LIFE+ LIMNOPIRINEUS: CONSERVATION OF AQUATIC HABITATS AND SPECIES IN HIGH MOUNTAINS OF THE PYRENEES

TECHNICAL REPORT

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CONSERVATION OF AQUATIC HABITATS
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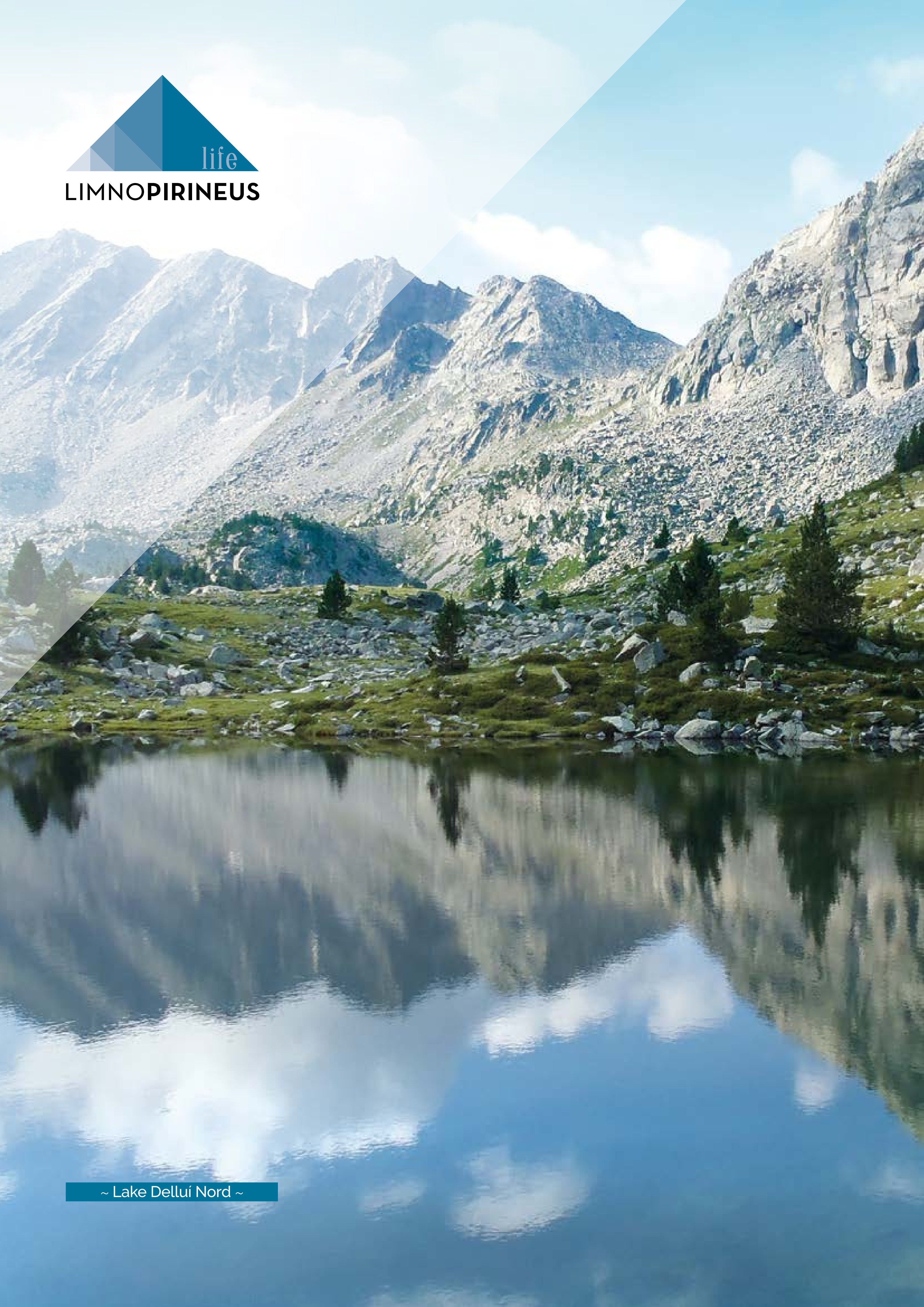

~ Lake Delluí Nord ~

CHANGES IN LAKES AFTER THE REDUCTION OF FISH DENSITIES

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ABSTRACT

In this study we analyse the time series of monitoring data for the 5 years of the LimnoPirineus project. The results show that the group with the clearest response to fish eradication are the macroinvertebrates of the littoral zone, with an increase in taxon richness over time and a convergence of macroinvertebrate composition to that of natural lakes. The change in the biomass of periphytic algae is less affected and only occurs where minnow alone were present or where they were accompanied by salmonid species. In the pelagic system, lakes where minnow were the only fish species present showed an increase in the abundance of pelagic crustaceans and a decrease in phytoplankton biomass after eradication. All these changes and those of physical-chemical parameters (water transparency and nutrients) did not, however, seem to clearly affect the composition of phytoplankton or periphytic algae in the lakes studied.

INTRODUCTION

There are currently a number of fish (salmonids and cyprinids) in high mountain lakes in the Pyrenees as a result of a historical process of introduction and exploitation that goes back centuries and has been accelerated in the last 60 years. Salmonid species introduced in the Catalan Pyrenees include common trout (*Salmo trutta*), rainbow trout (*Oncorhynchus mykiss*) and brook trout (*Salvelinus fontinalis*) (Miró & Ventura, 2013). The minnow (*Phoxinus sp.*) is a small cyprinid that has also been introduced in many of the Pyrenean lakes, but in this case, its arrival is related to its use as live bait to catch trout (Miró & Ventura, 2015).

The introduction of fish in high mountain lakes where there were none before results in a series of effects that propagate through the food web (Carpenter et al., 2001). Direct predation by the fish can affect larger zooplankton and produce an indirect top-down effect that alters the community and the biomass of smaller zooplankton as well as phytoplankton (Buchaca et al., 2016; Sarnelle & Knapp, 2005; Schindler et al., 2001). Benthic and nektonic macroinvertebrates may be locally wiped out after the introduction of fish while those that live half buried in the sediment are not affected and may even be indirectly favoured. (Knapp et al., 2001; Tiberti et al., 2014; Tiberti et al., 2018). The introduction of fish is also often an ecological exclusion factor for amphibians (Bradford et al., 1993; Knapp et al., 2016; Tiberti & von Hardenberg, 2012; Vredenburg, 2004). In the Pyrenees, minnow is only found where trout has been introduced (Miró & Ventura, 2015). This small cyprinid has a very negative effect on amphibian populations since amphibians are not able to recolonise the lakes where minnow has managed to displace trout (Miró et al., 2018). In addition to all these effects that occur within the aquatic environment, introduced fish can also alter the flow of resources, in the form of emerging insects and amphibians, offered by the aquatic system to the environment (Pope et al., 2009; Tiberti et al., 2016) and indirectly affect land predators such as birds, reptiles, amphibians or bats (e.g. Epanchin et al., 2010). The resistance and resilience of lake fauna to the introduction of fish in high mountain lakes where previously there were none has been studied by different authors (Epanchin et al., 2010; Knapp et al., 2001; Pope et al., 2009; Tiberti et al. 2018). However, all these studies have focused on the impact of the presence of salmonids. As a result, we have no record of similar studies that evaluate the impact of the presence of cyprinids in high mountain lakes.

