Online Resource 2: Fish capture methods and eradication efforts

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

Journal name: Biological Invasions

Rocco Tiberti*, Giuseppe Bogliani, Stefano Brighenti, Rocco Iacobuzio, Kevin Liautaud, Matteo Rolla, Achaz von Hardenberg, Bruno Bassano

*Corresponding author. DSTA, Dipartimento di Scienze della Terra e dell'Ambiente, Università di Pavia, Via Ferrata 9 27100, Pavia; e-mail: rocco.tiberti@gmail.com

In Online Appendix 2 we provide a detailed description of the fish capture methods and efforts and of the methods for estimating fish population biomasses. We also discuss which ecological factors could have affected the eradication efforts.

Fish eradication

Intensive gill-netting and electrofishing have been used as eradication methods (Knapp and Matthews, 1998; Knapp et al., 2007).
Two types of nets have been used: i) multi-mesh sinking monofilament gill nets (36 × 1.8 m, each with six 6 m panels with bar mesh sizes of 10, 12.5, 18.5, 24, 33, and 38 mm; manufactured by Oy Lindeman AB, Raippaluoto, Finland), and ii) pelagic sinking monofilament gillnets (from 36 × 1.8 m to 50 × 10 m, with a fixed mesh-size = 25 mm, placed in the central part of the lakes; manufactured by La Sebino Reti SNC, Monte Isola, Brescia, Italy). Pelagic gill nets were deployed only in the central part of the lakes to catch larger fish (Fig. OR2.1). Twelve to 50 gill nets were deployed continuously in each lake during the fish eradication period (including the ice-cover season), with the number of gill nets used being a function of lake surface area (Knapp et al., 2007). The nets have been held vertically and fixed to the shore with ropes along several fixed transects, bearing 1-6 nets. Their vertical displacement was regulated using floaters. All these nets represent the *fixed capture devices*. Their position in the lakes has been accurately mapped (Fig. OR2.1), and an individual alphanumeric code (net-ID) has been assigned to each of them. Settling the *fixed capture devices* in the lakes -while removing fish- took 8-45 days (depending on the lake size: 8 days in Lake NER, 15 in Lake DJO, 41 in Lake DRE, 45 in Lake LEY). During the ice-free season (June–October), captured fish were regularly removed from nets: every day -when capture rates were high- and at progressively longer intervals when they decreased. Each fall, just prior to when the lakes froze over, we moved all nets to deep water and allowed them to fish under the ice unattended for the entire winter. The following spring, the nets were cleaned of captured fish immediately after ice-out. Gill netting was continued at a lake until catch rates fell to zero for at least an entire year (see Knapp and Matthews, 1998; Knapp et al., 2007).

Electrofishing (with a ELT62 II 160 GI backpack equipment) and some additional movable multi-mesh gillnets were used - with different intensity depending on the lake features- in the littoral area (e.g. among littoral vegetation) or along the tributaries and the emissaries to support the eradication efforts.

Removed fish were transported over the shoulder till the nearer road or directly sunk in the lakes. The former were fish in good conservation status (quickly removed from the nets, mainly at the beginning of the eradication process, when a large part of the fish biomass was removed). They were i) given to charitable agencies, ii) sampled for scientific purposes (e.g. stomach content analyses; Tiberti et al., 2016), or iii) frozen within twelve hours and used as food for a captive Eurasian otter, *Lutra lutra* (L. 1758). The otter was maintained in captivity in the Educational Centre for Aquatic Ecosystem (Rovenaud, GPNP) within an education and research project carried out by GPNP (Peracino et al., 1996), as a member of the IUCN Otter Specialist Group. The fish which were sunk in the lakes were mainly small fish in advanced state of decomposition, usually captured late in the eradication process.

Personnel involved in the field work

A single field crew composed by 3-5 people was responsible for all the eradication and monitoring actions. In 2013-2014 the field crew was composed by one principal investigator, 2 field assistants, and 1-2 pre-graduate fellows; in 2015 by one principal investigator, one field assistants, and 1-2 pre-graduate fellows; in 2016-2017 by one principal investigator and 1-2 pre-graduate fellows. GPNP wardens devoted special attention to the surveillance of treated lakes and equipment.

