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

TECHNICAL REPORT

LIFE13 NAT/ES/001210 LIMNOPIRINEUS

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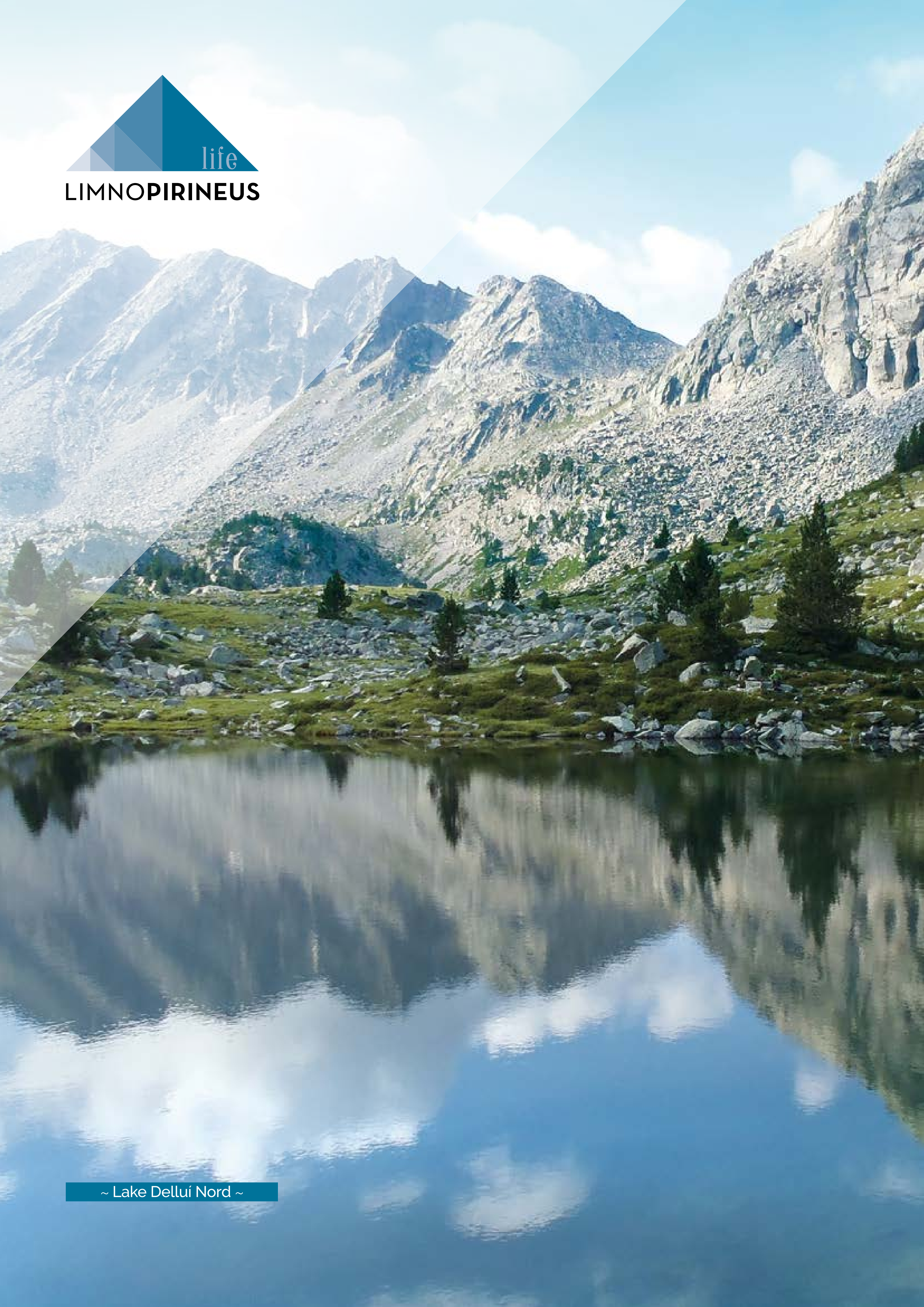

~ Lake Delluí Nord ~

~ Lake Naorte ~

REMOVAL OF INTRODUCED FISH AS A RESTORATION MEASURE IN HIGH MOUNTAIN LAKES

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ABSTRACT

Among the objectives of the LIFE+ LimnoPirineus project (2014-2019) was the ecological restoration of eight Pyrenean lakes, and the recovery or improvement of populations of various species of community interest in these lakes, through the eradication or intensive control of up to four species of introduced fish. In most of the lakes, the operational objective was eradication, while in only one of them the objective was simply to carry out intensive control to reach at least a 75% reduction of the initial population. At the end of 2019, these objectives were fully achieved for five of the lakes, while in the three remaining lakes, they are close to being achieved probably before the end of 2020. Fish extraction methods were conducted based on the combination of up to three main catch techniques; nets, traps and electrofishing. It has shown that the eradication of fish is feasible in many high mountain lakes, through sufficient investment in material and human resources, and with proper planning of operations. When eradication is not feasible, intensive control through a sustainable effort over time is also an alternative to consider in the medium term.

INTRODUCTION

The high mountain lakes of the Pyrenees are isolated from the rivers of the main valleys by hydrographic barriers that have prevented natural colonisation by the fish (Pechlaner, 1984). Despite this, especially during the twentieth century and linked to the practice of sport fishing, fish have been introduced to the point that many lakes currently maintain stable fish populations (Miró, 2011). The two most widespread species in the Pyrenean high mountain lakes are the common trout (*Salmo trutta*) and the minnow (*Phoxinus* sp). Other species introduced in the lakes of the southern Pyrenean slope, but less widespread than common trout and minnow, are brook trout (*Salvelinus fontinalis*) and rainbow trout (*Oncorhynchus mykiss*) (Aparicio et al., 2016, SI-EXOAQUA, 2019, SIBIC, 2019). Currently, between 35% and 85% of the Pyrenees lakes are occupied by fish, depending on the valley (Miró & Ventura, 2013; 2015). It seems clear that the motivation for the introduction of salmonids has always been their value as target species of traditional fishing in the past and sport fishing in modern times. However, in the case of minnow, its introduction began later, probably from the 70s of the twentieth century, when its use as live bait and forage fish was increased.

The effects of the introduction of fish in alpine lakes originally free of ichthyofauna range from the removal of other wildlife species, both invertebrates and vertebrates, to radical changes in the structure and dynamics of the lake ecosystem, even ultimately affecting the ecological status, or the quality and visual appearance of water (Buchaca et al., 2016; Knapp, 2005; Knapp et al., 2015; Maxwell et al., 2011; Miró et al., 2018; Reissing et al., 2006; Tiberti & Von Hardenberg, 2012; Ventura et al., 2017).

Along with the broad evidence of the impact caused by introduced species on the original biodiversity, both locally and regionally, there is also a broad consensus that the best measure to avoid or minimise these impacts is prevention and monitoring to avoid new introductions, be they deliberate or involuntary (Wittenberg et al., 2001; García-Berthou et al., 2005; Simberloff et al., 2013). Alongside this, it is recognised that the active management of populations of native species already established, whether to eliminate them or at least to contain them or mitigate their effects, is almost always technically unfeasible or economically unattainable, despite the fact that in some situations actions can be planned with certain chances of success. In this sense, it is pertinent to concentrate action efforts on isolated populations of introduced species, as is the case for the Pyrenean lakes, even more so where a high potential for ecological recovery is combined with a high conservation interest due to uniqueness or the biogeographical value of the habitats or native species potentially present.

Outside Europe, numerous projects or programmes for the active management of exotic fish species have been carried out, either to eradicate established populations or to initiate stable intensive control programmes, especially in North America (per ex. Tyus I Saunders, 2000; Hill & Cichra, 2005), but also in other areas such as Australia, New Zealand or South Africa (per ex. Lintermans, 2000; Thomas et al., 2006). Among these, it is worth mentioning the programmes conducted in the high mountains of North America, in the Rocky Mountain Range, with an extensive history of successful experiences.

In Europe, on the other hand, the history of active management of exotic fish species is still rare, although they are gradually increasing (per ex. Britton et al., 2009, Campos et al., 2013). But in the specific case of high mountains, known projects are very scarce, although often successful (Tiberti, 2018). Despite this, so far they have focused mainly on salmonids, and only very occasionally on small cyprinids such as minnow.

In this context, the LIFE+ LimnoPirineus project (LIFE + Natura LIFE13 NAT/ES/001210) included actions to eliminate exotic fish nuclei in a selection of mountain lakes in the Alt Pirineu Natural Park (APNP) and the Aigüestortes and Estany de Sant Maurici National Park (AESMNP). The conservation objectives directly linked to these actions were 1) the restoration of their structural quality and ecological function, 2) the recovery of lake populations of threatened amphibians (*Rana temporaria, Alytes obstetricans, Euproctus asper* and *Lissotriton helveticus*) for the improvement of its local metapopulations, and 3) the improvement of populations of various threatened mammals (*Galemys pyrenaicus, Lutra lutra, Rhinolophus hipposideros* and *Plecotus macrobullaris*) within these protected natural spaces.

METHODS

All methodological, operational and planning details regarding the elimination of fish introduced into the lakes under the LIFE+ LimnoPirineus (LLP) project have been included in two protocols specifically written for this purpose (Pou-Rovira, 2015a, Pou-Rovira, 2015b), which include more information than that provided here.

Action area and work plan

Eight lakes were selected by the LLP for the elimination of introduced fish nuclei, of which three were located in Alt Pirineu Natural Park (APNP) and the rest in Aigüestortes and Estany de Sant Maurici National Park (AESMNP) (Table 1, Figure 1). The three lakes located in APNP initially contained only minnow populations. In contrast, the starting situation in the lakes selected in AESMNP was more diverse. The five lakes in the national park contained salmonid, while two of these also contained minnow.

For organisation purposes, and mainly due to the capacity of workforces specifically intended for the task of fish extraction, the start of operations in each lake was staggered. By way of exception, at Lake Closell fish elimination work began in 2013, in the context of a previous experimental project to begin evaluating the most appropriate possibilities and methods to deal with the eradication of minnow in high mountain lakes. These tests continued during 2014, within the framework of a parallel project, and extended to other lakes not selected within the LLP (Pou-Rovira, 2015c). Finally, in 2015 the definitive work began at 4 of the initially planned lakes, leaving the start at the three other lakes for 2016 and 2017 (Table 1).