One of the main conservation actions being conducted within the framework of the LIFE+ LimnoPirineus project is intensive fishing in 8 lakes referred to as target lakes, 5 of them located in the Aigüestortes i Estany de Sant Maurici National Park and another 3 in the Alt Pirineu

Natural Park, in order to eradicate fish populations or drastically reduce their density. With this type of action, the aim is to reverse the effect of introducing fish on the function of the ecosystem as a whole. Eradication in the lakes of the National Park began in two of the lakes in the Dellui valley (2015) that had common trout and minnow, then in Subenuix (2016) where there was brook trout, Cap del Port de Peguera (2016) where there was common trout and finally in Cabana (2017) where there was rainbow trout. The lakes in Alt Pirineu Natural Park only contained minnow. In 2013, the summer before the start of the LIFE project, work began at Closell as a pilot feasibility test. At Naorte, the work began in 2015, while at Rovinets it began in 2016. From the beginning of the project, limnological monitoring of the target lakes has been conducted in order to know the status, structure and quality of the habitat and to verify the scope of the actions carried out there.

In this chapter we present the results of analysing the time series of data obtained during the 5 years of monitoring. Data has been collected regarding nutrients, biomass and composition of phytoplankton, zooplankton, macroinvertebrates and algae from the periphyton of the littoral zone. The results related to amphibians are presented in another chapter in this same volume.

MATERIAL AND METHODS

The lakes studied are located in the Aigüestortes i Estany de Sant Maurici National Park and the Alt Pirineu Natural Park. We selected 8 lakes that we refer to as target lakes, which are those where we are acting within the framework of the LIFE+ LimnoPirineus project and 19 further lakes where no action was taken and there are no fish. Within the set of lakes, 19 are lakes without fish (NATURAL), 2 have salmonids (SALM), 3 have minnow (PPH) and 2 have salmonids and minnow (SALM + PPH).

The lakes were sampled between July and August 2014, 2015, 2016, 2017 and 2018. The 'target' lakes were also sampled in September. Information was collected to study the composition of pelagic and littoral system organisms, as well as environmental variables to characterise the physical and chemical environment. The sample to analyse the water chemistry was taken from the outlet of each lake or from the boat at the central sampling point when the lake had no outlet. The analyses were performed following the methodology described in Ventura et al. (2000). Water mass transparency was measured by estimating the light extinction coefficient in the water column (Kd; m^{-1}) from the Secchi disk depth measurement. In lakes where the Secchi disk reached the bottom, a constant extinction coefficient of 0.2 m^{-1} was used (Buchaca, 2009).

Eradication of exotic fish

A combination of three catching techniques was used in the eradication of exotic fish: loose gillnets of different mesh size (5 to 43 mm), electric fishing on the shore, and small mesh creel-type traps (4 mm). Each of these techniques has a variable efficiency depending on the species and the time of the year, among other factors. The first two methods had already been previously tested in high mountain lakes in California and the Italian Alps, and we knew their efficacy for eliminating salmonid nuclei (Knapp & Matthews, 1998; Tiberti et al., 2018). The tubular creel-type traps, almost submerged and resting on the rocky or muddy bed of the shore, had been quite effective in catching minnow in a pilot project conducted at Closell. Electric fishing, despite its poor efficiency in this type of environment due to the typical low conductivity of the waters, appears the best technique in lake tributaries, and also for the catching minnow on the shore at certain times of the year.

The review and emptying of nets and traps were carried out on a daily basis at the start of the tasks in each lake and on a weekly basis once the catches decreased. Electric fishing on the shore was carried out approximately once a week in each lake during the first summer of action, with this frequency changing as of the second year based on results.

Fish abundance was estimated using relative abundance indices, CPUEs (Catches Per Unit of Effort) and BPUEs (Biomass Per Unit of Effort). Effort was standardised for each catching technique based on the time of use, and eventually also units related to its volume of exposure: net metres or installed parts. Consequently, the units are different for each catching technique, causing that the relative abundance indices derived from each technique to not be comparable to each other. However, they allow an easy comparison between locations and dates.

Macroinvertebrates

Littoral macroinvertebrates were characterised by sampling the shore of the lakes to about 80 cm deep. Samples were collected using the sweep net sampling method following the procedure used in other studies (Knapp et al., 2001; Tiberti et al., 2018) and comparable to that performed in the Pyrenees (de Mendoza et al., 2015): the diversity of habitats and substrates of the littoral area was sampled, at around 80 cm deep, and previously characterised *in situ* throughout its perimeter according to the dominance of silt, sand, gravel, shingle and stone as well as the coating of macrophytes and mosses. In each locality, a total of 30 one-metre-long passes with the 250 μm mesh size sleeve, were distributed in proportion to the abundance of habitats present in order to obtain a representative sample. Additionally, two submerged rocks were inspected and the organisms attached were collected, as they could not be so easily caught by conventional sleeve sampling. The samples gathered by sweep net sampling were included and preserved with absolute ethanol to a final concentration of 70%. Sampling was equivalent between lakes in terms of effort and area covered. Each sweep net sampling covered an area of approximately 10 m of shore. Before separating the samples in the laboratory, the shoreline material was screened using a 1 mm mesh. The macroinvertebrates were selected in the laboratory and stored in absolute ethanol for later observation with a stereomicroscope. The individuals collected were classified to a taxonomic gender resolution, except for the Hydracarina clade, the Oligochaeta class, the Ceratopogoninae subfamily and the Chironomidae family. For this last group, the resolution used was the rank of tribe, with the exception of the Orthocladiinae and Prodiamesinae subfamilies which, according to Wilson and Ruse (Wilson and Ruse 2005) do not currently have accepted divisions in tribes and the Podominae subfamily. Individuals were identified according to available literature (Vergon & Bourgeois, 1993).

Macrozooplankton

Planktonic crustaceans were collected with a Hensen-type net of 0.027 m^2 and 200 µm mesh size. Sampling was carried out by boat from the deepest area including three vertical trawls. The samples were preserved in lugol's iodine. The determination of the crustacean species present was made by separating adult individuals from each of the species (at least one of each sex) under the binocular magnifying glass. Subsequently, individuals were observed under stereomicroscope, dissecting them to observe their characteristics and identifying them according to various authors (Dussart, 1969; Einsle, 1992; Keifer, 1978) for copepods, and Alonso (Alonso, 1996) for cladocera. To obtain abundance (N), a minimum of 250 individuals per aliquot were counted or the total sample was counted if the material was scarce. The count was performed under an inverted microscope.

Phytoplankton and periphyton

Samples to study phytoplankton were collected by boat from the deepest point of the lake, at a depth of 1.5 the Secchi disk depth, using a UWITEC-type sampling equipment. In those lakes where the Secchi disk bottomed out the sample was taken between 1 and 2 m above the sediment. From this sample a known volume of water (between 1.5 and 2 litres) was filtered using a manual vacuum pump and GF/F filters (47 mm in diameter). The filter was kept wrapped in aluminium foil in a refrigerator until it reached the laboratory where it was frozen.