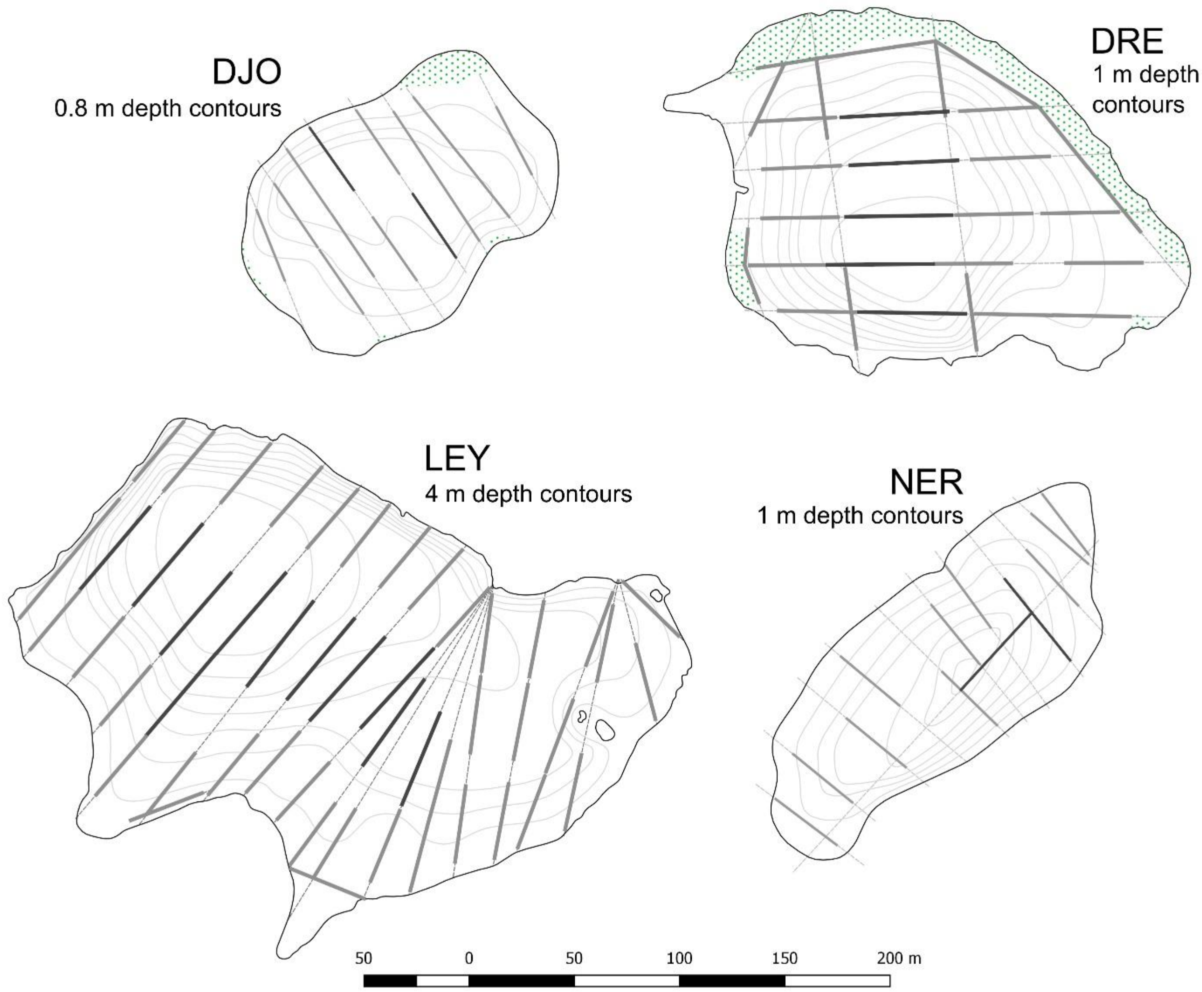

Fig. OR2.1 Gillnets positioning scheme in four lakes treated for fish eradication in the Gran Paradiso National Park. DJO: Lake Djouan; DRE: Lake Dres; LEY: Lake Leynir; NER: Lake Nero. Dashed grey lines: transects bearing the gillnets; gray bold lines: multimesh gillnets; black bold lines: pelagic gillnets; green dots: littoral emerging vegetation.

Fish abundance, size, and biomass

The number of fish captures was accurately recorded during the eradication in all the lakes. However, due to the poor conservation status of many captured fish and to the very large number of small fish captured during some capture sessions, it was sometimes impossible/impracticable to measure all the fish with the same precision. We therefore divided the captured fish into four groups based on the accuracy with which they were measured; the same method was already used in a previous study on the spatial distribution of brook trout within the lakes treated for fish eradication (Tiberti et al. 2017).

- Group 1: 4762 brook trout (23.5% of the total number of fish captures) for which both the total length (accuracy ± 1mm) and the body weight (accuracy ± 1g) were recorded.
- Group 2: 9267 brook trout (45.8%) for which only the total length was recorded.
- Group 3: 5940 brook trout (29.3%) which were not accurately measured, but which were assigned to a size class (four classes from <15 cm to ≥ 25 cm, at 5 cm intervals), e.g. when all the non-measured fish were young of the year, they were assigned with certainty to the size Class-1.
- Group 4: 301 brook trout (1.5%) in very bad conservation status, for which there are not information about their size.