When planning operations to eliminate fish nuclei or other aquatic fauna in inland bodies of water, there are several methodological options of value. These include strategies based on the temporary desiccation of the body of water, or alternatively on the use of ichthyocides, especially rotenone or its derivatives (Finlayson, 2010). Although very effective, desiccation is feasible in very few situations. Regarding the use of ichthyocides, this represents a management tool with great practical potential, but unfortunately it still has numerous limitations, both at the operational and legal levels. Consequently, the LLP project concentrated efforts on a strategy based on the planning of intensive fishing campaigns, i.e. a massive and persistent fish catch until the established operational objectives were reached. In fact, there are numerous precedents for fish elimination projects through the application of intensive specimen capture only (per ex. Bio et al., 2008; Pou et al., 2013; Pou- Rovira, 2013; Pou- Rovira, 2017), among which, it is worth noting here the projects for the removal of salmonid nuclei introduced in high mountain lakes (Bosch et al., 2019; Tiberti et al., 2018; Knapp & Matthews, 1998). These projects have constituted a reference base for the planning of similar operations set of salmònid removal forth by the LLP. On the other hand, at the time of initiating the LLP, there were no known precedents of intensive control or eradication experiences of minnow or similar species in high mountains by persistent catching. This was a factor for the LLP, which led to the implementation of an adaptive methodological strategy, to improve the methodology as results were gradually observed and recorded.

In order to achieve the objectives of ecological recovery established by the LLP with respect to the lakes, the operational objective at each of them was the complete eradication of fish, in order to leave the lake free of fish by the project deadlines (Table 1). However, at Lake Naorte, due to its size and the initial uncertainty regarding the case of minnow, the initial operational objective was lowered to intensive control in order to achieve a significant population reduction of at least 75%, that is easily sustainable in the medium term through subsequent efforts.

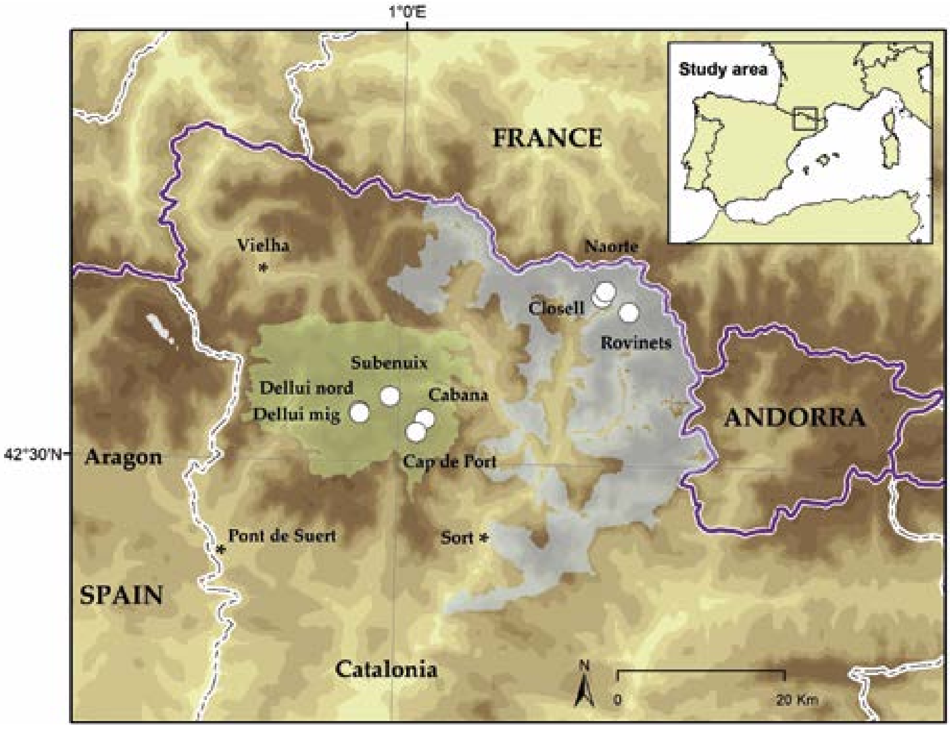
Catch techniques:

Within the framework of the initial tests carried out to choose the most efficient and appropriate catch techniques for each situation, various techniques were discarded since, although they result in catches, their performance is scarce, making them not even useful as a complementary method in most work scenarios for LLP. These are, for example, the use of cast nets, small trawlers and seine fishing, small bottle-type traps, baited traps, large traps, landing nets, or trammel nets. Some of these techniques have proven useful in other exotic aquatic fauna control projects (Rytwinski et al., 2018), but for now they have proved ineffective or very inefficient in high mountain lakes.

Finally, then, the three main catch techniques used for the elimination of fish in the lakes have been 1) the use of multi-mesh gillnets (Figure 2), 2) the use of fyke net traps (Figure 3), and 3) electrofishing (Figure 4). In any case, each of these techniques showed a certain variable effectiveness depending on the species and the time of year (Table 2).

Name (code)[a]	Protected area[b]	Maximum depth (m)	Surface area (ha)	Altitude (M.A.S.L.)	Fish species present[c]	Operational objective	Elimination of fish: start year
DEN (1831)	AESMNP	6.7	0.35	2306	STR, PPH	Eradication	2015
DEM (1838)	AESMNP	6.2	1.09	2314	STR, PPH	Eradication	2015
SUB (2066)	AESMNP	11	2.64	2194	SFO	Eradication	2016
CPO (2213)	AESMNP	31.7	7.35	2521	STR	Eradication	2016
CAB (2259)	AESMNP	11.7	2.33	2376	OMY	Eradication	2017
CLO (2468)	APNP	3.7	0.75	2074	PPH	Eradication	2013
NAO (2479)	APNP	14	3.94	2150	PPH	Intensive control	2015
ROV (2654)	APNP	5.4	0.37	2223	PPH	Eradication	2016

▲ **Table 1.** Descriptive features of the eight target lakes, fish present and operational objective.
a DEN: Dellui Nord. DEM: Dellui Mig. SUB: Subenuix. CPO: Cap del Port. CAB: Cabana. CLO: Closell. NAO: Naorte. ROV: Rovinets.
b AESMNP: Aigüestortes i Estany de Sant Maurici National Park. APNP: Alt Pirineu Natural Park.
c STR: Common trout (*Salmo trutta*). OMY: Rainbow trout (*Oncorhynchus mykiss*). SFO: Brook trout (*Salvelinus fontinalis*). PPH: Common minnow (*Phoxinus sp*).



▲ **Figure 1.** Location of the eight target lakes (white dots). Aigüestortes i Estany de Sant Maurici National Park is shown in green and Alt Pirineu Natural Park is shown in gray.

	EFFECTIVENESS BY SPECIES *			
Catch technique	**STR**	**OMY**	**SFL**	**PPH**
Multi-mesh gillnets	High	High	High	High
Fyke net traps	Low (lakes) Medium (streams)	Low (lakes)	Medium (lakes)	High (lakes, June to August) Low (lakes, August to October)
Electrofishing	Medium (streams) Low (lakes)	Low (lakes)	Medium (streams) Low (lakes)	Medium / High (lakes, June to October) Low (lake, July to September)

▲ **Table 2.** Efficacy of the main catch techniques used, by species. * See codes in table 1.

Nets

The multi-mesh nets used are made of translucent nylon. After testing other nets, the preferred provider for the supply of quality nets was Lindeman (Finland). Mesh sizes were as follows: 5.6, 8, 10, 12.5, 15.5, 19.5, 24, 29, 35, 43 and 55 mm. These meshes are part of the sequence of meshes of European standard multi-mesh nets for sampling fish in lakes (CEN 14757, 2005). Depending on the target species, a different combination of meshes was used. All were used for salmonids, while only mesh under 10 mm was used for minnow. Each piece consisted of a combination of different panels of different meshes or a single uniform meshed panel, but together, in each lake a proportionally balanced surface area of different mesh sizes was used, always depending on the subject species and progressively achieved results. Most installed networks had a height of 1.5 m and a length of 30 m, and were placed forming a grill that uniformly covered each lake. This grill was anchored with ropes on the lake shore, so that adjusting the tension of the ropes made it possible to regulate the operational depth of the pieces. Some central nets were of greater height, 3 m and occasionally even 4 m. Their position in the water column was varied according to their effectiveness and also to conditions such as the presence of submerged blocks or logs that could break them, although the aim was for them to reach the bottom. In addition, nets of low height (0.75 m or 0.5 m), and shorter length (15 m), generally of small mesh size (<8 mm), were also used for installation on the shore, a strategy that has proven to be effective especially for catching minnow. A key aspect of the use of nets is their regular and frequent review, to remove the catches accumulated and clean the deposits that accumulate on the nylon filament, rapidly decreasing its effectiveness. In initial phases of work at each lake, they were reviewed at least once a week. Later, once catches had decreased by approx. 10% compared to at the beginning, reviews were more spaced out, while aiming to maintain reviews no later than every 15 days.

A noteworthy aspect refers to the quality and durability of fishing materials, especially nets, and to a lesser extent traps. The climate conditions in high mountains, with notable variations in water temperature, and especially the high incident solar radiation, considerably shortens the useful life of nylon or polyamide mesh with which this fishing equipment was made. We observed notable variations in its durability, from scarcely one season in the case of some nets, up to a maximum of 4 continuous seasons of use. Everything indicates that the life of these materials depends mainly on the quality of the materials used by the manufacturer and supplier, but also on the local conditions at each lake, and the use made of them. This aspect significantly affects fishing performance, and may compromise the viability of planned multi-year campaigns, if not considered.