Samples to study the periphyton were collected from the top of 4-6 stones per lake. The stones were collected from different points of the littoral area of the lake at a depth of between 0.5 to 1 m. The periphyton was removed from the stones using a brush and the collected material (ca. 100 ml) was stored fresh until it reached the laboratory where it was frozen. The brushed surface was estimated using a sheet of aluminium foil where the surface was drawn and then it was weighed in the laboratory and its surface area was calculated using a regression equation between surface and weight. The frozen material was lyophilised before analysis.

The characterisation of the composition of plankton and periphyton cyanobacteria and algae was carried out by analysing the composition of marker pigments with chromatographic methods.

The pigments were extracted from the sample using 90% acetone and sonicating the sample for 2 minutes. The extract obtained was filtered (0.1 µm) and in the case of plankton samples it was concentrated 17 times with a TurboVap. The pigments were analysed following the method described by Buchaca et al. (2016). The UHPLC system (Acquity Waters, Milford, MA, USA) was equipped with an UPLC HSS C18 SB column (dimensions: 2.1 x 100 mm; particle size: 1.8 µm) and with PDA (λ: 300-800 nm). The PDA channel was set at 440 nm for pigment detection and quantification. After a sample injection (7.5 µL), the pigments were eluted with a linear gradient from 100% solvent B (51:36:13 methanol:acetonitrile: MilliQ water, v/v/v 0.3 M ammonium acetate) to 75% B and 25% A (70:30 ethyl acetate: acetonitrile, v/v) for 3 min, followed by 0.45 min of isocratic hold at 75% B and 2 min of linear gradient to 100% of solvent A. The initial conditions (100% B) were linearly recovered in 0.65 min. The flow rate was 0.7 mL min-1. Pigments were identified by comparison with a library of pigment spectra obtained from extracts of pure algae cultures from the Culture Collection of Algae and Protozoa (CCAP, Oban, Scotland, UK) and pigment standards (DHI Water and Environment, Hørsholm, Denmark). Pigment molecular weight was obtained from the literature (Jeffrey et al. 1997). The pigment concentration was expressed in nmols L^{-1} (phytoplankton) or in nmols cm^{-2} (periphyton). Of the total pigments identified, those with a higher taxonomic affinity were selected.

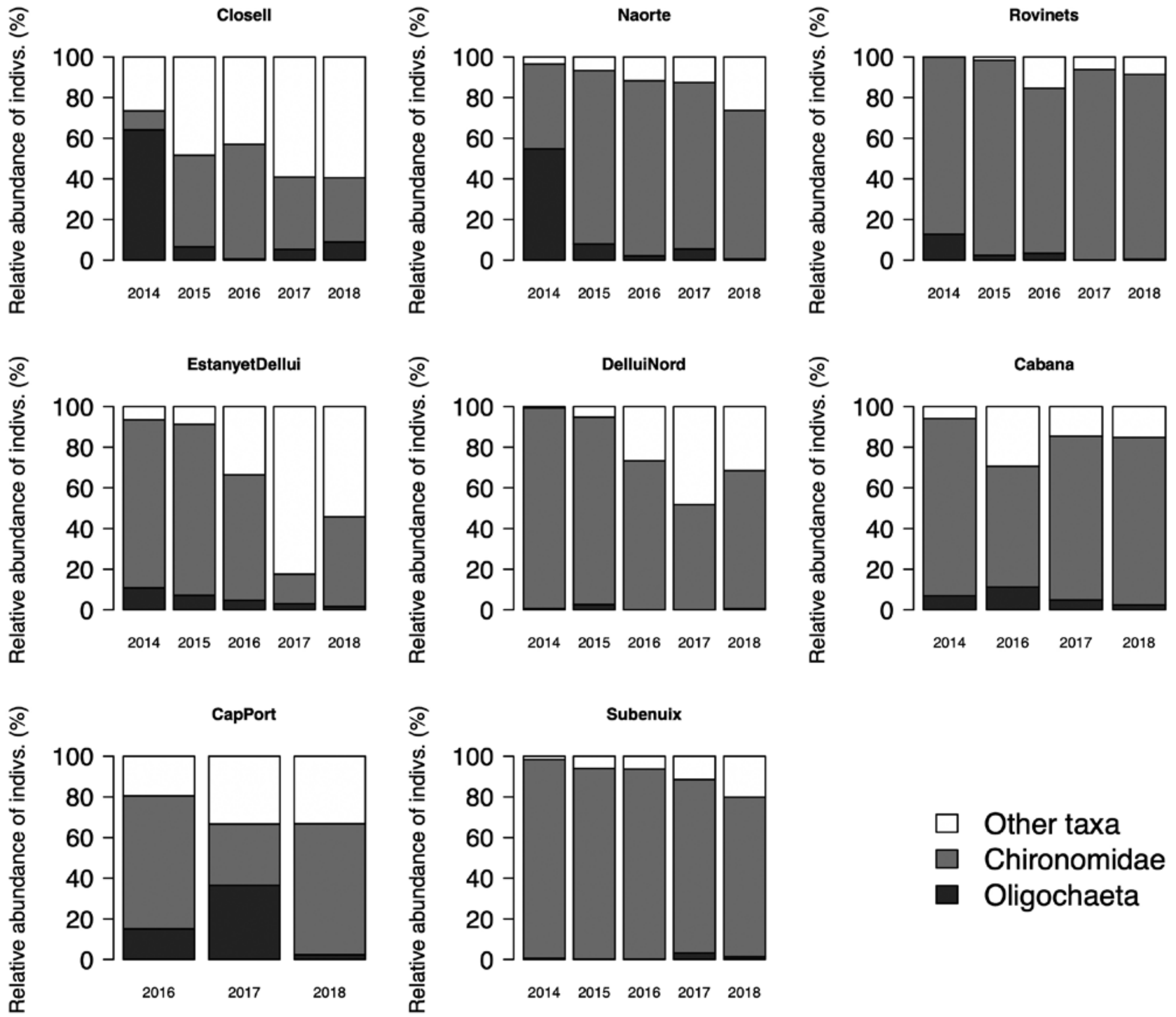
The CHEMTAX program was used (Mackey et al., 1996) to estimate the proportion of Chl-a of chlorophytes, chrysophytes, diatoms, cryptophytes, dinoflagellates and cyanobacteria following the methodology described by Buchaca (2009).

