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Recovery of high mountain Alpine lakes after the eradication of introduced brook trout *Salvelinus fontinalis* using non-chemical methods

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Abstract. Fish stocking is a serious threat to originally fishless mountain lakes. We used non-chemical eradication methods (i.e. gillnetting and electrofishing) in four high mountain lakes in the Gran Paradiso National Park (Western Italian Alps) to eradicate alien brook trout *Salvelinus fontinalis*. Data of amphibians, macroinvertebrates, zooplankton, chlorophyll-a, nutrient concentrations, and water transparency were used as indicators of the recovery process. All treated lakes were returned to their original fishless condition in spite of their different sizes and habitat complexity, without permanent negative side-effects for native species. Several ecological indicators showed that many impacts of introduced fish can be reversed over a short time period following eradication. The present study adds to a still growing body of specialized literature on the recovery of habitats after the eradication of alien species and provides further evidence that physical eradication methods are effective and can be part of a more general strategy for the conservation of high mountain lake biota.

Keywords. Gillnetting, electrofishing, ecological restoration, bycatch, Gran Paradiso National Park.

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Introduction

The ecological effects of invasive species have been described as “immense” and usually irreversible (IUCN 2000). The prevention of new introductions and the eradication of invasive species are the most important measures to contrast biological invasions (Clout and Veitch 2002). Eradication is considered an effective conservation strategy during the early stages of the biological invasions (before the invaders uncontrollably spread their range e.g. Bertolino and Genovesi 2003; Rout et al. 2009) and when the invaders are constrained by ecological barriers within isolated habitat patches. Habitat isolation, as well as being a state which promotes evolutionary processes leading in turn to unique endemic species, prevents recolonization and is in fact a key factor enabling a permanent eradication of established invasive species. Indeed, most successful eradication attempts have been carried out on islands (Bomford and O'Brien 1995; Clout and Veitch 2002). Eradications in this context have a better chance of success and are prioritised in isolated habitats where many unique biota of high conservation value, need conservation action (Whittaker and Fernández-Palacios 2007).

Most of what we know about the possibility of eradicating alien species –with particular emphasis on vertebrates– comes from several studies carried out on islands (e.g. Howald et al. 2007; Clout and Veitch 2002). However, for many aquatic taxa, similar levels of habitat isolation can occur in inland waters. High mountain lakes in particular are examples of where physical barriers can prevent many aquatic organisms from colonising headwaters from lowland habitats. The island-like nature of high mountain lakes allows for the development of greater localised genetic differentiation and adaptation (e.g. for bacteria and crustaceans; Casamayor 2017; Bellati et al. 2014). The awareness that unique and cryptic taxa may inhabit these high-altitude islands poses several issues for the conservation of their ecosystems against anthropogenic impact.

The introduction of fish associated with recreational fishing has been carried out in the majority of lakes over large mountain ranges (Miró and Ventura 2013; Ventura et al. 2017), including in remote mountain areas (Ventura et al. 2017). Introduced fish (mainly Salmonidae and small Cyprinidae, used as live baits i.e. minnows), which are likely to establish viable populations (Armstrong and Knapp 2004), are perhaps the most important threat to mountain lake biodiversity (Knapp et al. 2001). Notably, due to the presence of natural barriers such as waterfalls, alpine lake communities evolved in the absence of fish and are therefore very sensitive to fish predation. Fish stocking in once fishless lakes is commonly associated with the decline or disappearance of native aquatic species (e.g. invertebrates and amphibians; Knapp et al. 2001; Denoël et al. 2005; Tiberti et al. 2014a) and can have indirect effects on both entire ecosystems (Schindler et al. 2001; Eby et al. 2006), as well as their connection with the surrounding terrestrial habitats (Matthews et al. 2002; Pope et al. 2009; Epanchin et al. 2010; Tiberti et al. 2016).

The eradication of introduced fish from high mountain lakes is a realistic conservation measure. Whilst past eradication attempts have used chemical eradication methods (e.g. rotenone, See Ling 2002 for a review), more recent studies in the USA (Knapp and Matthews 1998; Knapp et al. 2001; Vredenburg 2004; Knapp et al. 2007), Canada (Parker et al. 2001; Pacas and Taylor 2015), and Europe (Toro et al. 2006) show that gill-netting, often combined with electrofishing, can be an effective and less invasive eradication method in mountain lakes. Whilst gillnetting is a mechanic and highly lethal eradication technique, the natural absence of native fish means that this method can be applied without concern for native fauna. Furthermore, due to the high conservation value of mountain lake biota, non-chemical methods are preferable as they reduce the impact on non-target organisms (Knapp and Matthews 1998).

The Gran Paradiso National Park (GPNP, Western Italian Alps) recently undertook an eradication campaign of brook trout, *Salvelinus fontinalis* Mitchill 1814 from four high altitude lakes, aiming to reduce the negative ecological effects of introduced fish. Almost all of the previously mentioned post invasion impacts were also documented in the GPNP lakes (Tiberti and von Hardenberg 2012; Magnea et al. 2013; Tiberti et al. 2014a; Tiberti et al. 2016; Rolla et al. 2018). Brook trout was introduced in several lakes in the 1960s and, by the time angling and fish introductions were banned in the 1970s, self-sustaining trout populations established in several lakes. Considering that brook trout reach sexual maturity at 1-4 years (Alessio et al., 1987; FishBase 2016), some tens of generations had followed one another since fish introduction, suggesting that the studied populations were well naturalized.

The present study has two main objectives: i) describe and test the effectiveness of the eradication process and the techniques used, provide information of the effort required when considering lakes with different characteristics (size and habitat complexity), and quantify any side effects (accidental death/capture) on non-target species (bycatch); ii) describe the post-eradication ecological resilience and quantify the short-term resilience potential of high altitude lakes. The former objective is addressed with a descriptive approach; the latter is based on three study hypotheses: that fish eradication should reverse the direct (H1) and indirect (H2) ecological effects of predation and that the ecological state of the fish-removal lakes should gradually resemble that of naturally fishless lakes (H3). Amphibians, littoral macroinvertebrates, pelagic zooplankton, chlorophyll-a concentration, water transparency, and total phosphorus concentration were used as indicators to monitor the recovery. Based on literature data and previous results, the three study hypotheses imply a series of predictions to be tested.