Traps

We reverted to the preferential use of front opening, river traps, with two, small mesh (4 mm), foldable, internal funnels without any bait. The total length of these traps was approx. 3 m, and the height at the entrance was 40 cm. The main suppliers were located in Andalusia, where local artisan producers manufacture them with quality and sufficient strength to supply the large continental crustacean fishery in southern Spain. These traps were useful for serial placement on the shore of the lakes, mainly for catching minnow. Occasionally, we also used larger traps of this type with larger mesh size, for catching salmonids in certain microhabitats, such as tributary streams, or operationally complicated shoreline sectors. As in the case of low-lying nets located on the

Figure 2. ▶
Gillnets used for intensive fishing in the lakes: A, review of a net placed in a lake; B, breakdown of a 0.5 m high net placed on the shore; C, "grid" arrangement of various nets.


A
PLASTIMO


B


C

shore, the traps regularly changed sector every month or two, or at least their position within the sector where they were located was changed, in order to reduce the "fatigue" phenomenon observed when they are permanently maintained in the exact same location, which translates into a drastic decrease in catches with respect to the expected efficiency. The review and emptying of traps were done regularly, at least once a week when they were very efficient, at the beginning of the fish elimination campaigns, and in general at the beginning of summer. Later, when their efficiency dropped considerably but they continued to catch along the shore, reviews could be spaced up to once a month, thanks to the fact that fish catches remain alive within the trap. However, it should be considered that this catch technique can accidentally catch other aquatic fauna species, some of which may also eventually drown. Therefore, depending on the incidence of this problem at each location and stage of work, it was necessary to adjust the frequency of review accordingly, and if the placing of the traps was appropriate.

▲ **Figure 3.** Small mesh fyke net traps used for intensive fishing in the lakes: A, general view of a trap placed on the shore; B, battery arrangement of various traps along the shoreline.

Electrofishing

This catch technique is very effective in most low or medium depth lotic systems. However, it is not very or not at all effective in open lentic systems, except along the shore, where its effectiveness depends on multiple key factors, such as habitat structure, fish behaviour, and especially turbidity and electrical conductivity of the water. In the LLP target lakes, with extremely low conductivity, always below 50 mS/cm, its efficiency was consequently very low. In any case, with good operational deployment, electrofishing proved to be moderately effective for catching minnow on the lake shore, especially while the water temperature remained below approx. 18 °C. In addition, it was also partially effective, and at the same time almost the only viable option, for catching salmonids in the tributary streams of some of the target lakes that also needed to be covered. Up to three types of electric fishing equipment were used from the Hans Grassl brand: EL65 IIGI, ELT62-IIF, ELT62IIH, listed from greater to lesser power. The devices of the first model were heavy, stationary, with extension for the anode, while the others were compact, lighter, backpack equipment. Each model had two devices, for simultaneous and strategic use in the various sectors of the LLP's area of action.

In the streams, electrofishing was conducted by making thorough passes upstream combing the entire bed, which were repeated regularly. In contrast, on the lake shores, fishing was operationally adapted to each location based on the orographic conditions. On the most accessible banks, the coastline was tracked using portable equipment. In contrast, in other cases it was convenient to fish with the help of a rowing boat, a fact that limits effectiveness. Throughout the LLP, as practical experience was gained in these types of environments, operational tactics improved, increasing the efficiency of electrofishing. Thus, for example, when the density of fish

▲ **Figure 4.** Electrofishing in the lakes: A, use of heavy equipment; B, use of portable equipment with the operational help of a vessel and in combination with the strategic placement of shore nets.

on the shoreline was already very low, it was more effective to catch “looking out” for the remaining shoals of fish, to deal with them suddenly, rather than continuing to comb the shoreline. At the same time, it was observed that in some key sectors of the shore where minnow specimens tended to concentrate, a combined use of traps, shore nets and intensive electrofishing, increased the catches of each technique compared to any of these alone; a resource especially useful in the final stages of the elimination of the fish nuclei, when density was already very low.

Fishing effort distribution

Table 3 shows the distribution of the annual fishing effort by technique and mass of water finally applied throughout the LLP. At all times, efforts have been made to maintain the overall fishing effort stable until the established operational objectives were achieved, i.e. until the fish nuclei were eradicated in most of the target lakes. However, the effort applied did vary, mainly due to the adaptive review of the work plan for each lake. Thus, in some cases, such as at Cap de Port, the initial effort with nets was soon revealed to be insufficient, so it gradually increased. In the case of secondary techniques (e.g. traps in lakes containing only salmonids), the effort was always low and often quite variable.

In addition, when what was assumed to be the last specimen was removed from each lake, it was recommended to maintain the effort for at least one year, to ensure that there was really no isolated specimen, before dismantling the previously installed fishing equipment and stopping fishing.

Data analysis

All specimens caught in the LLP fish elimination campaigns were counted, identified at species level, and sexed when possible. In addition, furcal length and individual weight, where possible, was measured for all catches, in order to obtain subsequent estimates of the total biomass removed.

In addition, the effort units applied to obtain the catches, which depend on each catch technique, were also continuously recorded (see table 3). From these effort units it was possible to generate relative density values (CPUEs, Captures Per Unit Effort) by species, lake and catch period. Thus, apart from the temporal evolution of simple catches, expressed in absolute or relative name for each period of reference time, CPUEs were also used as the best indicator of the evolution of the target population, since it is standardised by actual effort applied. Although we calculated the relative density for all the catch techniques applied, for each species only CPUEs that were considered less subject to interannual variation, whilst more reliable and representative of their population dynamics are presented. Thus, in the case of salmonids, CPUEs were used based on the use of nets throughout the entire effective fishing season from June to October, while in the case of minnow, only CPUEs based on use of river traps from June to mid-August were used.

Finally, we also calculated the fishing performance expressed simply as a ratio between the catches obtained and the work effort (working hours used), understanding that we always worked with equivalent fishing teams, sufficiently equipped and sized to apply the catch techniques with the maximum possible efficiency at each

		ANNUAL FISHING EFFORT BY TECHNIQUE AND MASS OF WATER*							
Catch technique		CLO	NAO	ROV	DEN	DEM	SUB	CPO	CAB
Nets	Mean	766.3	1633.0	1343,5	896.6	1560.,8	2457.8	2564.8	2634.3
(Units: net · day)	Maximum	1.238	2492	1800	1762	2181	2999	3450	3014
	Minimum	210	1046	847	560	1048	1806	782	2239
Traps	Mean	3401.0	2046.0	2349.5	1745.2	1744.4	99.,3	87.5	49.0
(Units: trap · day)	Maximum	5.860	2817	2500	2100	2412	337	186	102
	Minimum	1.740	1084	2156	1359	1100	0	0	0
Electrofishing	Mean	51.0	115,4	61.0	139.4	135.0	43.8	66.8	10.0
(Units: hours)	Maximum	114	190	82	210	341	91	96	18
	Minimum	0	30	38	21	28	6	36	0
Working hours	Mean	24.3	45.6	24.3	50.8	36.2	21.0	27.3	19.3
	Maximum	38	59	32	60	46	43	37	29
	Minimum	11	34	19	40	25	10	19	12

▲ **Table 3.** Summary of the fishing effort applied to each target lake. * See codes in table 1.

time and place of work. This fishing performance index is an additional indicator of the temporal evolution of the target population in each lake, and therefore also of the degree of success of fish elimination campaigns.

RESULTS

The operational objectives established for the elimination of fish nuclei introduced in the LLP target lakes (see table 1), were achieved in most cases, and everything indicates that they will be achieved within a short time for the rest. Specifically, the following fish nuclei were eradicated: minnow in the lakes at Closell (APNP) and Dellui Mig (ASEMNP), common trout in the lakes at Dellui Mig and Dellui Nord (ASEMNP), brook trout in the lake at Subenuix (ASEMNP), and rainbow trout in the lake at Cabana (ASEMNP). In all these lakes, the last specimen was caught in 2018 or earlier, and the fishing effort was maintained during 2019, generating zero catches and thus certifying that the fish nuclei were effectively eradicated. In addition, in the lake at Naorte (APNP), the initially planned population reduction of at least 75% for minnow was also exceeded.

In the case of the minnow populations of the lakes at Rovinets (APNP) and Dellui Nord, everything indicates that the remaining population in mid-summer 2019 is residual, with an absolute density probably less than a few dozen individuals, or even less. Also, in the lake at Cap de Port (ASEMNP), the common trout population was also almost gone by mid-summer 2019. Therefore, predictably the last specimen in each of these three lakes will be captured in 2019 or at the latest in early 2020. In any case, it will be necessary to maintain the fishing effort for at least an additional year until the end of 2020, in accordance with the provisions of the specific protocols.

Minnow

Table 4 and Figures 5-A, 5-B and 5-C show the results of the minnow elimination actions in the target lakes. For this species, the total number of specimens extracted can only be considered as an approximation to the absolute density before beginning the fish extraction, since over the various years of fishing campaigns, recruitment continues, although at a decreasing rate.

The size structures observed for the eliminated minnow populations indicate that they were well structured populations, with regular, although perhaps quite variable, recruitment in some lakes from one year to another, and also a low mortality that would explain a remarkable relative density of very large sized specimens (Figure 6). The differences observed in the total catches and biomass extracted, once weighted according to the surface area of each lake, are still quite remarkable, indicating a clear diversity of starting situations between the target lakes. The maximum density and initial biomass of minnow occurred in the lake at Dellui Nord, probably thanks to the structure of its littoral habitats that favoured this species, among other factors not yet analysed. However, the minimum initial density occurred in the lake at Dellui Mig, a situation that could be related to the presence, until then, of a stable trout nucleus in this lake.

The population dynamics of minnow in each lake, once the intensive fishing work began, was quite variable, partly due to adjusted methodological approximations as results were recorded. In fact, one of the lakes with a more erratic trajectory of catches extracted was the first where work began at Closell. In any case, in all the lakes, the decrease in both the average relative density and the fishing yield were gradual and sustained, until the ultimate eradication was achieved or approached. For all the target lakes for which the operational objective of eradicating minnow was established, this milestone was reached (or will most likely be reached) within a period of between 4 and 6 years of continued work. In the lakes Closell and Dellui Mig, where the species was already eradicated, the last catch was obtained after almost a year without any catches. During the last years of the campaigns, recruits were not observed on the shore, a fact that indicates that the remaining nucleus has failed to reproduce.