RESULTS

Progress of catches in target lakes

Eradication in the lakes of the National Park began in June 2015 in two of the Dellui lakes and in two river sections of the same sector, containing trout and minnow. During the first year, a reduction in the initial stock was achieved of over 90% in the lakes and up to 40% in the river sections. In 2016, the last trout specimens were caught in the lakes of the Dellui valley, while in the river stretches in 2017, a 90% reduction of the initial stock was reached. In accumulated values, a total of 3,739 individuals of *Phoxinus sp.* with a total biomass of 19 kg, and 431 individuals of *S. trutta* representing a total biomass of 55.5 kg were caught. At Nord de Dellui the number caught amounted to 18,322 individuals of *Phoxinus sp.* (53.9 kg of biomass) and just 7 Individuals of *S. trutta* (3.4 kg of biomass). In the river sections, a total of 1,295 individuals of *S. trutta* with 22 kg accumulated biomass were caught. In June 2016, fishing began at Subenuix where there was brook trout (*Salvelinus fontinalis*). At the end of October of the same year, about 90% of the initial stock had already been caught. In accumulated values at Subenuix, 4,995 specimens of *S. fontinalis* equivalent to a total biomass of 184.8 kg were caught. Most of the 2017 catches were small or medium sized individuals suggesting that all adult reproductive specimens were eliminated at this location. At Cap del Port de Peguera, where there was trout, individuals were first caught in August 2016 both in the lake itself and in a stretch of effluent river. A total of 1,608 specimens of *S. trutta* with a total biomass of 182.4 kg were caught in the lake while up to 652 specimens of *S. trutta* (4.8 kg of biomass) were captured in the river stretches.

Finally, the last target lake of the National Park where fishing began in 2017 was La Cabana where there was rainbow trout, catching 521 specimens with a total biomass of 112.3 kg. An aspect to highlight in the set of lakes of the National Park are the differences in the population size reached in each lake according to the species. Brook trout can reach densities between 10 and 20 times higher than those of common trout or rainbow trout. In the Alt Pirineu Natural Park, all the lakes where work took place had minnow and did not have any salmonid species. In 2013, the summer before the start of the LIFE+ project, work began at Closell as a pilot feasibility test. In accumulated values at Closell, 16,708 individuals, equivalent to a total biomass of 46.4 kg were caught. At Naorte, action began in 2015, and until the autumn of 2017 a total of 85,388 individuals with an accumulated biomass of 219.3 kg were caught. Finally, at Rovinets, action began in 2016 and until the autumn of 2017, 5,680 individuals with a total biomass of 20.9 kg were caught. Population reductions were significant since the first intervention. A complete description of results of fish catches can be found in the first chapter of this volume.



▲ **Figure 1.** The evolution over time of the relative abundance of three main fractions in macroinvertebrate samples: oligochaetes, chironomids and other groups.

RESPONSE OF THE ORGANISMS TO ERADICATION ACTION

Littoral macroinvertebrates

The evolution over time of the relative abundance of three main fractions in macroinvertebrate samples has been studied: oligochaetes, chironomids and other groups (Figure 1). This grouping has been established taking into account that both oligochaetes and members of the Chironomidae family, in a broad sense, are organisms generally present and abundant in aquatic environments, whether altered or not. However, the category that includes the rest of aquatic organisms includes groups of special interest that have a lower abundance of individuals in the samples, but constitute a greater abundance of represented species. In addition, it is this last category of organisms that includes the majority of groups considered vulnerable to the impact of introduced fish as they are potential prey.

In general terms, there is an increase in the significance of the fraction that includes organisms that are neither oligochaetes nor chironomids over time (Figure 1). Once the density of fish in the lakes is sufficiently reduced, the relative abundance of this fraction increases. This change is especially noticeable in the lakes containing minnow, a predator with a greater impact on the community.

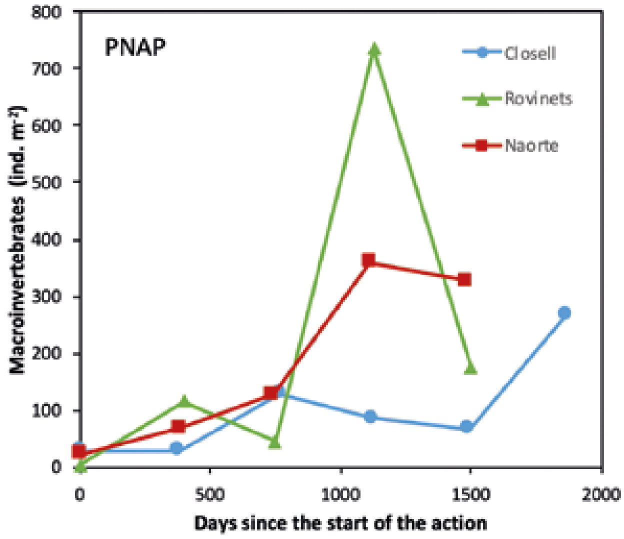
The fraction that includes oligochaetes tends to reduce in significance over time, although the pattern of evolution of this fraction compared to that of chironomids is more erratic and is not as directly influenced by predator fish.

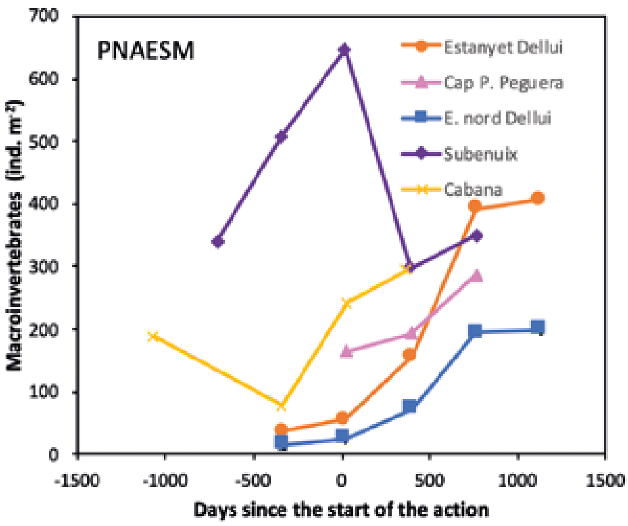
The absolute abundance of individuals also increased over time from the action starting (Figure 2). This increase is generally relevant and has an exponential trend in those lakes where there were high densities of minnow at the beginning of the time series. Some lakes present a more pronounced change than others in absolute abundance after several years since the fishing started. This was the case of the lakes at Naorte, Estanyet Dellui del Mig, Nord de Dellui and Rovinets. Regarding the abundance of taxonomic groups, there is a progressive increase of this over time that has clearly not yet stabilised for lakes such as Nord de Dellui, Naorte or Rovinets (Figure 3).

This increase in the number of taxa is mainly due to the catching of new organisms vulnerable to the presence of introduced fish that have colonised the system or have increased their abundance once the pressure of predation on them has decreased.