H1 implies the recovery of large zooplankton, littoral macroinvertebrates, and amphibians (Knapp et al. 2001; Tiberti et al. 2014a). In particular, we predict: i) an increase in the biomass of large zooplankton taxa, ii) an increase in the abundance of macroinvertebrate taxa and ecological groups exposed to direct fish predation (non-borrower taxa); iii) a recovery/increase in amphibian populations. Expected indirect impacts related to H2 include top-down effects within the zooplankton and macroinvertebrate communities (favoring small-bodied zooplankton and borrower

macroinvertebrates; Knapp et al. 2001; Tiberti et al. 2014a) and cascading effects (increasing primary production and nutrient availability; Schindler et al. 2001). For the testing of H2 we therefore predict: i) a decrease of the biomass of small zooplankton (Schabetsberger et al. 2009; Tiberti et al. 2014a), ii) a decrease of the abundance of borrower macroinvertebrates (Knapp et al. 2001; Tiberti et al. 2014a), iii) an oligotrophication of the lakes (Schindler et al. 2001). H3 is related to the short-term (5 years) restoration potential after the eradication. We predict that, if ecological resilience is complete or near-to-complete, the indicators of ecological resilience in the lakes treated for fish eradication should overlap the values recorded in a set of similar, never-stocked control lakes.

Methods

Study lakes

All the study lakes (4 fish-removal and 14 fishless control lakes) are located within the GPNP, at between 2000 and 3000 m a.s.l. (see Online Resource 1 “Study lakes” for a detailed description of the study lakes). Fish eradication was carried out between 2013 and 2017 in four lakes (Djouan - DJO, Dres - DRE, Leynir - LEY, and Nero – NER; Table 1). All fish-removal lakes were originally fishless but were stocked with brook trout in the 1960s, in the course of a single fish stocking campaign. Only in Lake DRE, which was incorporated in the protected area in 1979, fish introductions continued probably until the early 2000s (when a legal dispute over the park border was ended). Fish-removal lakes have a varied size, morphology, habitat complexity and ecological connections with other aquatic habitats. These differences may influence the effectiveness of eradication methods, the efforts needed to complete the eradication, and the recovery potential of the lakes. In particular, when comparing the four lakes, fish eradication was expected to be more difficult in LEY (Table 1) due to its larger size (Pacas and Tylor 2015) and in DRE due to its showing a higher habitat complexity (abundant aquatic vegetation and 380 m of permanent tributaries colonized by brook trout).

Fish eradication

Eradication methods and efforts

Intensive gill-netting and electrofishing were used as eradication methods (Knapp and Matthews 1998; Knapp et al. 2007). In addition, just before the start of the eradication campaign, two days of experimental intensive angling had already substantially contributed to the decline of the population in Lake DRE (Tiberti et al. 2017a). A variable number of gill-nets were deployed continuously in each lake during the fish eradication period, including the ice-cover season

(Knapp et al. 2007). These nets represent the fixed capture devices. The captures from the fixed capture devices were used to describe the brook trout population trends with a Capture Per Unit Effort - CPUE index of abundance (expressed as: No. of fish × $hour^{-1}$ × m^{-2} of net). Electrofishing and some additional movable gillnets were used to support the eradication efforts. Additional information on the fish capture methods, on the ecological factors which could have affected the eradication efforts, and other methodological aspects related to fish removal (i.e. fish biomass estimates) are reported in the Resource 2 "Fish capture methods and eradication efforts".

Declaring the eradication end

Based on literature (Knapp and Matthews 1998; Knapp et al. 2007), one year without fish captures is considered sufficient to declare an eradication successful if the same gillnetting effort is maintained, i.e. net surface. We extended this empirical assumption to the tributaries of Lake DRE, where we repeated several electrofishing sessions during the year following the last capture. We declared the end of the eradication when the time elapsed following the capture of the last fish reached one year. However, we tested this assumption maintaining the nets in the water and performing some electrofishing sessions even after the expiry of the one-year period, up to two years after the capture of the last fish.

Bycatch

We took note of all the animals accidentally captured in the nets during the entire eradication project. Captured individuals were counted and identified at the species/genus level, and their position in the net (net-ID) was recorded. We also took note of their survival (i.e. dead or alive when recovered in the net), but we cannot account for hidden capture traumas (e.g. internal traumas and stress) and post-release mortality.

Ecological resilience: sampling and analytical methods

Recovery indicators and sampling history

Amphibians, littoral macroinvertebrates, pelagic zooplankton, chlorophyll-a, nutrient content, and water transparency were used as indicators of the recovery process. Samples and surveys were repeated (from 1 to 8 times per year) in all the study lakes at different times during the ice-free period to account for the strong seasonality governing alpine lakes (Tiberti et al. 2013).

- Amphibians. The only amphibian inhabiting the study area is *Rana temporaria*. The presence/absence of *R. temporaria* (any stage) and of breeding populations (i.e. presence of eggs, tadpoles, and froglets) has been

monitored since 2006. From 2013-2017, the population trends of the frogs inhabiting the fish-removal lakes were monitored by counting the egg-masses.

- Macroinvertebrates. Littoral habitats were sampled for semiquantitative macroinvertebrates estimates with a standard d-frame net following Knapp et al. (2001). Macroinvertebrates were grouped into three ecological categories: swimmers, clingers, and burrowers (see Knapp et al. 2001; Tiberti et al. 2014a).
- Zooplankton. Zooplankton biomass estimates were obtained from samples collected at the deepest point of each lake with a conical plankton net.
- Chlorophyll-a and nutrients. Water samples were averaged over depth with a horizontal Van Dorn bottle and analyzed for Total Phosphorous (TP) and Chlorophyll-a concentrations.
- Water transparency. The Photosynthetic Active Radiation – PAR vertical extinction coefficient (k) was estimated for each lake from vertical profiles of light intensity.

In Online Resource 3 "Recovery indicators: sampling and analytical methods" we provide a detailed description of the sampling and analytical methods.

Ecological recovery: data analysis

In the absence of pre-stocking data, we used a quasi-experimental approach and a series of fishless control lakes as a reference against which recovery is compared. Fish removal-lakes should shift from their impacted state to a new post-impact state, resembling that which is observed in control lakes. All the statistical analyses were performed using R version 3.4.0 (R core team 2017). To test the study hypotheses and in order to compare the fish-removal and control lakes we used generalized linear mixed models (GLMM) with a Bayesian Markov Chain Monte Carlo (MCMC) approach implemented in the R package MCMCglmm (Hadfield 2010). This approach allowed us to model the response of the ecological indicators (dependent variables) to fish eradication, while accounting for repeated sampling in the lakes. Lake identity and year of sampling were fitted as additive random terms to account for repeated measurements and avoid pseudoreplication. Zooplankton biomass (log+1 transformed to approximate normality), TP, PAR attenuation coefficient (k), and Chlorophyll-a concentration were modelled using a Gaussian distribution; macroinvertebrate abundances with a Poisson distribution (family = "Poisson") and presence/absence of *R. temporaria* with a binomial distribution (family="Categorical"). Several independent variables were added to model the temporal and spatial variability of the response variables. Fixed terms to avoid collinearity Fixed terms include a series of relevant experimental and environmental variables:

- DAY: an integer variable indicating the number of days elapsed from the 1st of June and accounting for the strong seasonal variations (Tiberti et al. 2013).