In the specific case of the lake at Naorte, the largest of the target lakes with a presence of minnow, where the initial operational objective was only to achieve a significant reduction in the initial population, the population trend has also been regressive, although with a lower gradient or rate. It is the only lake where minnow fishing performance never dropped by 5% compared to the first year, despite having gradually decreased.

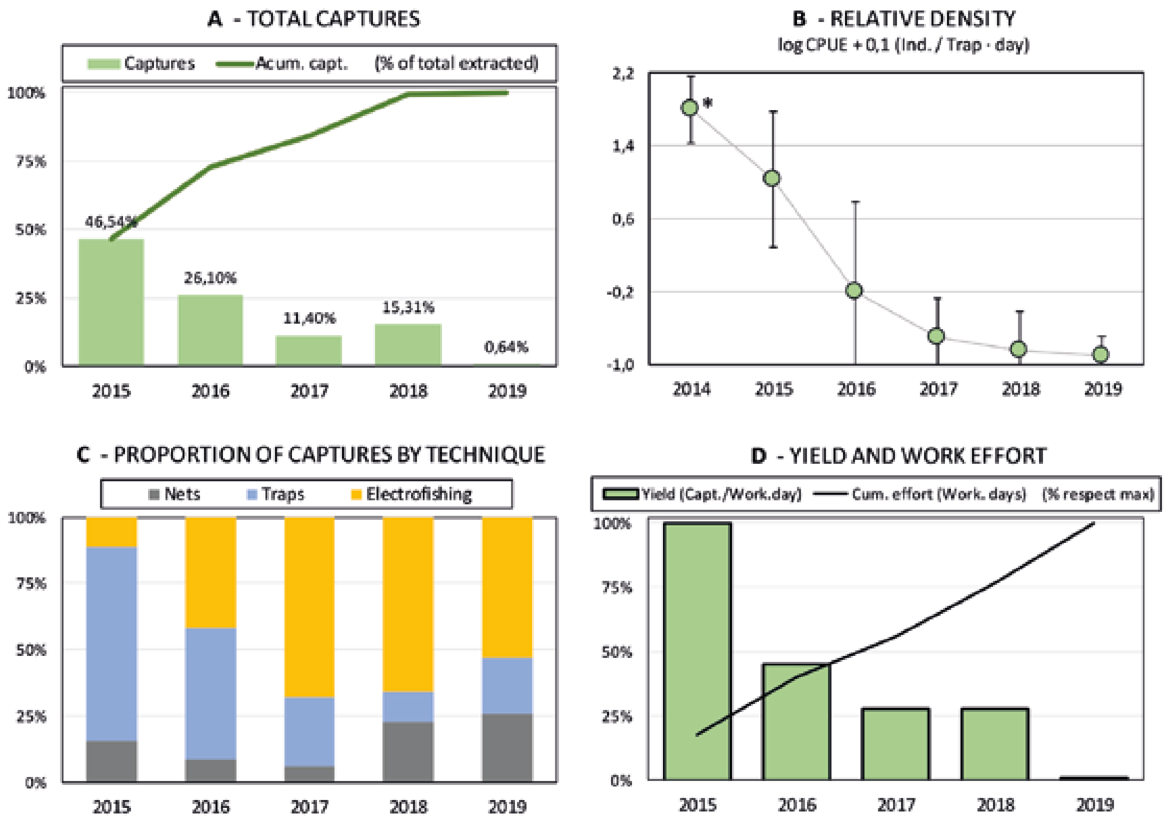
The various catch techniques produced proportionally quite different results between lakes and years, partly depending on the physical conditions of each lake, but also on the changes and improvements that were gradually introduced in fishing gear or equipment and way of using them, especially with regard to electrofishing and nets. In any case, in all the lakes the three main catch techniques (nets, traps and electrofishing), combined, played a key role throughout the eradication or intensive control process of this species.

	SPECIMENS CAUGHT (Ind.), BY YEAR AND WATER MASS				
Species:**	PPH	PPH	PPH	PPH	PPH
Year	Dellui Nord	Dellui Mig	Closell	Naorte	Rovinets
2014	-	-	4,053	-	1,905*
2015	9,954	2,315	6,169	46,009	-
2016	5,582	1,338	152	22,747	3,111
2017	2,439	50	3	8,557	664
2018	3,275	3	1	6,505	119
2019	137	0	0	3,800	27
TOTAL CATCHES (Ind.)	21,387	3,706	10,378	87,132	5,826
TOTAL CATCHES (Ind./ha)	61,106	3,400	13,837	22,114	15,574
TOTAL BIOMASS (kg)	53.99	18.80	35.43	212.13	21.28
TOTAL BIOMASS (kg/ha)	154.26	17.24	47.24	53.84	57.50

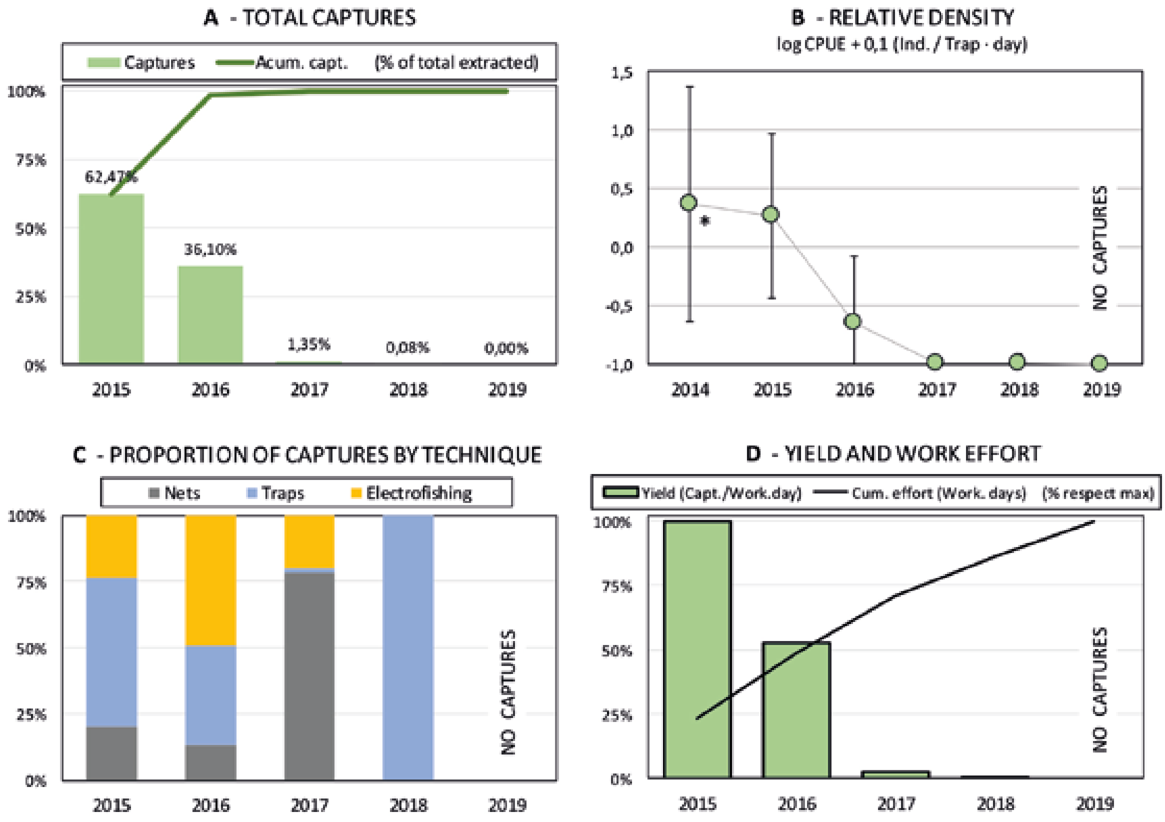
▲ **Table 4.** Summary of minnow (*Phoxinus sp*) catches in the target lakes. *Catches produced by a initial sampling; ** See codes in table 1.

Minnow (*Phoxinus* sp.)

LAKE DELLUÍ NORD - Minnow (*Phoxinus* sp)

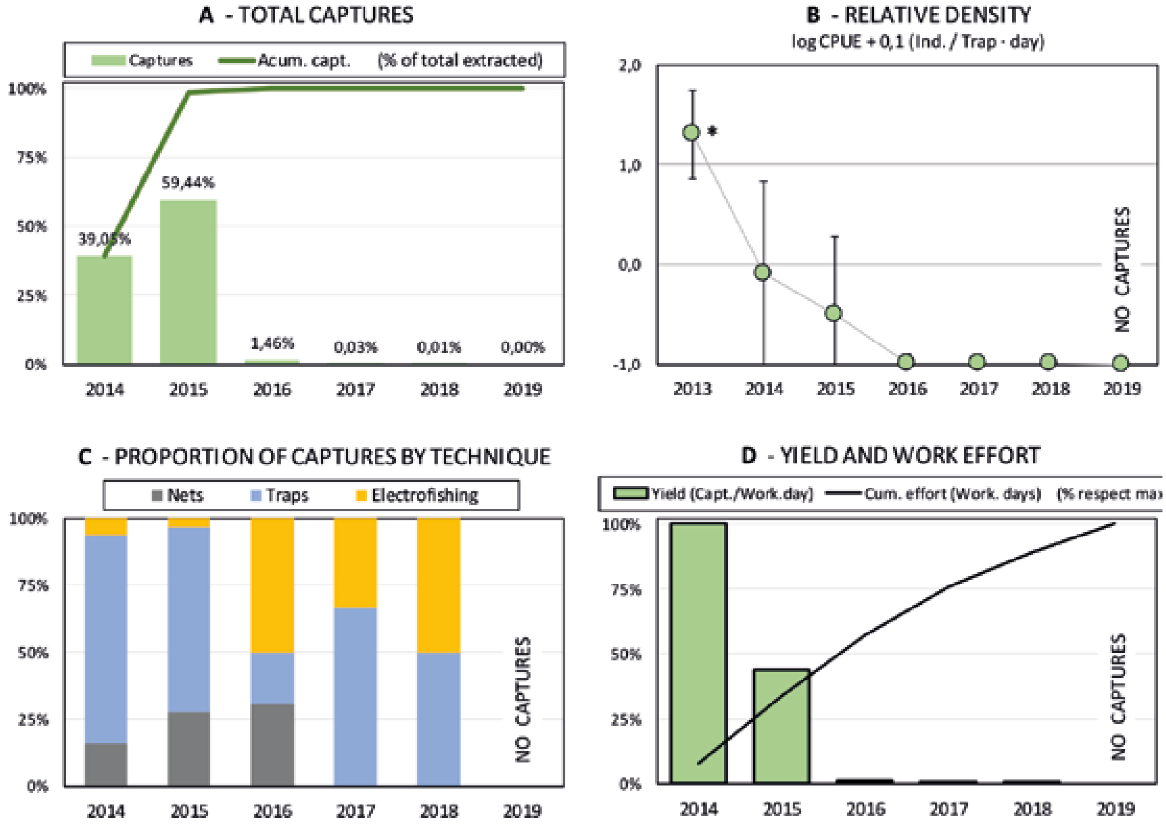


LAKE DELLUÍ MIG - Minnow (*Phoxinus* sp)