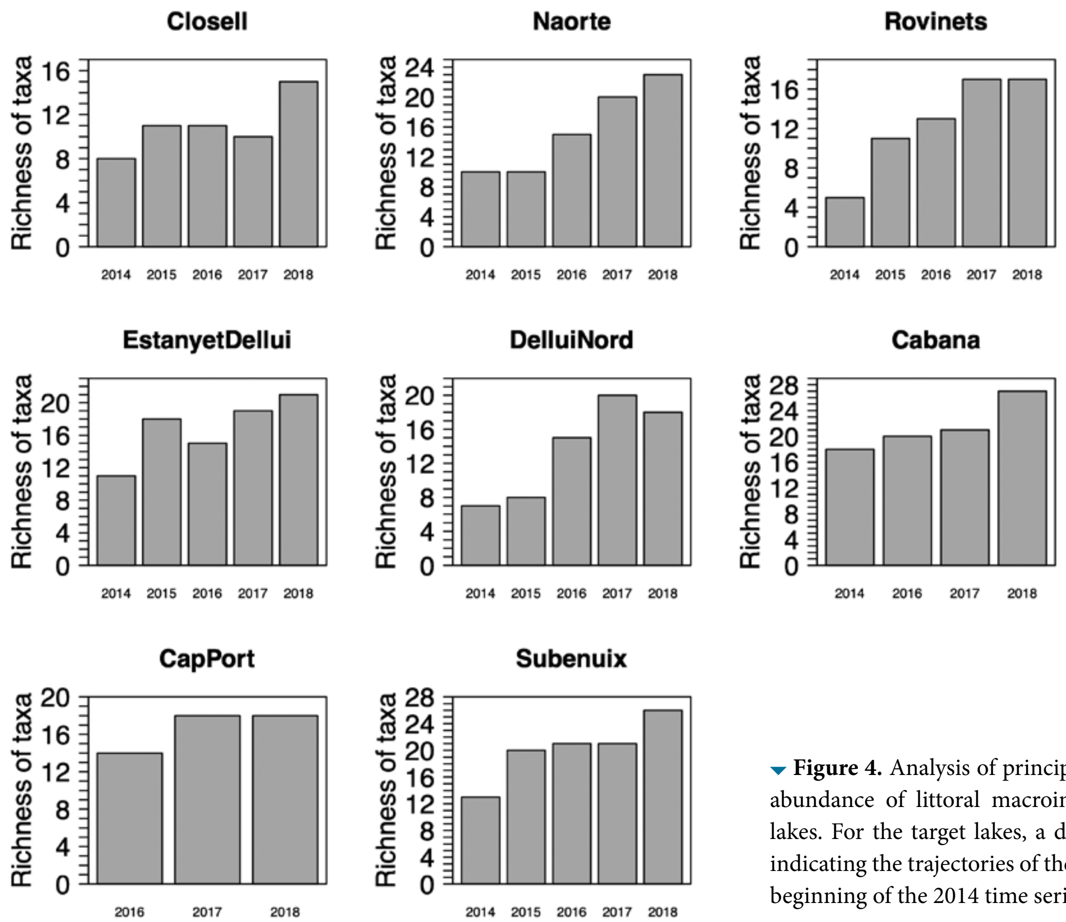
The change in the composition and structure of the macroinvertebrate community as a whole has been studied with a Principal Coordinate Analysis on the abundance of taxa data. Oligochaetes and some chironomidae subfamilies have been excluded in the analysis because they are organisms generally present and not vulnerable to the presence of fish (Figure 4).

Ephemeroptera nymph of the genus *Cloeon*.



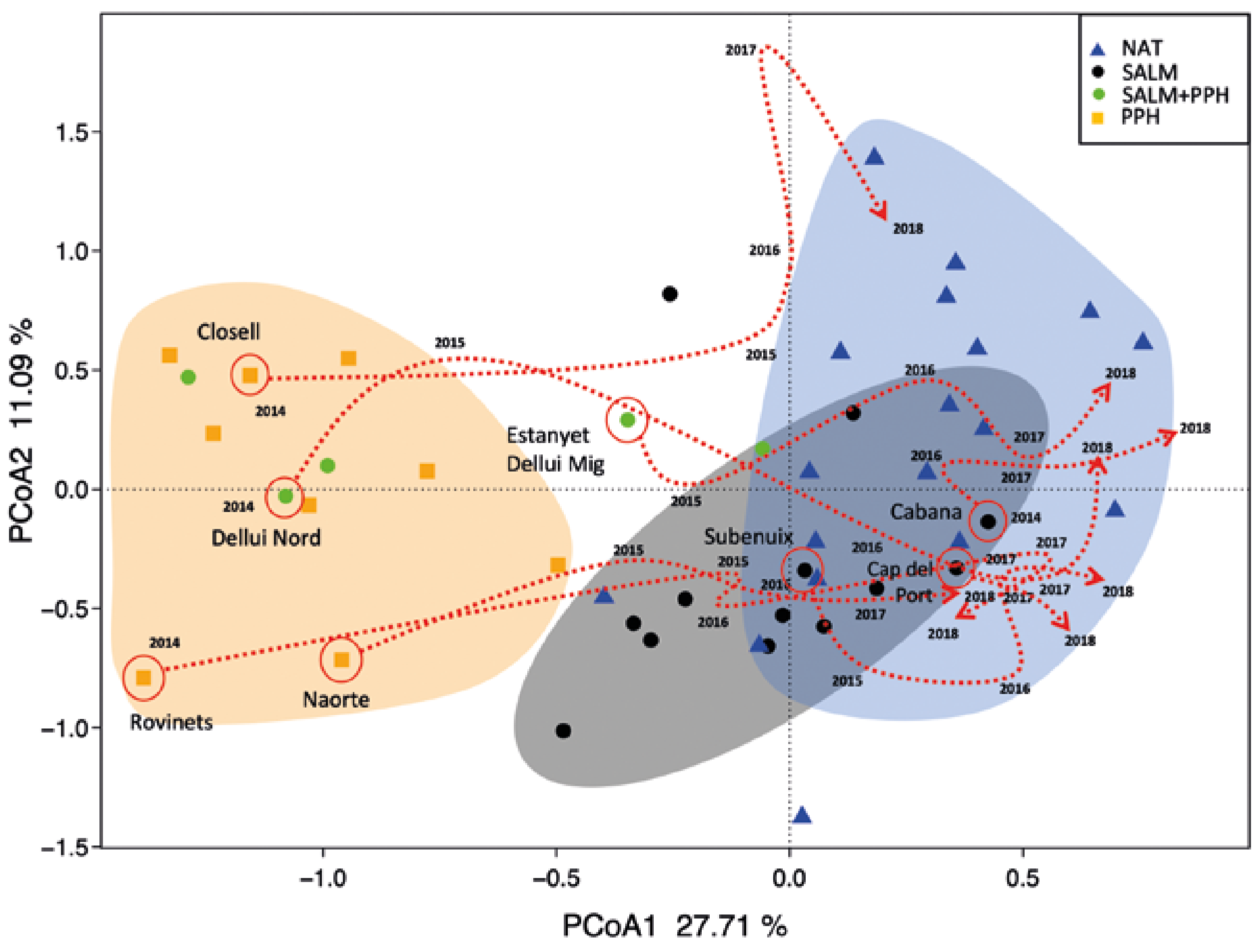


▲ **Figure 2.** Absolute abundance of macroinvertebrates (ind. m^{-2}) according to the days since the start of the action.



◀ **Figure 3.** Evolution of the richness of taxonomic groups over time.

▼ **Figure 4.** Analysis of principal coordinates on data for the abundance of littoral macroinvertebrate taxa in the target lakes. For the target lakes, a dashed line has been included, indicating the trajectories of the data series that goes from the beginning of the 2014 time series to the 2018 data.