- TIME: an integer variable assuming value 0 for all the samples collected before 2013 (starting year of the eradication campaign) and ranging from 1 (for 2013) to 5 (for 2017) along the subsequent monitoring period.
- TREATMENT: a two-level factor distinguishing between fish removal lakes and fishless control lakes.
- ALTITUDE: the altitude of each lake.
- DEPTH: the maximum depth of each lake, accounting for lake size, e.g. there is a positive correlation between lake area and dept (Spearman's rho = 0.87; $p < 0.001$).
- GEOLOGY: a two-level factor indicating if the lake catchments are entirely composed by acidic gneiss (AG) or at least partially covered by calcareous shists (CS): this is a very important environmental feature controlling the hydrochemistry of the study lakes, in particular, their acidity (Tiberti et al. 2010).
- TIME×TREATMENT: the interaction between TIME and TREATMENT.

A test of H1 and H2 is provided by the interaction term TIME×TREATMENT. Assuming that in the control lakes the ecological indicators should remain unaltered along with the study period, the positive and negative significant effects of TIME×TREATMENT respectively indicate an increase and a decrease of the ecological indicators. H3 is related to TREATMENT. The estimates of the effect of TREATMENT are provided at TIME = 0 (before treatment, when the fish impact on the indicator should be higher) and at TIME = 5 (at the end of the monitoring, when the fish impact should be lower): non-significant differences at year 5 indicate a rapid and satisfactory recovery. To evaluate the recovery potential of each lake treated for fish eradication the same models were refitted replacing TREATMENT with a 5-level factor distinguishing the four fish-removal lakes (1 level per lake) from all the fishless control lakes.

MCMC models were run for 130,000 iterations with a burn-in period of 30,000. The level of non-independence between successive samples in the chain was checked for all models with the "autocorr" function in the MCMCglmm package, while the convergence of the chains was checked by visual inspection. Reported credibility intervals (CI) are the 95% highest posterior density intervals. We considered a fixed effect to be significant if its CI did not include 0. We report MCMC significance P values (pMCMC) which correspond to twice the proportion of iterations in which the posterior distribution is positive or in which it is negative (whichever of the two is the smallest) (Baayen et al. 2008). Models were fitted only on common taxa/ecological groups, whose frequency of occurrence in the samples exceeded 20% (see the paragraph "Faunal assemblage" in the result section).

Results

Fish eradication

More than 20,000 fish were removed from all four lakes during the eradication campaign. Almost all fish were *S. fontinalis*, but four marble trout *Salmo marmoratus*, one brown trout *Salmo trutta*, and one minnow *Phoxinus* sp. were also captured in Lake DRE. The abundance, biomass and size distribution of removed brook trout varied considerably among lakes, as did the efforts needed to complete the eradication (Fig. 1a; Table 1). Brook trout population size and eradication efforts (in terms of number of deployed gillnets, electrofishing sessions and field surveys) were larger in Lake LEY and DRE, providing the larger and more complex habitats respectively. Following initiation of intensive gill netting, fish populations were quickly reduced, i.e. CPUE trends showed high initial values which rapidly decreased (Fig. 1b-e). A large part of the fish were caught in the early stages of the eradication process in all the fish-removal lakes, but in lake DRE many small fish were captured during the second year (Fig. 2). Complete eradication of all fish was accomplished after 1-3 years of continued gill netting (Fig. 1). Continued gill netting during subsequent 1.1-2.0 years confirmed the complete eradication of each of these fish populations.

Fish inhabiting the stream sections above and below Lake DRE were eliminated completely by the third year of the eradication process. Repeated electrofishing sessions (from 10 to 12 in each stream section) without fish captures along all the tributaries confirmed the complete eradication of brook trout. Fish remain in the stream below Lake DRE, but their reinvasion is prevented by an impassable waterfall on the outlet stream 80 m below the lake. Electrofishing was also used as a complementary eradication method in the littoral area of lakes DJO, DRE and LEY. Its contribution to fish eradication was substantial in Lake DRE, while in lakes DJO and LEY the proportion of fish captured by electrofishing was lower (Fig. 1f).

The minimum size of free swimming brook trout was 2.4 cm. Electrofishing allowed the removal of fish of any size, including smaller fish, while the minimum size of gillnetted brook trout was ca. 6-7 cm (Fig. 2). Different mesh sizes were highly selective for differently sized brook trout (Fig. 2). Most large-sized, potentially reproductive brook trout were quickly removed by intensive gillnetting (Fig. 2). The large majority of >15 cm fish (100.0 % in DJO; 99.6% in DRE; 95.5% in LEY; 98.9% in NER) were captured during the first summer. The fish captured during the following field seasons (2014-2016) were essentially young fish (Fig. 2), hatched from their nests during the previous or the same season.

The capture of recently hatched (≈ 3 cm long) brook trout during the ice-free season was indicative of the fact that some individuals reproduced during the previous breeding season (October-November). Following initiation of intensive gill netting, reproduction was soon avoided in Lakes DJO and NER. In Lake LEY, reproduction occurred during the 2013 breeding season as demonstrated by the capture of recently hatched brook trout in July 2014. In Lake DRE recently hatched brook trout were captured in June-July 2014 and 2015. In the latter case, a single nest was found

in a secondary tributary; the river section was isolated from the lake using plastic nets (1 mm mesh size), the nest was destroyed, and all the free-swimming fries were captured by electrofishing.

Bycatch

Besides introduced fish, 5 other vertebrate taxa were trapped in the gill-nets during eradication (Table 2). Their abundance was low compared to the number of captured fish (ratio 1:197). Bycatch occurred in all the fish-removal lakes, excepted Lake NER. Bycatch started from the second ice-free period (2014). Most of the accidental captures were concentrated in the nets placed closer to the shoreline. Despite bycatch mortality, all taxa persisted along the eradication process, as demonstrated by their capture in the last year of eradication (Table 2). Mortality was 82% for amphibians, 92% for birds, and 100% for water shrews. Surviving individuals were released as soon as possible close to the capture location. During the electrofishing sessions, many invertebrates (e.g. aquatic insects) and frogs were electrocuted without visible negative effects.

Faunal assemblage

Rana temporaria is the only amphibian inhabiting the lakes of the GPNP. The littoral macroinvertebrate communities were composed of several taxonomic groups including burrower, swimmer and clinger macroinvertebrates. The zooplankton communities consisted of copepods, cladocerans, and rotifers, including large (≥1.25 mm at reproduction) and small zooplankton species (<1.25 mm). The taxonomic groups divided by ecological groups are listed in Table 3.