Due to these missing data, both measured and estimated weights were used to obtain a realistic estimate of the fish biomass removed along the eradication campaign from each lake. The length-weight relationships (data from Group 1) were estimated separately for each lake fitting an exponential curve (Tiberti et al., 2017). For all the fish belonging to the Group 2, the parameters of the equation of the curves were used to calculate their expected weights. These measured and estimated weights were subsequently used to calculate the mean weight of the fish belonging to each size class in each lake, and these means were used as an estimate of the weight of all the fish of Group 3. Finally, we used all the previous measured/estimated weights to calculate the mean fish weight in each lake and these means were used as an estimate of the weight of all the fish of Group 4. This procedure enabled the direct measurement or estimation of the weights of all the captured fish, which were summed up to obtain an estimate of the total fish biomass removed from each lake.

Fish eradication efforts and environmental factors

Fish-removal lakes contrast for their morphology and habitat complexity. These factors may influence the effectiveness of the eradication methods and the efforts needed to get the complete eradication. In particular DJO, DRE and NER are considerably smaller than LEY -where fish eradication was therefore expected to be harder (Pacas and Tylor, 2015)- and DRE shows a higher habitat complexity (abundant aquatic vegetation and 380 m of permanent tributaries colonized by brook trout).

The small sample size (four treated lakes) prevented us using any statistical inferences on the ecological factors which could have affected the eradication efforts. As a consequence, we provide simple graphical descriptive statistics to explore the relationships among three groups of variables related to i) eradication efforts (duration of the eradication process, number of field surveys), ii) fish populations (total abundance and biomass), and iii) lake features (altitude, area, and trophic state - TP).

The duration of the eradication process and the eradication efforts varied considerably among lakes. A description of how eradication efforts varied in relation to the features of fish populations and treated lakes is provided in Fig. OR2.2. Scatter diagrams suggest that the variables related to the eradication efforts might present some positive linear relationships with lake size and trophic state, and with brook trout abundance/biomass.

The number of deployed gillnets was proportional to the lake surface (according to Knapp and Matthews, 1998) and their density was similar among lakes (8-12 nets × ha^{-1}). We experimented for the first time the use of pelagic gillnets (Online Appendix 2). Pelagic gillnets have different size depending on the lake depth, have fixed mesh size (24 mm, to target adult brook trout), and are placed in the central part of the lakes. The rationale for using large pelagic gillnets was to keep the ratio between total net surface and lake volume (0.018-0.038) comparable among lakes, rather than calculating the number of needed multi-mesh gillnets based only on lake surface. Because of their mesh size, pelagic nets cannot capture small fish. However, young brook trout are exclusively littoral, and only adults can thrive in the lake centre, where smaller mesh size were inefficient (see Tiberti eta al, 2014; Tiberti et al., 2017). The use of pelagic gillnets could help to quickly remove the adult reproductive fraction of the population, which is a milestone to achieve the complete eradication (Pacas and Tylor, 2015).

The time needed to complete the eradication (removal of the last fish) in lakes and river sections varied between less than one year (Lake DJO) and almost three years (Lake LEY). This time encompasses 2-4 consecutive summers. Compared to usual duration of eradication attempts using physical methods (up to 6 years for lakes and up to 10 years for streams; NPS 2013), the time needed to eradicate brook trout from the GPNP lakes was relatively short. In the case of stream dwelling brook trout (Lake DRE), a rapid eradication was certainly favoured by the short total length of colonized river sections (380 m). In lakes, a rapid eradication was achieved despite the very large number of fish removed from some lakes, and the large size (Lake LEY) and high complexity (Lake DRE) of the treated lakes.

Some salmonid populations (e.g. from DJO and NER) can be quickly eradicated in 1-2 years from small lakes with little habitat complexity. Although very abundant populations from relatively large and complex lakes (e.g. lake DRE and LEY) can be successfully eradicated as well, it is necessary to put in place remarkable capture efforts (present study; Pacas and Tylor, 2015). As well as the lake size (Knapp and Matthews, 1998; NPS, 2013), efforts may be related to the trophic and thermal state of lakes, likely influencing the abundance/biomass of fish populations. Eradicating fish from river sections needs additional efforts (Pacas and Tylor, 2015; NPS, 2013).

