically in separate plots separating the unshared fraction of variance that each factor explains (Figures 3 and A3). 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 species 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 the fish species was, in a relative sense, most likely to be detected at sites at intermediate elevations and less likely to be detected at sites of both low and high elevations (Knapp et al. 2003). All regression-related calculations were conducted using *R* (R Development Core Team 2010) using the function library *gam*.

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Table A1. Results of generalized additive models developed for each fish species introduced in the Pyrenean lakes. 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))x100 (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 ($P > 0.01$).

Parameter	Species					
	<i>S. trutta</i>		<i>O. mykiss</i>		<i>S. fontinalis</i>	
Null deviance	721		240		246	
Degrees of freedom (null model)	519		519		519	
Model deviance ^a	345		197		210	
Degrees of freedom (full model)	495		517		517	
Explained deviance (% of total)	52		18		15	
<i>Deviance increase</i>						
Helicopter stocking	63.6	(16.9)***		NS		NS
Active fish management	42.0	(11.2)***	19.1	(43.8)***	7.2	(20.1)**
Surface area	38.8	(10.3)*		NS		NS
Altitude	32.3	(8.6)***		NS		NS
Location	19.9	(5.3)*		NS		NS
pH < 5.5	18.9	(5.0)**		NS		NS
Walking distance	11.2	(3.0)***		NS		NS
Forestry road	11.3	(3.0)***	20.8	(47.5)***	25.6	(71.3)***
Hydroelectrical Power	1.4	(0.4)***		NS		NS

^a Sometimes referred to as “residual” deviance.

Table A2. Pearson correlation among continuous variables. * denote correlations significantly different from zero ($P < 0.05$).

	SURF	ADD	WALK	POP	HOT
ALT	-0.125*	-0.715*	0.605*	-0.128*	-0.063
SURF		0.313*	-0.090*	-0.020*	-0.042
ADD			-0.407*	0.124*	0.001
WALK				0.118*	0.173*
POP					0.519*

Figure A1. Box plots showing elevation, surface area, accumulated degree days above 7.8 °C, walking distance (in minutes), total catchment, the ratio of total catchment to lake surface area, and the walking distance from the nearest town to each lake, for fishless lakes, stocked <1900 and stocked >1900. 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).

Figure A2. Relative importance of the factors affecting salmonid introductions in the southern Pyrenean lakes during the last century. Only the deviance increase explained by each factor and for each decade is plotted.

Figure A3. Estimated effect of each of the highly significant ($p \leq 0.01$) predictor variables on the probability of occurrence of (a) *Onchorynchus mykiss* and (b) *Salvelinus fontinalis*, 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 A1 for model details.