Ecosystem recovery

In the next paragraphs we will present the results shown in Fig. 3, which provides a graphical summary of the statistical analyses (extensively presented in the Online Resource 4 “MCMCglmm Model outputs”). Before fish eradication, fish presence produced most of the expected direct ecological effects (i.e. affecting common frogs, large zooplankton species, and non-burrower macroinvertebrates; see estimates at year 0 in Fig. 3, Models a). These effects were consistent among the lakes treated for fish eradication (see estimates at year 0 in Fig. 3, Models b). Indirect impacts such as higher numbers of burrower macroinvertebrates and higher biomasses of small zooplankton species were observable, but less consistent among fish-removal lakes before eradication (see estimates at year 0 in Fig. 3, Models b). Several ecological indicators show significant trends along the eradication process (see TREATMENT x TIME

estimates in Fig. 3 and Fig, 4). Said trends are always consistent with H1 and H2 excepting the increasing trend of *E. serrulatus* in Lake DJO and the increase of chlorophyll-a concentration. These trends also usually bring the ecological indicators back to a level which is not significantly different from the estimates for fishless control lakes, as predicted by H3 for a satisfactory recovery (see estimates at year 5 in Fig. 3, Models a and b). Other covariates helped to explain the variability attributable to spatial and temporal gradients. For example, seasonal dynamics were an important significant factor for many indicators and altitude negatively affected several populations such as the common frog, Corixidae, *Cyclops abyssorum*, and *Daphnia longispina*.

R. temporaria showed a general increase in the fish-removal lakes confirming H1 predictions (Fig 3, Models a), but its recovery dynamics varied (Fig. 3, Models b). In Lake LEY, where the common frog colonized the lake and reproduced for the first time since 2006, all the recovery indicators show a significant increasing trend. In Lakes DJO and DRE, where some common frogs had already bred before fish eradication, the trend of the presence/absence indicators provided little information, but the increase of egg-masses counts (Fig. 5) provides evidence that here the recovery is better described by a numerical increase in populations rather than by presence/absence data. In Lake NER, the common frog has not bred since 2006 and only the presence of a few adult frogs suggest that a recolonization process is occurring. At the end of the monitoring period the probability of finding *R. temporaria* was similar in treated lakes and in never-stocked lakes (Figure 3, Model a).

Littoral macroinvertebrates rapidly responded to the fish removal. A general and conspicuous increase of abundance of the sensitive ecological groups (i.e. swimmers and clingers) and taxa was observed from the early stages of the eradication project in all the treated lakes, confirming H1 predictions (Fig. 4). H2 was partially confirmed by the decreasing trend of some burrower taxa (i.e. Oligochaeta), but burrower Diptera and *Pisidium* sp. did not decrease. At the end of the monitoring, the abundance of sensitive macroinvertebrates was similar in treated and control lakes, confirming H3 prediction.

When considering large zooplankton species affected by fish predation, the biomass of *Daphnia longispina* and *Cyclops abyssorum* showed a general increase after fish eradication, confirming H1 (Fig. 3, Models a and b). However, their increase did not produce an increase of the total biomass of large zooplankton species (Fig. 3). Evidence of H2 is less consistent among lakes; even if small cladocerans and *Polyarthra* sp. showed a general decrease over all treated lakes, (Fig. 3, Models a) several small taxa decreased only in some treated lakes (Fig. 3, Model b). The biomass of *Daphnia* sp. at the end of the eradication process was similar in treated and control lakes (partially confirming H3) due to a prominent increase of *Daphnia longispina* in Lakes DJO, DRE and LEY. The recovery of *Cyclops abyssorum* was less prominent and some populations are still present at low biomasses. The zooplankton community of Lake NER did not recover.

The predicted oligotrophication did not occur in the treated lakes and the only significant trend is represented by an unexpected and general increase of chlorophyll-a concentration. However, the estimated effects of fish presence before fish eradication (at year 0) did not include any serious eutrophication event and the trophic state of all the treated lakes was similar in treated and control lakes, excepting Lake DJO, which showed lower transparency and higher chlorophyll-a concentrations.

Discussion

Fish eradication

As already reported in previous studies (Knapp and Matthews 1998; Parker et al. 2001; Vredenburg 2004; Toro et al. 2006; Knapp et al. 2007; Pope et al., 2009; Pacas and Taylor 2015), gillnetting is an effective eradication method for introduced salmonids in high mountain lakes. Complementary methods such as electrofishing and rod angling were necessary or helpful to complete the eradications. The combined use of gillnetting and electrofishing had already been used in similar projects (Knapp et al. 2007; Pacas and Taylor 2015). In addition, intensive rod angling was experimentally used as a complementary eradication method in Lake DRE, enabling a substantial reduction of the initial density of adult fish (see Tiberti et al. 2017a).

In our study, lake size was similar to that of the lakes where previous eradication actions using mechanical techniques were successful. However, Lake LEY (area: 4.56 ha; depth: 22.1 m) is among the largest lakes ever treated for fish eradication (Knapp et al. 2007; Pacas and Taylor 2015). Some of the removed brook trout populations were very abundant, demonstrating that physical methods can also be effective for large populations. The large number of removed fish is mainly due to the exceptional fish abundance in Lake DRE (>15,000, including fish recruitment occurring during the eradication period). Fish abundance in Lakes DJO and LEY (Table 1) was also higher than that which is commonly observed in mountain lakes treated for fish eradication (Knapp and Matthews 1998; Parker et al. 2001; Knapp et al. 2007). In Lake DRE a very large number of small fish were captured during the second year of eradication. Due to competitive release and reduced cannibalism, the removal of large fish can enhance the survival rates of young fish and produce a recruitment spike (Hall 1991; Ludgate and Closs 2003). The occurrence of a recruitment spike only in Lake DRE could be due to the availability of shelter from cannibalistic fish (i.e. aquatic vegetation) and to the large patches of habitat suitable for reproduction such as gravel and pebbles in the littoral area and rivers.

As with previous successful eradications, the present study was also carried out within a protected area, a common socio-political context which provides some necessary regulatory/logistical tools such as the prohibition of fishing and fish stocking as well as surveillance personnel in a position to enforce it. Such measures are necessary to keep the risk of sabotage low (e.g. fish reintroduction; Ventura et al. 2017). Other than socio-political constraints, there are economic, logistical, and environmental limitations to the applicability of physical eradication methods (NPS 2013). Economic and logistical constraints largely depend on the cost of equipment/field crew and on their safe transport/stay at remote lakes. These constraints can limit the number, size and complexity of lakes treated. Environmental constraints are related to the lake size and to their habitat complexity (e.g. presence of aquatic vegetation, tributaries colonized by fish). The exact extent of the limits is not yet fully understood. Compared to initial beliefs (Knapp and Matthews 1998), physical eradication methods were effective also in relatively large and complex lakes (Pacas and Tylor 2015). In the present study, fish-removal was effective in all of the lakes, regardless of their size and habitat complexity. However, eradication efforts - including the amount of equipment, time, and work needed to complete the eradication - varied considerably among lakes. For further discussions on the deployed eradication efforts and on the factors influencing them see Online Resource 2 “Fish capture methods and eradication efforts”.