The lakes that contained minnow, with or without the additional presence of salmonids, are concentrated on the left part of the graph in Figure 4. The lakes with salmonids are distributed along the horizontal axis on the lower part of the graph. In addition, lakes without fish are distributed on the far right. For the target lakes, a dashed line has been drawn that joins the points of the time series and allows us to visualise the path that each target lake follows over time. We can see how, progressively, the lakes move to the right of the graph, indicating that, as the eradication actions in these lakes progress, they are more like natural lakes in terms of composition and abundance of taxa. Again, it can be seen how the displacement is more relevant for those lakes where there was minnow, but it is also evident where there were salmonids.

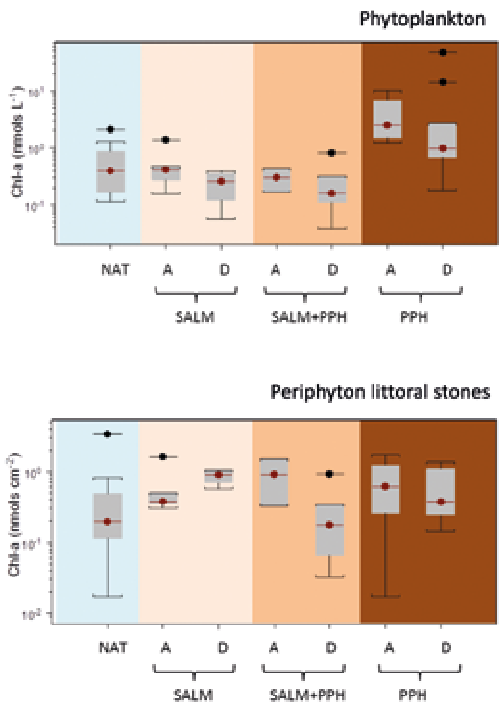
Macrozooplankton

Crustacean populations are very seasonal and have a maximum abundance that can occur both in summer and autumn, depending on the year and/or the lake. The interannual variability has been seen to be high, and for this reason we have represented the data taking into account the values of the summer (July) and autumn (September) campaigns. The values represented in Figure 5 have been obtained by calculating the average of the two dates and representing their range of variation.

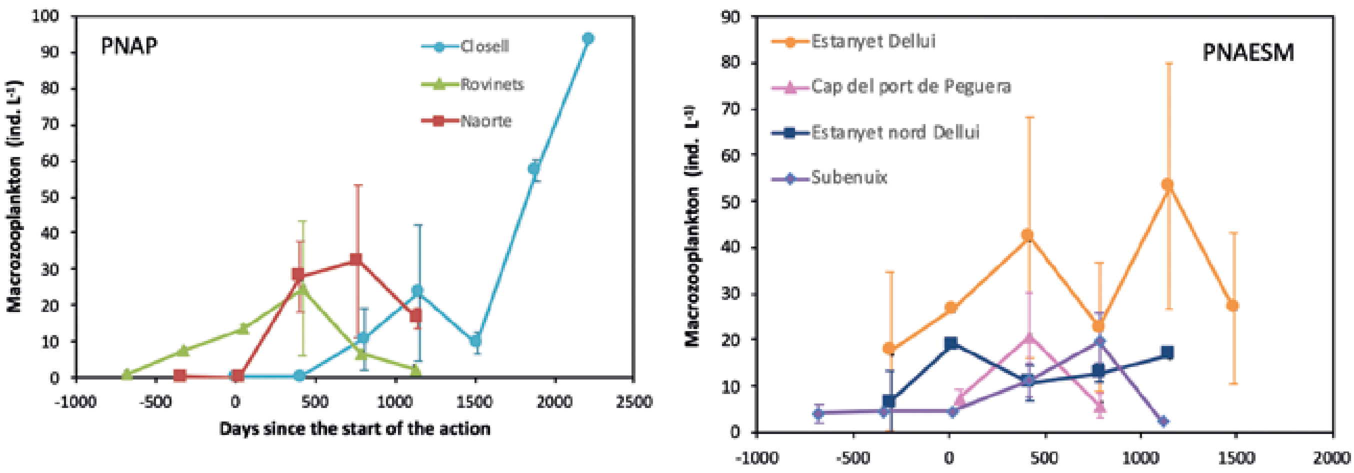
After starting the actions, the crustacean abundances increased very clearly in the lakes where the minnow had been present as the only species (Closell, Naorte and Rovinets). The crustacean populations of the lakes with salmonids also began to respond (Subenuix and Cap del Port de Peguera). In contrast, the abundance values of crustaceans in the lakes where salmonids and minnow (Dellui lakes) lived together are more erratic (Figure 5).

Biomass and composition of phytoplankton and periphyton

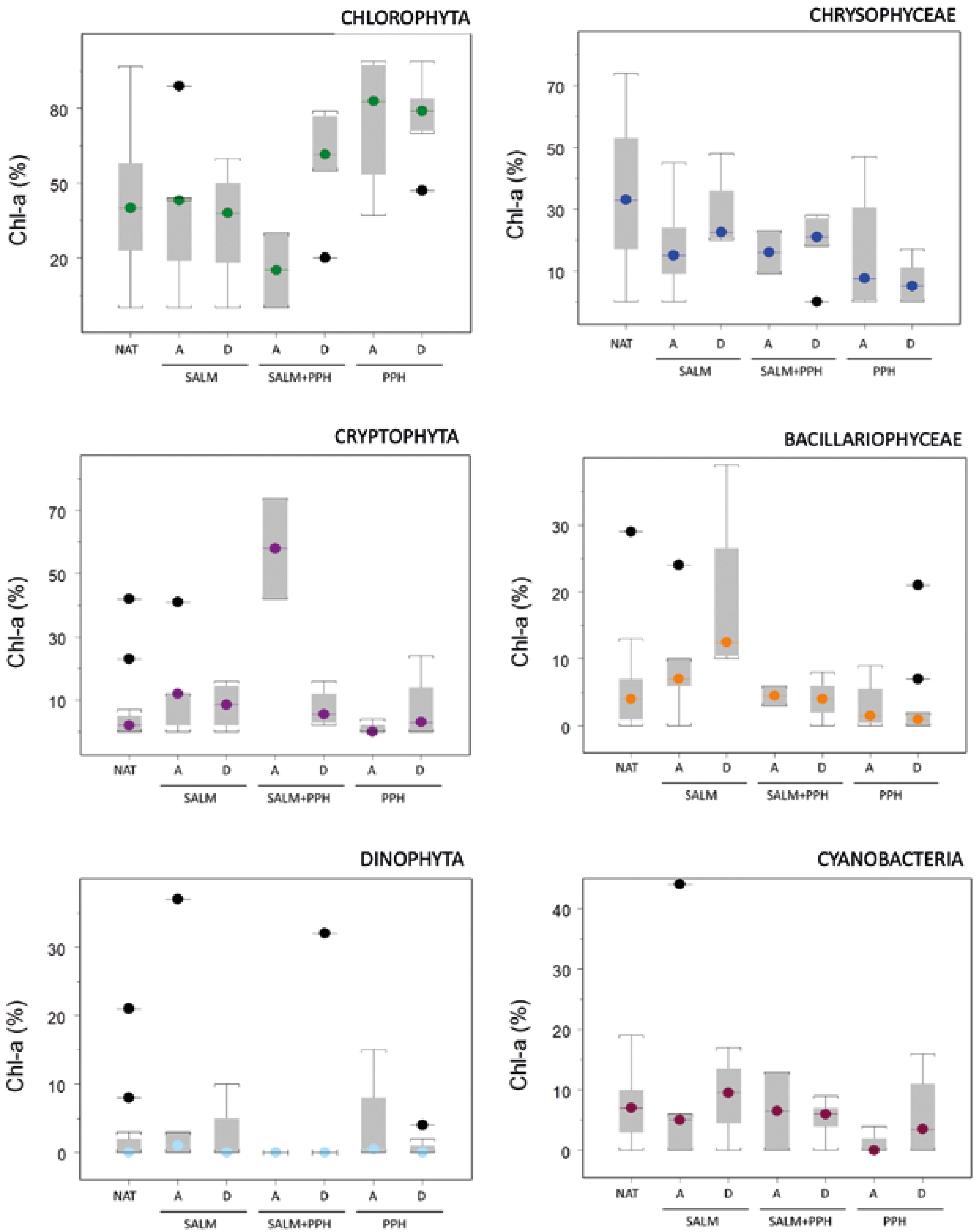
Chlorophyll-a (Chl-a) in the water column, used as an estimate of phytoplankton biomass, shows a slight decrease after starting actions (Figure 6, upper graph).