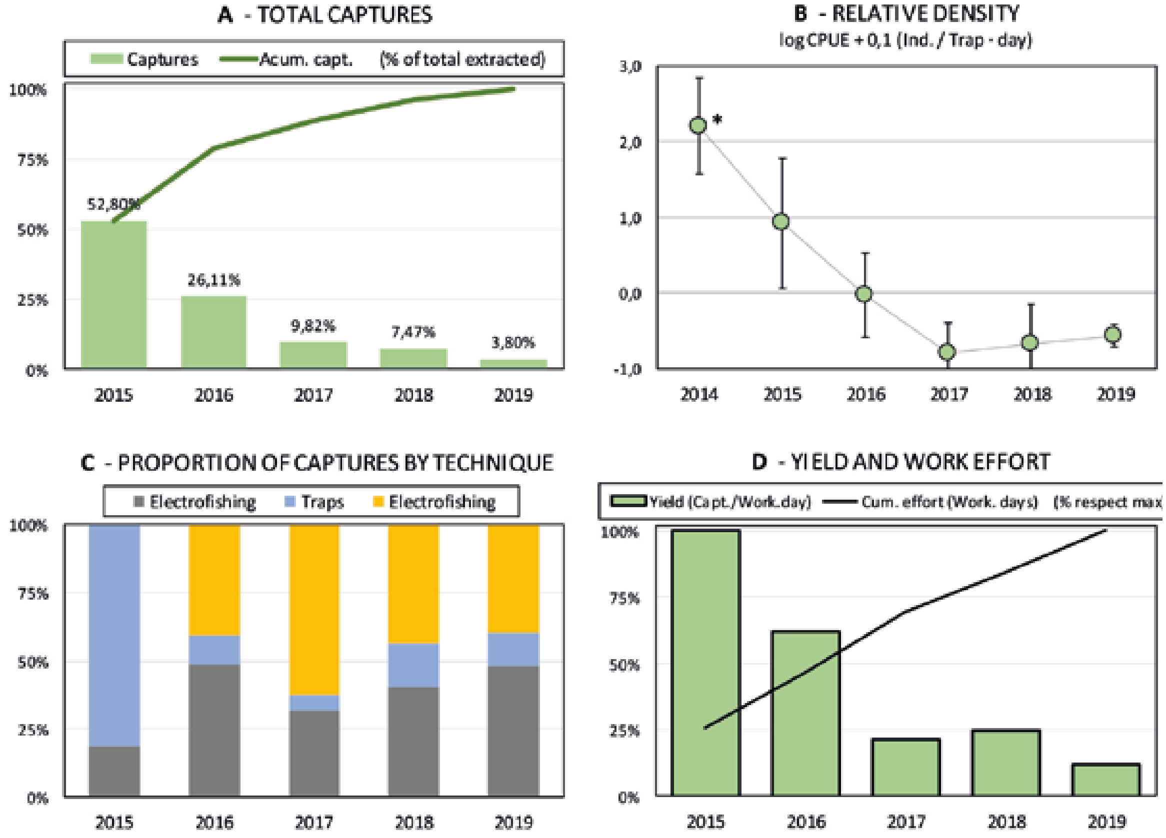


▲ **Figure 5-A.** Results of the control and eradication campaigns for minnow (*Phoxinus* sp) in the lakes. * Sampling prior to the start of campaigns. The bars represent the standard error.

LAKE CLOSELL - Minnow (*Phoxinus* sp)

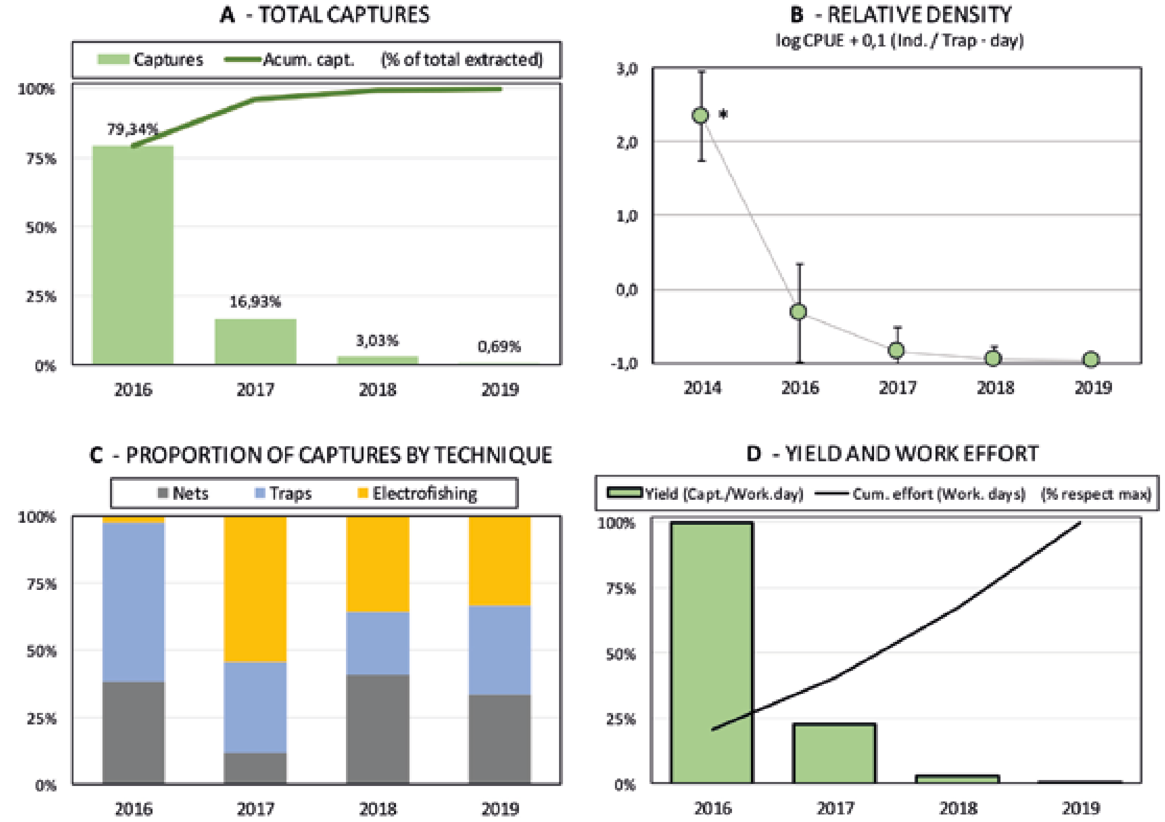


LAKE NAORTE - Minnow (*Phoxinus* sp)



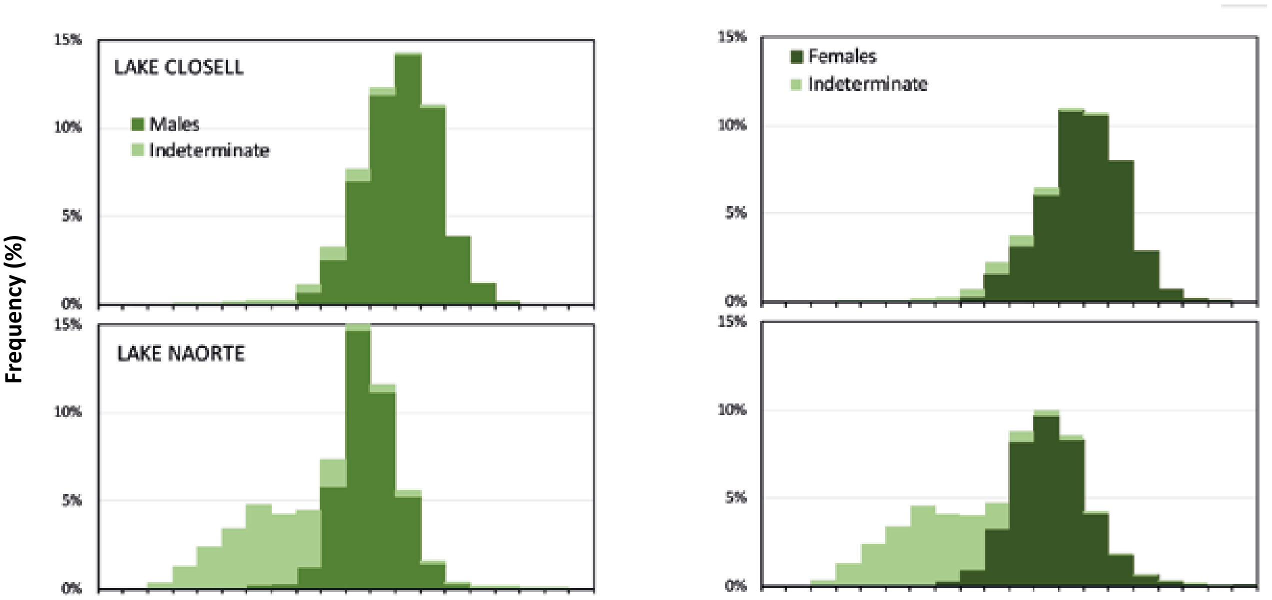
▲ **Figure 5-B.** Results of the control and eradication campaigns for minnow (*Phoxinus* sp) in the lakes. * Sampling prior to the start of campaigns. The bars represent the standard error.