Short-term ecological resilience

The introduction of brook trout into fishless high mountain lakes in the GPNP caused dramatic changes in the biotic communities, including direct and indirect impacts on the ecological indicators used in the present study (Tiberti and von Hardenberg 2012; Tiberti et al. 2014a). The effects of introduced trout on high elevation lakes are remarkably consistent across geographically disparate areas (Knapp et al. 2001) and the GPNP lakes are no exception. Comparisons of the faunal assemblages between fish-removal lakes and fishless control lakes show that, before fish removal, the former were heavily affected by most of the expected impacts, e.g. loss of amphibians, large zooplankton species, and non-burrower macroinvertebrates (Tiberti and von Hardenberg, 2012; Tiberti et al., 2014). Even over the shorter period of time, most common taxa recovered after the trout were removed. However, the extent of the recovery varied among ecological indicators (see next paragraphs). Our results are similar to those reported by the few studies that have evaluated the recovery of faunal assemblages in high mountain lakes after fish removal (Knapp et al. 2001; Parker et al. 2001; Knapp et al. 2005; Knapp et al. 2007).

On one hand, the recovery of directly impacted ecological indicators/compartments related to H1 (i.e. amphibians, large zooplankton, and clinger/swimmer macroinvertebrates) was evident and consistent among the treated

lakes. On the other hand, the recovery from indirect impacts related to H2 and its consistency among lakes was less clear. As a matter of facts, while direct predatory impacts affected all the treated lakes in almost the same way, the magnitude/occurrence of indirect impacts varied in fish-removal lakes before eradication (Tiberti et al., 2014). This partially explains why the expected results were obtained in some lakes (i.e. those affected by the expected impact) and not in others. Despite the substantial alteration of the biotic communities by fish presence, many ecological indicators recovered after fish were actively eradicated to levels typical of those in never-stocked lakes. These results are consistent with the existing literature and confirm the high recovery potential of high mountain lakes (Knapp et al. 2001, 2005).

The recovery of the common frog over the short period (5 years) is encouraging and suggests that impact caused by fish introduction can be quickly reversed by their removal. It is probable that amphibian populations are yet to completely recover and their presence in the fish-removal lakes is expected to further increase in the next years; Lake LEY, for example, currently supports as little as one single pair of reproductive frogs. Furthermore, Knapp et al. (2001) found that the Sierra Nevada yellow-legged-frogs *Rana sierrae* recovered to levels typical of never-stocked lakes, but only after the lakes lost their fish populations for >20 years and if there were adjacent frog populations. In other cases, amphibian population recovery was very rapid with few exceptions (Vredenburg 2004; Knapp et al. 2007; Pope 2008). In lakes DRE and DJO, where a residual population was still present, the recovery process was rapid (as documented by egg-masses trends). In these lakes, population persistence was probably facilitated by the presence of aquatic vegetation, providing an antipredatory refuge (Kenison et al. 2016). However, fish introduction often causes the local extinction of amphibians (Bradford et al. 1993; Orizaola and Braña 2006; Miró et al. 2018) and population recovery is aided by the presence of immigrant individuals (Vredenburg 2004). Because many amphibians have little dispersion capacity, survival after fish introduction and recolonization after fish removal can be influenced by the presence of alternative safe sites (Denoël et al. 2016; Knapp et al. 2016; Winandy et al. 2017; Tiberti 2018) and the proximity of nearby source populations (Knapp et al. 2001; Vredenburg 2004; Miró et al. 2018). In our case, the recovery in Lake NER seemed slower than elsewhere probably because it has no nearby source populations, but similar isolated conditions did not prevent the common frog from colonizing Lake LEY. To facilitate recovery, amphibian translocations and reintroductions are actions worth considering, especially in the case of endangered amphibians (e.g. see restoration projects of *Rana sierrae* in Sierra Nevada and King Canyon National Park; NPS 2013). Sometimes the extent of fish introductions can compromise habitat suitability at a higher metapopulation level, producing declines or extinctions in entire pond and lake networks (Bradford et al. 1993; Pilliod and Peterson 2001, 2010). In this case, restoring single lakes may not be sufficient to recover amphibian populations and reintroduction could fail. A last factor which could limit the recolonization of treated lakes is the close proximity of Lakes NER and LEY to the altitudinal

limit for common frogs (i.e. 2799 m a.s.l., Lake Gias de Beu, Gran Paradiso National Park, Torino, Italy; Tiberti and von Hardenberg 2012). Considering the prominent role of introduced fish in the global amphibian decline (Kats and Ferrer 2003) and the large extent of fish introduction in mountain lakes (Ventura et al. 2017), our results provide a viable solution to limit the loss of amphibian biodiversity at least in mountain areas.

Macroinvertebrates were particularly resilient. Most common clinger/swimmer taxa recovered to levels at or above that of never-stocked lakes. This is almost identical to what has been reported in similar studies and whilst it may have been facilitated by the strong flying abilities of their adult stages, we must also consider that strictly aquatic taxa such as water mites also recovered quickly. Among non-burrower taxa, only *Crenobia alpina* (Tricladida) and nektonic Diptera (largely dominated by Chironomidae pupae) did not show the expected increasing trend. The former has a relatively low frequency of occurrence and has limited dispersion capacity, which may have influenced its resilience potential or the power of the model. The latter are mostly nektonic stages of otherwise borrower taxa (Chironomidae larvae); their larvae may be positively influenced by fish presence (Knapp et al. 2001; Tiberti et al. 2014a), producing an increase of their emergent pupae (Finlay and Vredenburg 2007; Pope et al. 2009; and Tiberti et al. 2016 for a study focused on the study lakes). Most burrowing taxa showed an increasing trend over TIME, which we consider a sampling artefact (because sampler expertise increased over the study period and inconspicuous taxa, such as small Chironomidae and Oligochaetes, were initially undersampled). Some burrower taxa (i.e. Oligochaetes) can be favoured by fish presence (Knapp et al. 2001; Tiberti et al. 2014a), and the observed decreasing trends during the eradication process can provide some evidence of lake recovery due to indirect top-down ecological impacts (Knapp et al. 2001). Such a conspicuous recovery is likely to cause an improvement in terms of functional diversity, involving shifts in the overall food-web functioning.