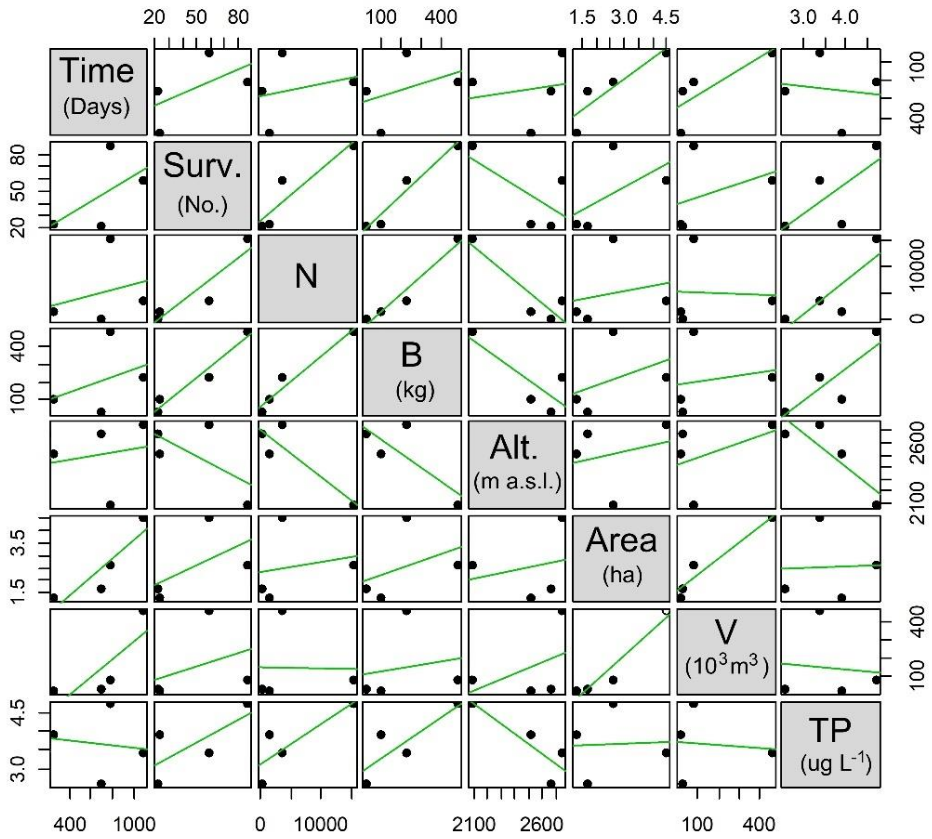

Fig. OR2.2 Relationships among variables (scatter diagrams and regression lines) related to i) the eradication efforts, ii) eradicated brook trout populations, and iii) lake environmental features. Time: duration in days of the eradication from the setting of the first net to the removal of the last fish; Surv: number of field surveys before the last fish was removed; N: total number of removed fish; B: total biomass of removed fish; Alt.: lake altitude; Area: lake area; V: lake volume; TP: total phosphorus concentration in lake water (average value from 28-32 measurements from 2008 to 2017). Data from brook trout eradication actions in Lakes Djouan, Dres, Leynir and Nero (Gran Paradiso National Park, Italy).

References

Knapp RA, Matthews KR. 1998. Eradication of nonnative fish by gill netting from a small mountain lake in California. Restoration Ecology 6:207-213.

Knapp RA., Boiano DM, Vredenburg VT. 2007. Removal of nonnative fish results in population expansion of a declining amphibian (mountain yellow-legged frog, *Rana muscosa*). Biological Conservation 135:11-20.

NPS [National Park Service]. 2013. Restoration of Native Species in High Elevation Aquatic Ecosystems Plan and Draft Environmental Impact Statement. Sequoia and Kings Canyon National Parks, Three Rivers, CA.

Pacas C, Taylor MK. 2015. Nonchemical eradication of an introduced trout from a headwater complex in Banff National Park, Canada. North American Journal of Fish Management 35: 748-754.
Peracino V. 1996. Progetto lontra Gran Paradiso. IBEX Journal of Mountain Ecology 4. Parco Nazionale Gran Paradiso, Torino, Italy.
Tiberti R, Acerbi E, Iacobuzio R. 2014. Preliminary studies on fish capture techniques in Gran Paradiso alpine lakes: Towards an eradication plan. Journal of Mountain Ecology 9:61–74.
Tiberti R, Brighenti S, Canedoli C, Iacobuzio R, Pasquini G, Rolla M. 2016. The diet of introduced brook trout (*Salvelinus fontinalis*; Mitchill, 1814) in an alpine area and a literature review on its feeding ecology. Journal of Limnology 75:488-507.
Tiberti R, Nelli L, Brighenti S, Iacobuzio R, Rolla M. 2017. Spatial distribution of introduced brook trout *Salvelinus fontinalis* within alpine lakes: evidences from a fish eradication campaign. The European Zoological Journal 2017: 73-88.