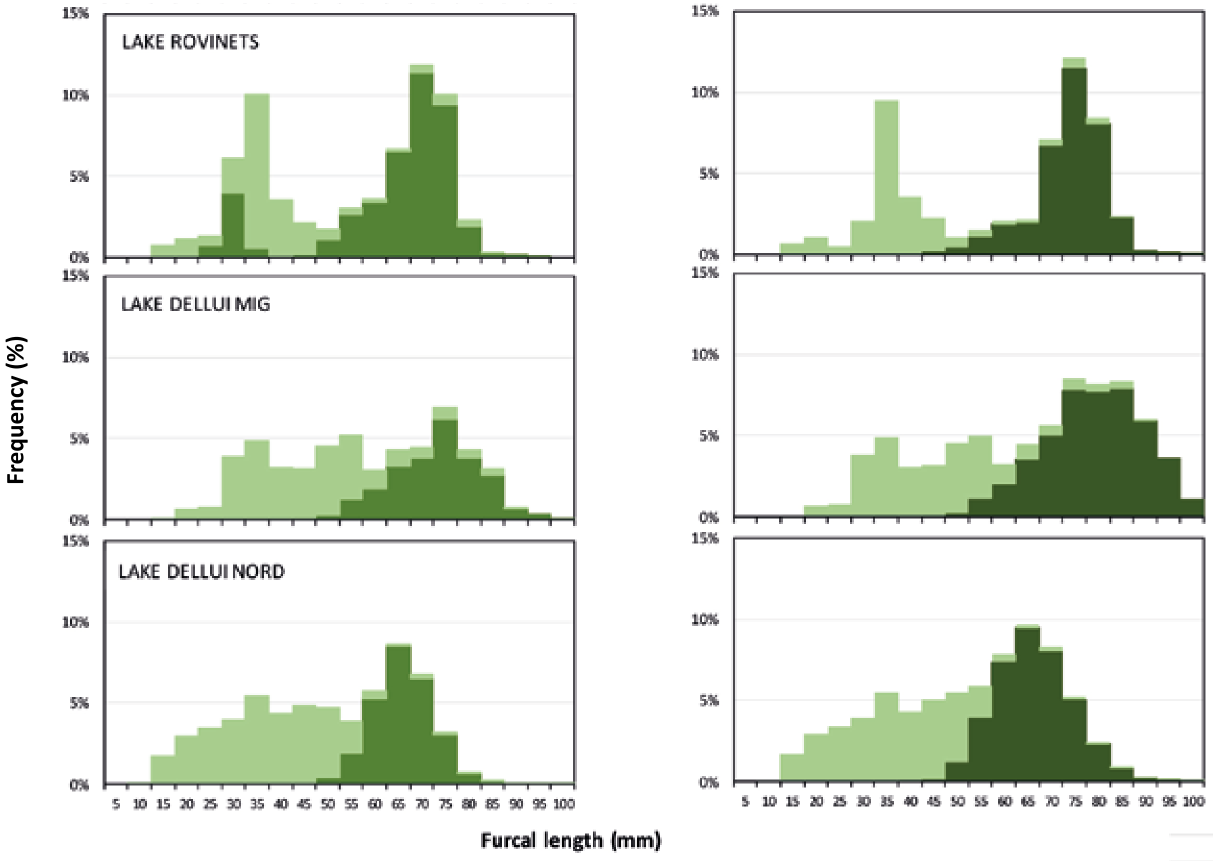
LAKE ROVINETS - Minnow (*Phoxinus* sp)



▲ **Figure 5-C.** Results of the control and eradication campaigns for minnow (*Phoxinus* sp) in the lakes. * Sampling prior to the start of campaigns. The bars represent the standard error.

▼ **Figure 6.** Initial size structures of minnow populations removed from the target lakes.





Salmonids

Table 5 and Figures 7, 8 and 9 show the results of the salmonid elimination actions in the target lakes. The total number of specimens extracted, which could be considered equivalent or very close to the initial population before starting the fish extraction activities, differs depending on the species and the characteristics of the lake. Brook trout had the maximum density and biomass observed in the 5 target lakes with salmonid populations. Rainbow trout and common trout had clearly lower density and biomass. In the case of common trout, in one of the lakes (Dellui Nord) density was already initially very low. In fact, everything indicates that the few specimens captured in this lake had arrived from the Dellui Mig lake, both interconnected by an intermittent stream.

The initial size structures observed for the eliminated populations show remarkable diversity (Figure 10). However, in the majority there is a clear dominance of medium and large sizes, i.e. the older specimens, over the small sizes corresponding to young specimens, indicating the existence of scarce and probably irregular recruitment from one year to another, added to a probable predation pressure of adults on young. The most extreme case of this situation was for rainbow trout in the lake at Cabana. In contrast, in the case of the lake at Cap de Port, this pattern of population structure did not occur, due to the existence of a small creek out of the lake where youngsters took refuge, a fact that guaranteed a greater relative presence in the whole of the population.

Common trout was eradicated in the lakes at Dellui Nord and Dellui Mig in just two years. In contrast, in the lake at Cap de Port with four years of action, complete eradication has not yet been achieved, although the rate of decline of the original population has been similar in the three lakes, with more than 75% reduction during the first year of action and a constant reduction in fishing performance, year after year. In fact, in the case of the lake at Cap de Port, the trends observed indicated the probable fishing of the last specimen at the end of 2018 or beginning of 2019. A fact has hindered the completion of eradication in this lake, the probable furtive introduction of some specimens during the last years of action. In 2019, it was found that at least one of the last adult specimens caught had been caught by sport fishing, an illegal practice within the national park.

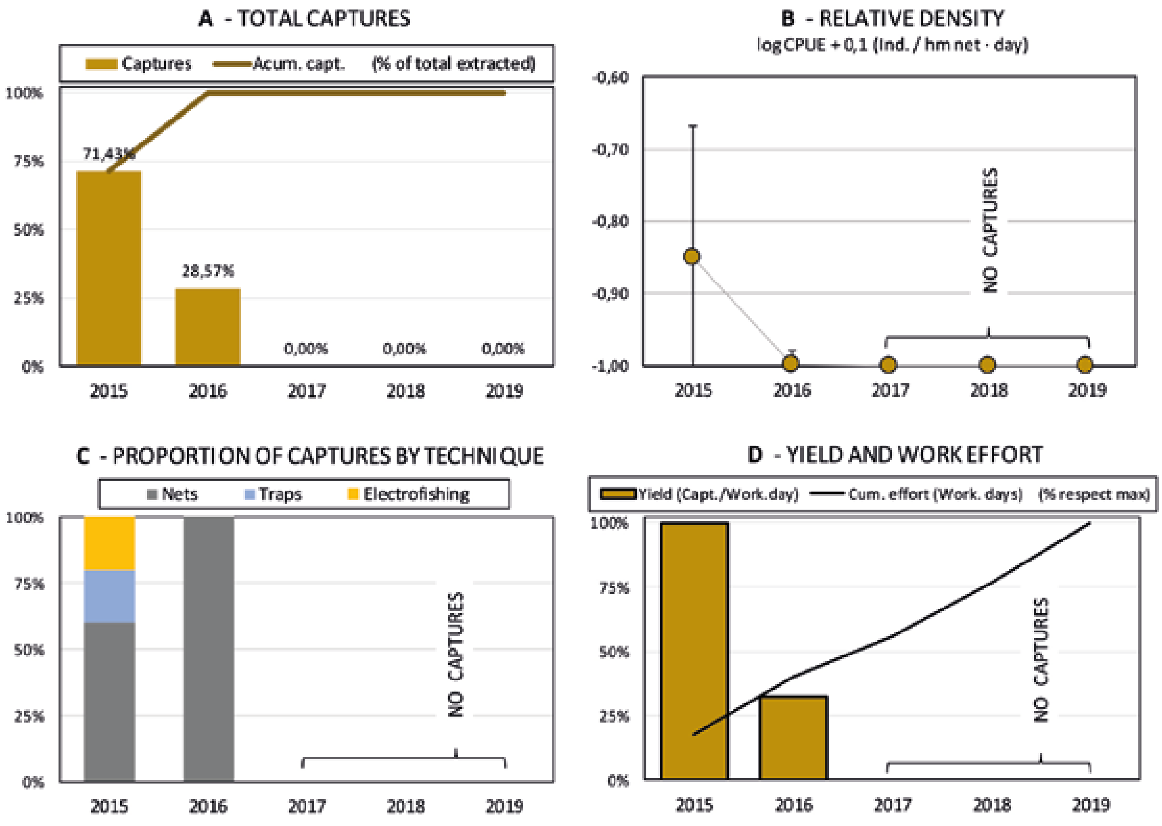
The population of rainbow trout in the lake at Cabana were also completely eliminated in just two years, with a rapid decrease in density already in the first year of work. In the case of brook trout in the lake at Subenuix, despite its high starting density, its population was removed with an even more pronounced trend than in any other salmonid treated population. Although eventually, the ultimate eradication of brook trout was achieved in three years, it should be noted that in the third year only two single specimens that had taken refuge in a small segment of an inlet stream were extracted.

As expected, the use of nets was the main catch technique that led to all or most of the salmonid catches, depending on the lake. However, electrofishing was key to the complementary catching of salmonids in some secondary habitats where the nets were not useful, especially small sectors of the shore where in some cases young fish were concentrated, and also inlet or outlet streams. The traps only had a role during the initial phase of elimination of the brook trout population, and in the elimination of common trout from streams.