After fish disappearance the biomass of larger zooplankton taxa (*Daphnia* sp. and *Cyclops abyssorum*) increased or even returned to levels characteristic of never-stocked lakes. Similar results have also been reported for zooplankton communities in other high mountain lakes (McNaught et al. 1999; Knapp et al. 2001; Parker et al. 2001; Schabetsberger et al., 2006; Knapp and Sarnelle 2008). However, the total biomass of large zooplankton species and the biomass of the large bodied copepod *Arctodiaptomus alpinus*, did not show a general increase in the fish-removal lakes. Parker et al. (2001) also observed that the recovery of some large bodied species did not produce an increase of the total zooplankton biomass and the same observation was made during a comparative study of GPNP lakes with and without fish. The recovery of *Daphnia longispina* is particularly significant from an ecological point of view, because *Daphnia* is considered a key species in high mountain lakes. The rapid recovery of *D. longispina* after fish disappearance was likely facilitated by the relatively long-lived resting eggs characteristic of this genus (Parker et al. 1996; Sarnelle and Knapp 2004). However, the viability of resting eggs is limited in time: similar zooplankton recoveries from the so-

called sediment bank could therefore become less and less likely in the next decades. As a consequence, for some zooplankton species the natural resilience of their populations depends on fish-occupancy time (Knapp and Sarnelle 2008). For example, in the study lakes, fish were first introduced in the 1960s, and remained there for approximatively 50 years. This consideration raises important conservation issues concerning the need of a timely recovery of invaded lakes to their initial fishless conditions through active eradication projects or less permissive fish management (i.e. stocking) policies. Local extinctions can represent an irreversible loss of biodiversity for rare and unique taxa. Recent resurrection ecology techniques, however, could provide the disciplinary framework for the research into new techniques to recover ancient pre-stocking population- forming propagules (Orsini et al. 2013). The reduction of some small crustaceans and rotifers following fish removal was probably a consequence of the increase in the abundance of large bodied zooplanktonic competitors. These reductions provide some evidence of H2 and are consistent with literature data (Knapp et al. 2001).

Contrary to other studies that correlated planktivorous fish density and the trophic state of lakes (Vanni 1996; Schindler et al. 2001; Eby et al. 2006), there was little evidence for a trophic cascade in our study lakes. Although *Cyclops abyssorum* and *Daphnia longispina* biomasses increased, to date there has been no accompanying decrease of nutrients and chlorophyll-a concentrations or increase of water transparency. Similar results were also found by Parker et al. (2001). There are several possible reasons for the weak trophic cascade response. Among others, the total zooplankton biomass was not affected by fish removal, suggesting that any change to overall grazing pressure was limited. The unexpected increase of chlorophyll-a concentration discovered has no clear ecological explanation but could be accounted for by the fact that samples for chlorophyll-a analyses were collected only during the eradication process (2013-2017) and pre-eradication data is absent, possibly masking the effects of fish presence (which were already depleted for most of the chlorophyll-a sampling period). Alternatively, the co-occurrence of other anthropic impacts (i.e. livestock grazing in the lake catchments) could have altered the trophic state of the lakes.

Declaring fish eradication

Imperfect detection makes it difficult to tell whether an invasive species has been successfully eradicated (Rout et al. 2014; Rout et al. 2009). An underestimation of efforts needed to complete an eradication could lead to species re-invasion from residual individuals. In the particular case of fish eradication in high mountain lakes using physical techniques, a “wait and see” strategy is commonly adopted to declare the eradication complete. This is an empirical method which postulates that a certain period should pass without any fish capture (maintaining a high capture effort) before eradication can be considered as achieved. This period can vary among studies, from one summer to 1-1.5 years

(Knapp and Matthews 1998; Knapp et al. 2007; Daniel Boiano, personal communication). After the expiry of this monitoring period, natural reinvasion has never been reported among the sites treated with similar eradication techniques (Knapp and Matthews 1998; Knapp et al. 2001; Parker et al. 2001; Vredenburg 2004; Knapp et al. 2007; NPS 2013; Pacas and Taylor 2015).

In the present study, the required duration of the period without captures was one year, but monitoring was continued up to 1.1-2.0 years after the removal of the last fish as a field test of the "wait and see" strategy, which confirmed to be robust. Because brook trout mature earlier than most other salmonids, their populations can recover quickly when reduced but not eliminated (e.g., Cooper et al. 1962). We are therefore confident that the monitoring period was sufficient to declare complete eradication with reasonable certainty.

Does bycatch matter?

Side mortality and bycatch are a component of the ecological and ethical costs of each eradication attempt and should therefore be considered and monitored as an integral part of eradication actions. In the 1990s, intensive gillnetting and electrofishing began to be adopted in high mountain lakes as a safe/acceptable alternative to the use of chemical piscicides (Knapp and Matthews 1998) such as rotenone, which can produce ecological, legal, and social complications. Gillnetting in high mountain lakes is considered a safe eradication method for non-target species, mainly because mountain lakes usually do not harbor native fish. However, native species can include several semiaquatic organisms, which might be affected by gillnets (NPS 2013).

Intensive gillnetting and electrofishing have been successfully used in several restoration programs, apparently without noticeable problems to native biota (NPS 2013). However, there are no published data concerning bycatch of native species caused by physical eradication methods. In the present eradication campaign, the used methods were highly selective for introduced fish, with relatively few accidental captures of semiaquatic vertebrates in the gillnets. For some species such as the black redstart, bycatch is numerically irrelevant. For others, it is insufficient to counterbalance the positive documented effects of fish eradication, as is the case of *Rana temporaria*. For semiaquatic birds and mammals (i.e. mallard ducklings, dippers, water shrews), monitoring data on the possible positive effects caused by fish removal (predatory and competitive release, e.g. Epanchin et al. 2010) is not available and bycatch could locally/temporarily cause a reduction of their population size. This could concern conservation authorities, if semiaquatic vertebrates are endangered/protected species. As far as this study is concerned, mallards returned to nest at Lake DRE in 2017, and bycatch data confirm the persistence of dippers and water shrews. Their persistence suggests that populations of these taxa have the capacity to withstand short term bycatch mortality and to survive. We therefore

believe that ecological resilience can immediately or shortly compensate bycatch mortality. Our results would imply some cautionary limitations to the use of gillnetting in the presence of endangered semiaquatic vertebrates, such as diving ducks. This was seemingly not an issue of concern in the case of the critically endangered hooded grebe *Podiceps gallardoi* Rumboll 1974 (Casañas et al. 2013; BirdLife International 2016): some ongoing fish eradication attempts use gillnetting to restore a number of lakes in the bird's native Patagonian plateau but the hooded grebe is yet to recolonize the treated lakes.

Bycatch could be sizeably reduced by displacing problematic nets in cases where bycatch occurred almost exclusively in a few nets in the littoral area and through the earlier removal of nets, as prolonging the monitoring period after the capture of the last fish up to two years produced a 30% increase of bycatch mortality. Prolonging the monitoring period without fish captures too long could worsen bycatch, while providing insignificant higher probabilities of eradication. In conclusions, intensive gillnetting might provide sufficient evidence for concerns about the conservation of native species.