▲ **Figure 6.** Box diagrams showing the frequency distribution of chlorophyll-a in the water column (upper graph) and the periphyton of the littoral stones (lower graph) in the different scenarios for the target lakes. NAT: lakes with no fish; SALM: with salmonids; SALM+PPH: with salmonids and *Phoxinus* sp.; PPH: with *Phoxinus* sp. The initials A and D indicate before and during the action. The red line within the box diagrams corresponds to the median of the data, the box limits indicate the 25th and 75th percentile. The lines extending from the box indicate the 90th and 10th percentile. The black dots with the horizontal line indicate extreme values.



▲ **Figure 5.** Macrozooplankton abundance (ind. L^{-1}) according to the days since the start of the action. The value represents the average data and its range of values taking into account the summer and autumn data.



▲ **Figure 7.** The box diagrams showing the frequency distribution of the percentage of Chl-a of each algal group and of phytoplankton cyanobacteria of the target lakes and for the different scenarios. Abbreviations the same as in Figure 6.

The decrease was more pronounced in the lakes where there was only minnow before action started, although it can be seen that the dispersion of the data is vast. Regarding the periphyton of the stones, the dispersion of the data is also vast, but the trend is the same as in the case of phytoplankton in the lakes where there was only minnow before action started or where they were accompanied by a salmonid species (Figure 8, lower graph). However, the Chl-a of the stones even increases after the action in the lakes that had contained salmonids.

The phytoplankton composition was estimated as the Chl-a of each algal group and cyanobacteria using the CHEMTAX program algorithm.

The results obtained place chlorophytes and chrysophytes as dominant groups in the group of lakes studied. In general, there is a greater contribution of chlorophytes in lakes with minnow, of chrysophytes in natural lakes and diatoms in lakes with salmonids. Although there are no clear patterns of the effect of the actions (Figure 7). Regarding the composition of primary producers of the periphyton of the stones, there are also no clear patterns of the effect of the actions, although it can be said again that diatoms are more abundant in lakes with salmonids and that cyanobacteria are more abundant in the periphyton of the stones of natural lakes (Figure 8).

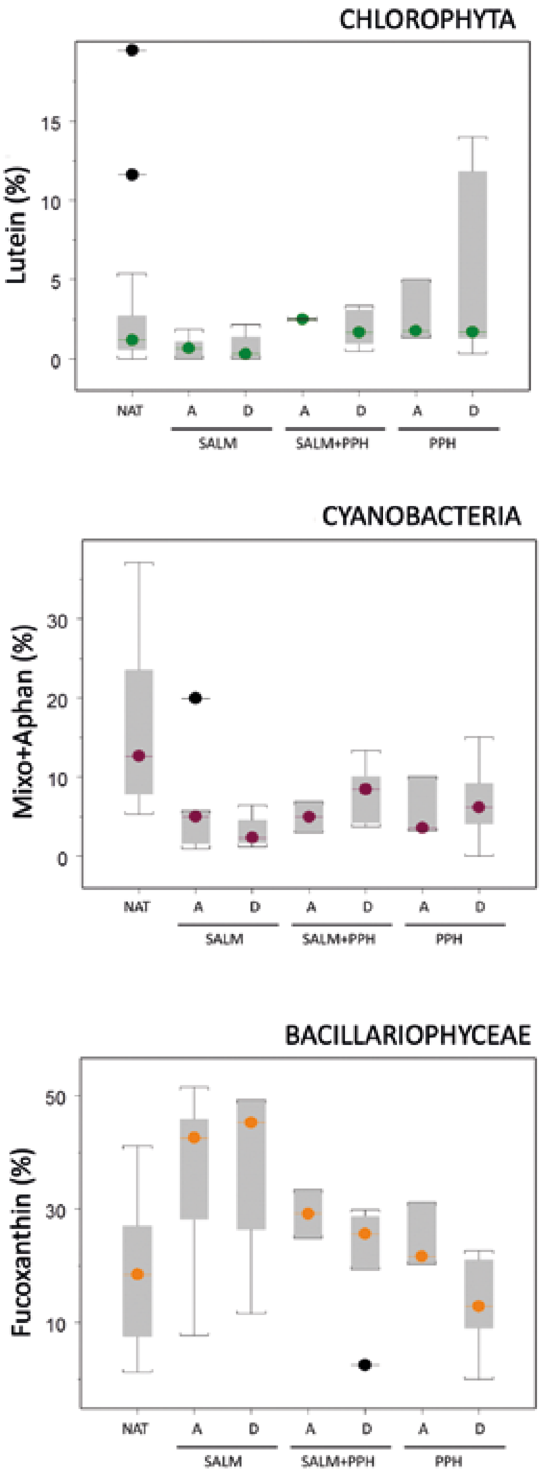


Figure 8. Box diagrams showing the frequency distribution of the percentage of carotenoids indicative of Chlorophyta (lutein), Bacillariophyceae (fucoxanthin) and Cyanobacteria (myxoxanthophyll and aphanizophyll) on the periphyton of the littoral stones of the target lakes and for the different scenarios. Abbreviations the same as in Figure 6.