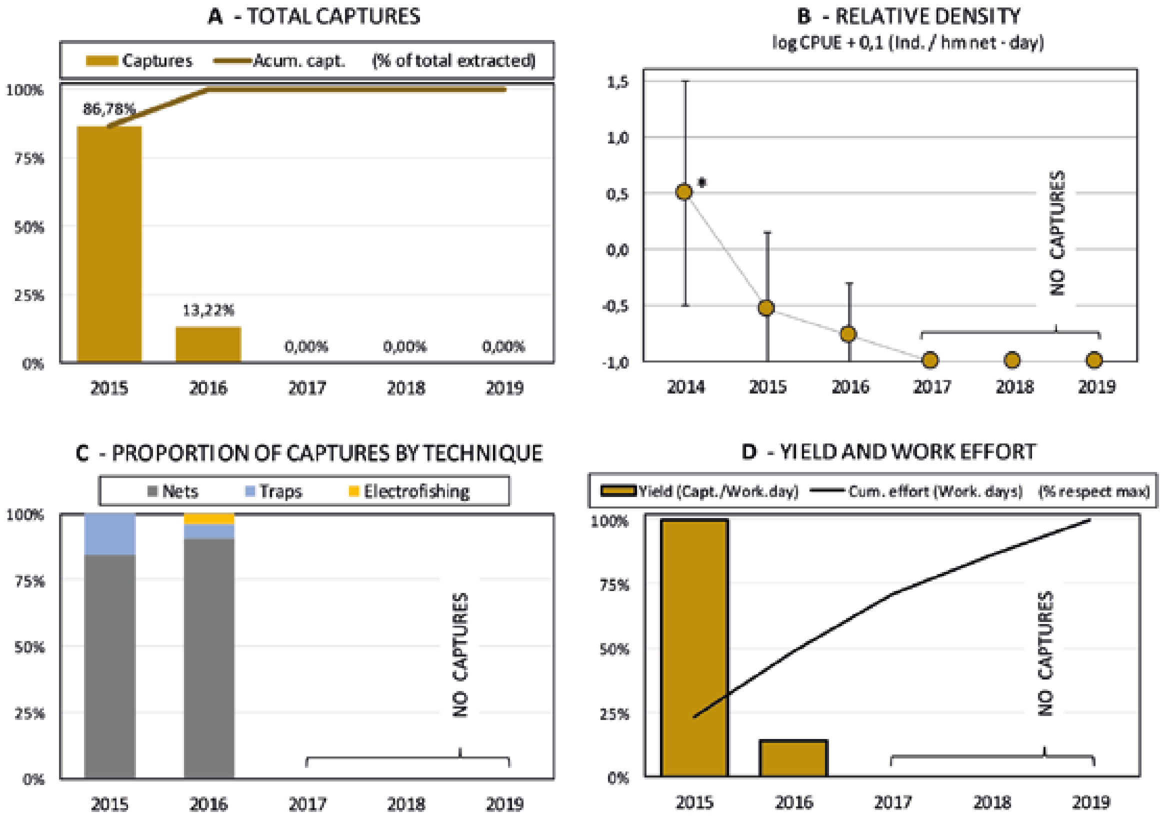
	SPECIMENS CAUGHT (Ind.), BY YEAR, WATER MASS AND SPECIES*				
Species*:	STR	STR	STR	SFL	OMY
Year	Dellui Nord	Dellui Mig	Cap de Port	Subenuix	Cabana
2015	5	348	-	-	-
2016	2	53	1,416	4,720	-
2017	0	0	192	275	501
2018	0	0	57	2	64
2019	0	0	4	0	0
TOTAL CATCHES (Ind.)	7	401	1669	4997	565
TOTAL CATCHES (Ind./ha)	20	368	227	1893	242
TOTAL BIOMASS (kg)	3.44	55.53	189.19	184.85	109.96
TOTAL BIOMASS (kg/ha)	9.82	50.95	25.74	70.02	47.19

▲ **Table 5.** Summary of salmonid species catches in the target lakes. * See codes in table 1.

LAKE DELLUÍ NORD - Brown trout (*Salmo trutta*)

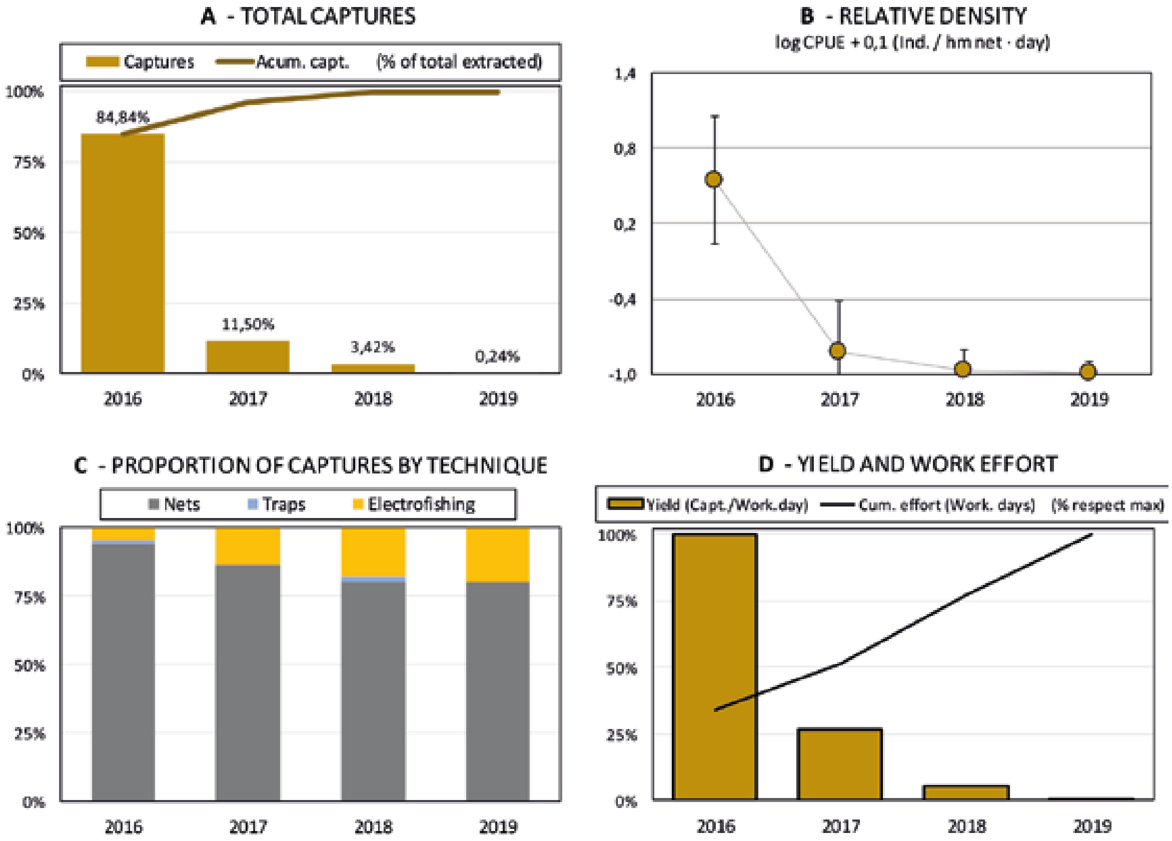


LAKE DELLUÍ MIG- Brown trout (*Salmo trutta*)



▲ **Figure 7-A.** Results of the control and eradication campaigns for common trout (*Salmo trutta*) in the target lakes. * Sampling prior to the start of campaigns. The bars represent the standard error.

LAKE CAP DE PORT - Brown trout (*Salmo trutta*)



▲ **Figure 7-B.** Results of the control and eradication campaigns for common trout (*Salmo trutta*) in the target lakes. * Sampling prior to the start of campaigns. The bars represent the standard error.

LAKE CABANA - Rainbow trout (*Oncorhynchus mykiss*)

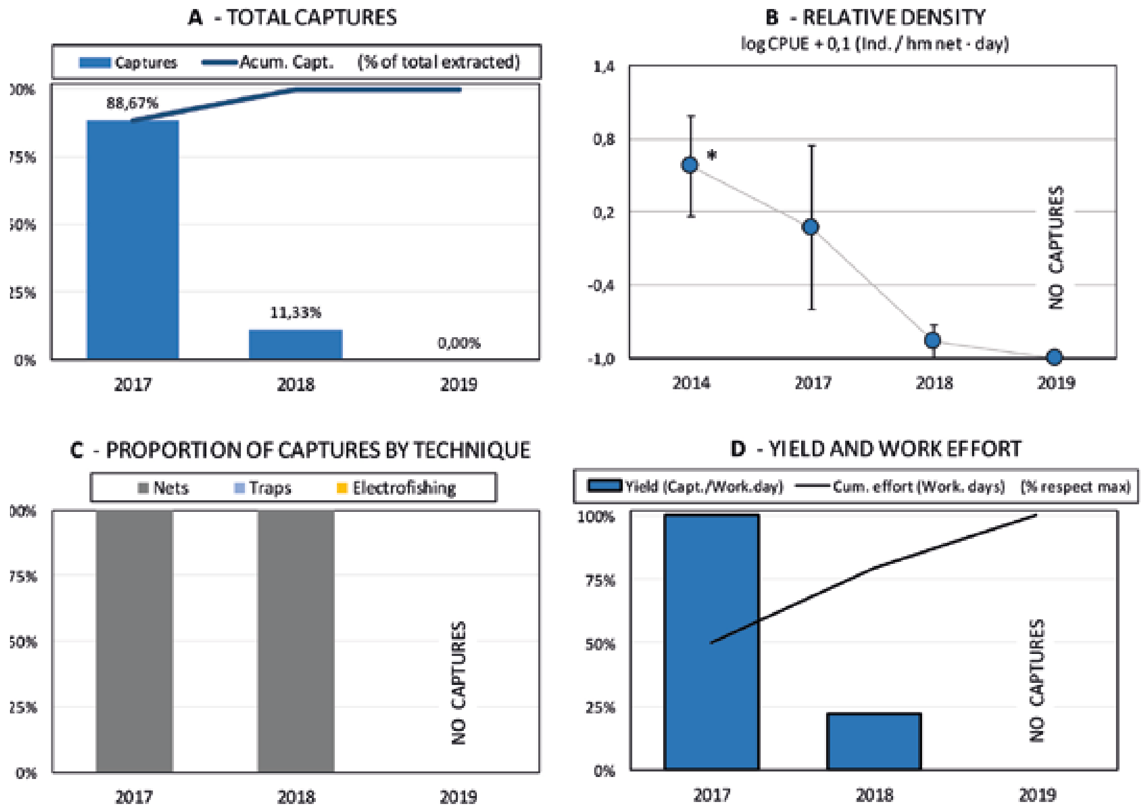


Figure 8. Results of the control and eradication campaigns for rainbow trout (*Oncorhynchus mykiss*) in the target lakes. * Sampling prior to the start of campaigns. The bars represent the standard error.