Conclusions

There is now a consolidated history of successful conservation projects adopting physical fish eradication techniques in mountain lakes. All these projects adopted similar methodologies in similar social contexts (i.e. protected areas) and provided similar conservation results. They were carried out by different operators from different regions, demonstrating consistency despite environmental variability and providing a robust scientific background. In addition, lake gillnetting expanded the range of habitat where mechanical eradication methods can be successful (i.e. drying up was effective for small ponds; see Denoël and Winandy 2015). Following the example of the Sequoia and Kings Canyon National Park (NPS 2013), physical eradication methods could form part of the ordinary strategies for the conservation and management of high altitude aquatic ecosystems. However, further research should be devoted to the development of eradication protocols and predictive models in order to minimise eradication efforts and better estimate efforts required before starting eradication processes. Predicting eradication efforts could help the development of quantitative criteria for the selection of basins for restoration actions, and for the optimization of economic and human resources.

High mountain lake ecosystems can recover following fish removal, but there are species-specific traits such as distribution, dispersal capacity and resting egg viability which can limit recovery potential. Recent resurrection ecology techniques could however help the recovery of ancient pre-stocking populations by using their propagules (Orsini et al. 2013). Some species forming metapopulations would require that fish are removed on a landscape scale, challenging

restoration efforts. Large scale recovery would probably require the adoption of a combination of management measures (fish stocking and fishing bans) and eradication actions. Physical eradication should be the first choice to minimize impacts on non-target species, but chemical eradication could be considered for very large lakes and complex ecosystems (NPS 2013). However, further research is needed to find effective methods to secure native biodiversity when applying chemical treatments.

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Tables

Table 1. Brook trout eradication from four high mountain lakes in Gran Paradiso National Park (Western Italian Alps): relevant lake features and summary of the eradication efforts and outputs. DJO: Lake Djouan; DRE: Lake Dres; LEY: Lake Leynir; NER: Lake Nero; MMG: Multi Mesh Gillnets; PG: Pelagic Gillnets

		DJO	DRE	LEY	NER
Relevant lake features					
Altitude	(m a.s.l.)	2515	2088	2747	2667
Area	(ha)	1.43	2.51	4.56	1.45
Perimeter	(m)	458	713	956	548
Volume	(10^3 m^3)	20.3	83.7	456.1	30.6
Maximum depth	(m)	3.0	7.4	22.1	6.0
Total Phosphorus[a] – TP ± SD	(µg L^{-1})	3.9 ± 1.5	4.7 ± 3.6	3.4 ± 1.4	2.6 ± 1.4
Littoral emergent vegetation	(%)	25	64	0	0
Littoral submerged vegetation	(%)	100	0	0	0
Length of colonized tributaries	(m)	0	380	0	0
Eradication history and efforts					
No. of nets (MMG, PG)		14 (12,2)	30[b] (25,5)	50 (39,11)	12 (10,2)
MMG size – length × height	(m)	36 × 1.8	36 × 1.8	36 × 1.8	36 × 1.8
PG size – length × height	(m)	36 × 1.8	50 × 4	50 × 10	50 × 4
Total net surface	(m^2)	921	2620	8027	1148
(No. of nets):(lake surface) ratio	(nets × ha^{-1})	9.8	12.0	11.0	8.3
(Net surface):(lake surface) ratio		0.064	0.104	0.176	0.079
(Net surface):(lake volume) ratio	(m^2 × m^{-3})	0.045	0.031	0.018	0.038
Number of field surveys[c]		23+16	87+16	59+6	22+8
Number of surveys of the gillnets		39	79	53	30
Number of electrofishing sessions		4	40	7	0
Date of settlement of the first nets		04/07/13	24/06/13	05/08/13	11/07/13
Date of removal of the last fish		14/06/14	11/08/15	05/07/16	07/06/15
Date of removal of the nets		17/06/16	09/06/17	06/09/17	03/07/16
Duration of the eradication process	(days)	346	781	1065	696
Duration of the monitoring period	(days)	733	668	428	392
Breakdown of the fish captures[d]					
Fixed capture devices					
Class 1 (< 15 cm)		206 (3.2)	4362 (57.4)	426 (6.8)	18 (0.2)
Class 2 (≥ 15 cm and < 20 cm)		112 (6.5)	593 (27.9)	425 (22.1)	1 (0.0)
Class 3 (≥ 20 cm and < 25 cm)		655 (65.2)	320 (33.9)	1744 (161.2)	14 (1.2)
Class 4 (≥ 25 cm)		149 (19.4)	114 (19.7)	68 (9.2)	161 (31.8)
Not available		2 (0.1)	31 (1.3)	261 (17.2)	1 (0.2)
Subtotal		1124 (94)	5420 (140)	2924 (217)	195 (33)
Electrofishing, angling, and movable nets					
Class 1 (< 15 cm)		241 (1.3)	7809 (89.6)	472 (4.8)	0
Class 2 (≥ 15 cm and < 20 cm)		1 (0.1)	263 (13.7)	9 (0.5)	0
Class 3 (≥ 20 cm and < 25 cm)		28 (2.9)	997 (98.2)	37 (3.2)	0
Class 4 (≥ 25 cm)		10 (1.3)	727 (133.9)	1 (0.1)	6 (1.4)
Not available		1 (0.1)	5 (0.2)	0 (0)	0
Subtotal		281 (6)	9801 (335)	519 (9)	6 (1)
TOTAL		1405 (100)	15221 (476)	3443 (225)	201 (35)
TOTAL × m^{-2}		0.108 (0.008)	0.585 (0.018)	0.077 (0.005)	0.012 (0.002)

[a] Mean ± SD values based on 29-32 measures taken between 2008 and 2017; [b] Seven additional MMG were added in the littoral vegetation; [c] reported as the number of field surveys before the removal of the last fish plus the number of field surveys performed in the following years to confirm the eradication; [d] biomasses (in kg) in brackets.

Table 2. Number of non-target species accidentally captured in four high altitude lakes (DJO, DRE, LEY, NER) treated for alien brook trout eradication between 2013 and 2017.

	DJO	DRE	LEY	NER	2013	2014	2015	2016	2017	Total
Amphibians										
Common frog *Rana temporaria*	29	27	-	-	0	16	7	28	5	56
Birds										
Dipper *Cinclus cinclus*	-	23	13	-	0	6	18	8	4	36
Mallard *Anas platyrhyncos*[a]	-	5	-	-	0	0	5	0	0	5
Black redstart *Phoenicurus ochruros*	-	-	1	-	0	0	1	0	0	1
Mammals										
Water shrew *Neomys* sp.	-	5	-	-	0	3	1	0	1	5

[a]all ducklings

Table 3. List of biotic indicators and taxonomic groups recorded in the lakes of the Gran Paradiso National Park. Numbers in brackets are the frequency of occurrence of each ecological and taxonomic group in the samples/field surveys. For holometabolous aquatic and semiaquatic insects the developmental stages are reported: l.: larva; p.: pupa; i.: imago.