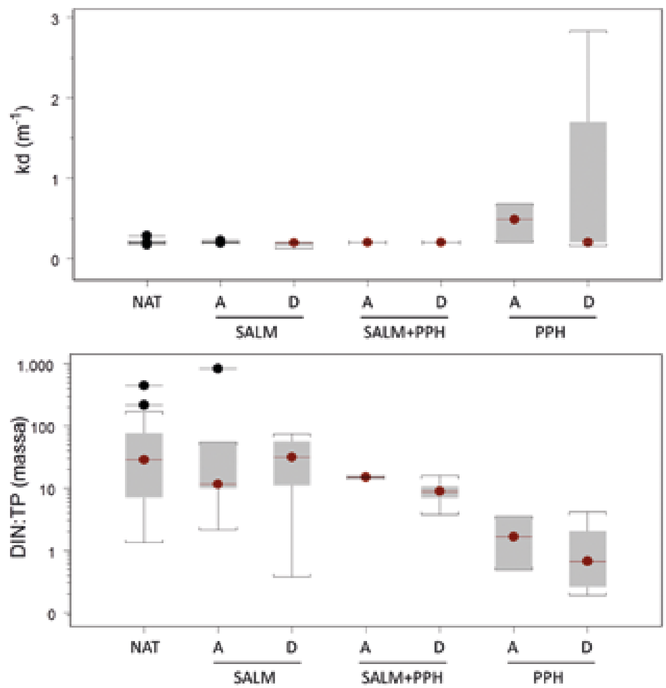


Figure 9. Box diagrams showing the frequency distribution of the light extinction coefficient (kd; m^{-1}) in the upper graph, and the mass ratio of dissolved inorganic nitrogen to total phosphorus (DIN:TP) in the lower graph in the different scenarios for the target lakes. Abbreviations the same as in Figure 6.

PHYSICAL-CHEMICAL PARAMETERS

The transparency of the water column, represented by the light extinction coefficient K_d was generally high (low K_d values) in all lakes except those with minnow (Figure 9). In the lakes where minnow had been removed, there was a slight recovery in the transparency of the water column indicated by the decrease in the light extinction coefficient.

Regarding nutrients, the DIN:TP ratio of both natural lakes and lakes with salmonids with or without minnow gave values between 10 and 30. The same ratio in lakes with minnow had much lower values, between 0.4-1.5. The ratio increased after starting actions in lakes where there had been salmonids and instead decreased where there had been minnow alone or accompanied by salmonids.

DISCUSSION

This work is a pioneer in highlighting the recovery of high mountain lake communities after decreasing densities of cyprinids such as minnow. In the set of lakes where the work was conducted, which include lakes with salmonids alone or accompanied by minnow and lakes that only had minnow, the recovery was clearer in general for all the indicators measured in the lakes where minnow had been present.

This more pronounced recovery is likely to be related to the greater degradation in the communities of lakes with minnow (Buchaca et al., 2016).

The group of organisms studied that presents a clearer response to the actions are the littoral macroinvertebrates that show a progressive increase in abundance of taxa over time, without having yet reached stabilisation. This increase occurs in all lakes, both those that had only salmonids and those that had salmonids with minnow or only minnow. After starting the work, the macroinvertebrate composition of the shore of the target lakes converged progressively with that of the natural lakes. This result coincides with that obtained in other studies after practicing salmonid eradication in both the Rocky Mountains of North America (Epanchin et al., 2010; Knapp et al., 2001; Pope et al., 2009) and in the Italian Alps (Tiberti et al., 2018). In these studies the recovery of the taxonomic composition and abundance of the community of organisms in general (amphibians, zooplankton and benthic macroinvertebrates) occurred in a period of 10-20 years after the eradication of fish (Knapp et al., 2001), or even in a shorter period of time, of only 5 years (Tiberti et al., 2018). Similar results have been obtained when studying the recovery of amphibious fauna from the same lakes (Miro et al., 2019 this volume), indicating that larger species have a greater facility to recolonise the ecosystem (Arribas et al., 2012).

The increase in biomass and the abundance of littoral macroinvertebrate taxa after starting the actions led towards expecting a decrease due to herbivory of the biomass of the periphyton that grew on the stones of the shore (Hillebrand & Kahlert, 2001; Ventura et al., 2016). This decrease was observed in all the lakes where there had been minnow although it was clearer in the lakes that had contained salmonids and minnow (2 target lakes in the Dellui valley). The lower response of the lakes that had minnow as the only species at the time the actions began could be explained by the particularities of each system, the ecological status and the composition of the starting community of each of them. In particular, at Rovinets, tadpoles had not yet entered, only newts that do not have roaming habits (Miró et al., 2019). At Naorte, tadpoles have not yet arrived either whereas Closell already had the common toad (*Bufo spinosus*) before action had started, but this is a species that is not affected by the presence of fish, and therefore it was difficult to find relevant changes. In addition, in the lakes where salmonids were the only fish species before starting the action, the periphyton biomass was even greater after the fishing began. In two of these lakes that had salmonids (Cap de Port and Cabana) eradications are still very recent (2016 and 2017) and in the third, Subenuix, the fauna of grazers, both of insects and amphibians, took a long time to recover (see Miró et al. 2019 in this volume). This delay in recovery at Subenuix is probably related to the species of salmonid that there was, brook trout (*Salvelinus fontinalis*). This salmonid reaches densities that can be 10 times higher than other species of the same family (Tiberti et al., 2018). Therefore, it was not until the end of 2017 that the density of brook trout that remained in the lake at Subenuix was low enough to have no negative ecological effects. Everything indicates that in this case it was still early to find clearer changes that would affect even the primary producers of periphyton.

The composition of primary producers of the periphyton of the stones, showed clear patterns of the effect of the actions. However, the results show a slight increase in the proportion of cyanobacteria and a decrease in that of diatoms and chlorophytes after starting the actions in the lakes where there was minnow alone or accompanied by salmonids. It will be necessary to follow the evolution of these communities for a longer period of time in order to verify it. If this trend were confirmed, the composition of the community could come to resemble that found in the periphyton of natural lakes. These changes in the composition of algal groups are consistent with those found experimentally by various authors who report a dominance of cyanobacteria compared to chlorophytes and diatoms under conditions of higher herbivory pressure (Hillebrand & Kahlert, 2001).

The change in the structure and biomass of the communities of pelagic organisms and the transparency of the water column of the target lakes only occurs markedly when minnow had been the only fish species in the lakes. In these cases (Closell, Naorte and Rovinets) the elimination of the minnow led to a marked increase in the abundance of crustaceans, a decrease in the phytoplankton biomass and an increase in the transparency of the water column. This recovery of crustaceans would not yet be complete, both with regards to the community and its biomass. The most notable case is that of the lake at Closell, where there have been oscillations in the transparency of water closely linked with oscillations of biomass of crustaceans of the *Daphnia* genus, following the theory of alternative stable states described in shallow lakes of the temperate zone with mesotrophic-eutrophic characteristics (Scheffer et al., 1993). The other two lakes with minnow, Naorte and Rovinets, have shown a more marked trend towards an increase in transparency. However, these changes did not seem to affect the phytoplankton composition.

The results indicated that the target lakes are in the process of recovering the communities of organisms that would be their own under natural conditions. However, it seems necessary to continue to monitor the evolution of these communities for a longer period of time in order to verify it.

The introduction of fish in high mountain lakes is a type of disturbance that has occurred over centuries across a very vast territory. The eradication of introduced fish allows us to study the resilience of the lake as an ecosystem against this type of disturbance and offers us the opportunity to demonstrate the impact that fish have on different biotic and abiotic variables of these particular systems.

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