Biotic indicators	Taxonomic groups
Amphibians 249 surveys from 18 lakes	*Rana temporaria* (0.62)
Macroinvertebrates 168 samples from 17 lakes	
Clinger (0.85)	Order Trichoptera l. (0.71): families Limnephilidae (0.71), Polycentropodidae (0.02), Philopotamidae (0.01), Brachycentridae (0.01), Sericostomatidae (0.01); Order Coleoptera (0.58): families Dytiscidae l. (0.54), Hydraenidae i. (0.04), Helophoridae i. (0.02), Dryopidae i. (0.02), Hygrobiidae i. (0.01); Subclass Acari (water mites) (0.32); Order Tricladida: *Crenobia alpina* Dana, 1766 (0.23); Order Plecoptera l. (0.23): genera *Nemoura* (0.09), *Leuctra* (0.09), *Perlodes* (0.07), *Nemurella* (0.07), *Isoperla* (0.02), *Protonemoura* (0.01), *Chloroperla* (0.01); Subclass Hirudinea: *Helobdella stagnalis* L., 1758 (0.08); Order Ephemeroptera l.: genus *Ecdyonurus* (0.04); Order Odonata: genus *Aeshna* (0.04); Class Gateropoda: genus *Lymnea* (0.02); Order Megaloptera l. (0.01).
Swimmer (0.79)	Order Coleoptera: family Dytiscidae i. (0.61); Order Hemiptera: family Corixidae (0.52); Order Diptera (0.30): families Chironomidae p. (0.28), Culicidae l. (0.02), Dixidae l. (0.01); Order Ephemeroptera l.: genus *Baetis* (0.02).
Burrower (1.00)	Order Diptera l. (0.97): families Chironomidae (0.96), Limoniidae (0.10), Tipulidae (0.02), Ceratopogonidae (0.01), Muscidae (0.01), Tabanidae (0.01), Stratiomydae (0.01); Order Bivalvia: genus *Pisidium* (0.50); Subclass Oligochaeta (0.90): families Lumbriculidae (0.71), Tubificidae (0.58), Haplotaxidae (0.24), Lumbricidae (0.02), Enchytraeidae (0.01), Naididae (0.01).
Zooplankton 291 samples from 18 lakes	
Large taxa (1.00)	Large copepods (0.99): *Cyclops abyssorum* Sars, 1863 (0.84), *Arctodiaptomus alpinus* Imhof, 1885 (0.85); large cladocerans (0.69): *Daphnia longispina* Leydig, 1860 (0.64), *Daphnia pulicaria* sensu Alonso (1996) (0.16)
Small taxa (0.99)	Small copepods: *Eucyclops serrulatus* Fisher, 1851 (0.35); small cladocerans (0.28): *Chydorus sphaericus* Müller, 1785 (0.20), *Alona* sp.[1] (0.13), *Acropaerus harpae* Baird, 1835 (0.11), *Scapholeberis mucronata* Müller, 1776 (<0.01); Ostracoda (0.01); Rotatoria (0.96): *Keratella* gr. *quadrata* Müller, 1786 (0.95), *Keratella cochlearis* Gosso, 1851 (0.49), *Polyarthra* gr. *vulgaris-dolichoptera* (0.40), *Notholca squamula* Müller, 1786 (0.24), *Notholca labis* Gosse, 1887 (0.08), *Synchaeta* sp. (0.08), *Hexarthra bulgarica* Wiszniewski 1933 (0.07), *Lecane* gr. *lunaris* (0.06), *Lecane* gr. *luna* (0.04), *Euchlanis* gr. *dilatata-parva* (0.02), *Ascomorpha* sp. (0.01), *Cephalobdella* sp. (<0.01).

[1] Both *Alona affinis* Leydig, 1860 and *Alona quadrangularis* Müller, 1776 were found.

Figure captions

Fig. 1 Progress of the brook trout eradication actions in four lakes of the Gran Paradiso National Park: a) number of annual captures (including under-ice captures, dark grey; numbers in brackets) in Lakes Djouan, Dres, Leynir and Nero; b) CPUE trends in Lake Djouan; c) CPUE trends in Lake Dres; d) CPUE trends in Lake Leynir; e) CPUE trends in Lake Nero; f) cumulative number of fish captured by gill-netting (black dots) and other capture techniques (electrofishing and rod angling; grey dots) along the eradication campaign in Lakes Djouan, Dres, Leynir and Nero. The y axis has been rescaled in panels c') and d') to highlight CPUE trend approaching zero. Black arrows in Panels c-e indicate the date of removal of the last fish; black flags indicate the date of removal of all the gillnets.

Fig. 2 Total length distribution of brook trout removed from Lakes Djouan (DJO), Dres (DRE), Leynir (LEY) and Nero (NER), with different methods (EF – electrofishing, GN – gillnetting, RA - rod angling), in different years, or gillnetted in different mesh sizes (number from 1 to 6 correspond to mesh sizes 10, 12.5, 18.5, 24, 33, and 38 mm).

Fig. 3 Graphical summary of the recovery process: response of i) amphibian occurrence, ii) macroinvertebrate abundance, iii) zooplankton biomass, and iv) trophic state indicators after fish eradication in four high-mountain lakes. Up and down arrows respectively indicate increasing and decreasing trends associated to different probability levels (pMCMC levels from MCMCglmm model outputs). Models-a compare fish removal lakes (FR) with a series of control fishless lakes (FL); models-b compare each fish removal lake (DJO, DRE, LEY and NER) with the same group of control fishless lakes (FL); TREAT.: treatment. Complete model outputs are provided in Online Resource 4 "MCMCglmm Model outputs".

Figure 4 Multi-annual trends of a selection of ecological indicators in the lakes treated for fish eradication. FL: fishless control lakes; FR: fish-removal lakes; Year-B: years before the beginning of the eradication actions (i.e. 2006-2012).

Figure 5 Number of egg-masses of common frog *Rana temporaria* laid in Lakes Djouan (DJO), Dres (DRE), Leynir (LEY) and Nero (NER) from 2013 to 2017.

Online Resources captions

Online Resource 1 Study lakes: a detailed descriptions of the study lakes

Online Resource 2 Fish capture methods and eradication efforts: a detailed description of the fish capture methods and efforts and of the methods for estimating fish population biomasses

Online Resource 3 Recovery indicators: sampling and analytical methods

Online Resource 4 MCMCglmm model outputs: complete outputs of the MCMCglmm models (generalized linear mixed models with a Bayesian Markov chain Monte Carlo approach)