LAKE SUBENUIX - Brook trout (*Salvelinus fontinalis*)

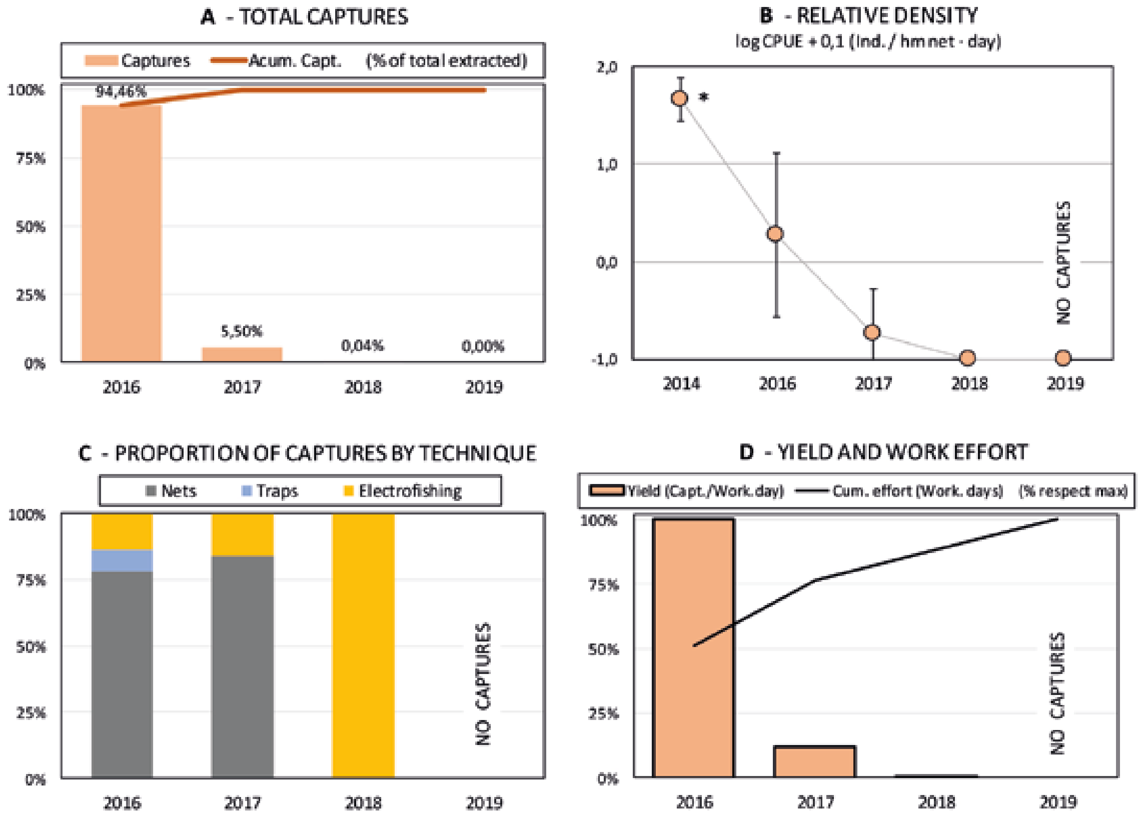
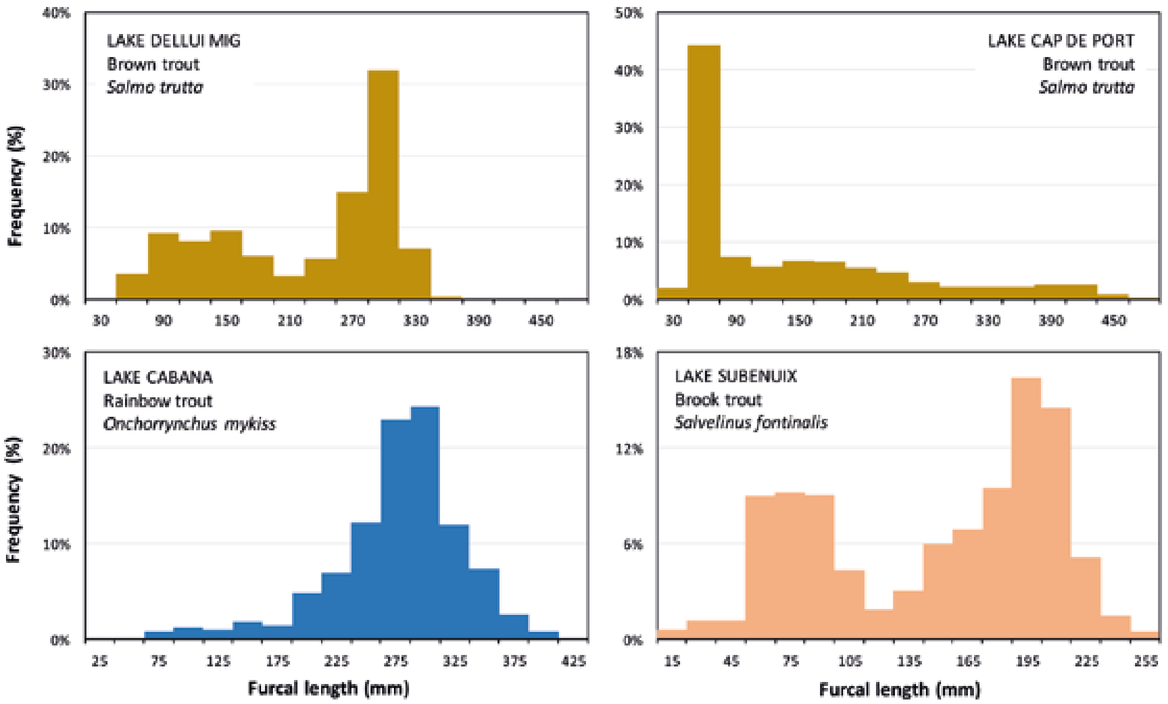


Figure 9. Results of the control and eradication campaigns for brook trout (*Salvelinus fontinalis*) in the target lakes. * Sampling prior to the start of campaigns. The bars represent the standard error.

LAKE SUBENUIX - Brook trout (*Salvelinus fontinalis*)



▲ **Figure 10.** Initial size structures of salmonid populations removed from the target lakes.

DISCUSSION

The results achieved within the framework of the actions of ecological restoration of the pyrenean lakes of the LLP, through the elimination of introduced fish populations, demonstrate firstly that the eradication of salmonid nuclei is effectively feasible by methodologies already established by previous works in similar environments (Knapp and Matthews, 1998; Tiberti et al., 2018), and secondly, that the challenge of eradicating nuclei of small cyprinids such as minnow is also feasible, despite the absence of similar precedents. In addition, they also allow the highlighting of the main conditions for the planning and execution of this type of operation.

Initially, the first condition is the biological and ecological characteristics of the species of fish to be eliminated. Although the fish populations of the high mountain lakes of the Pyrenees have been poorly studied, results and observations generated throughout the LLP project indicate a series of common characteristics for all the species: high population stability, high longevity and low mortality, compared to other populations studied in lower altitude systems (Cárceles, 2014; Busquets, 2015; Fernández, 2015; Fernández, 2016; Lapedra, 2016; Calderón, 2017; Dalmau, 2017; Llenas, 2018; Rodríguez, 2018; Campistol, 2019; Monclús, 2019; Ramada, 2019). However, everything indicates that recruitment, growth and productivity are very variable not only between species but also between lakes, probably based on a complex combination of various factors not yet analysed. Thus, it seems that species such as minnow and brook trout have regular, often high recruitment, and generally reach high densities and biomass in the lakes they occupy. In contrast, common trout and rainbow trout tend to have recruitment problems, and generally maintain low density populations.

The age of sexual maturation of lake fish is a key aspect in planning their eradication. Even though it is very variable, in the case of salmonids it seems to always be above two years. This gives enough room for the complete elimination of populations, if from the beginning of the campaigns sufficient intensity of effort is applied to quickly eliminate at least the adult specimens within one or two years. In contrast, in the case of minnow we have verified that, in most lakes, part of the specimens can already reproduce when they are only one year old. This fact, together with a lower catch efficiency for these fish compared to salmonids, largely condition the campaigns, since it does not allow rapid eradication of population nuclei. Only when it is possible to avoid reproduction from the first year, a rapid elimination process of the population is expected, but this will probably be feasible in very few cases. In any case, it is necessary to correctly determine its reproductive phenology and, above all, to know in which sectors or microhabitats it tends to occur, preferably in each lake. This allows better distribution of fishing efforts over space and time, to minimise the chances of the minnow population recovering even with intensive fishing campaigns.

On the other hand, the initial density of the fish nucleus to be eliminated does not seem to condition the results, as long as an effort is applied with sufficient intensity and continuity, always with the intervention of specialised and well-equipped workforces. The absolute density, i.e.

the initial population size, partly dependent on the size of the lake (within the range of target lakes; <10 ha), does not seem to condition the success of operations either.

However, other factors beyond those directly related to the biology of the fish species, also condition the operations: the general morphometry of the lake, the bathymetric profile, the structure of the lake shores and bed, the presence of submerged vegetation, the presence of streams or torrents connected to the lakes, and also the meteorology and hydrology of each work season. In fact, some of these conditions end up explaining a good part of the variability of the results obtained between lakes. Thus, for example, the minnow nuclei located in two small lakes with a surface area of less than half a hectare, Rovinets and Dellui Nord, turned out to be where the work was more complex, and ultimately longer than expected, due mainly to the orography of their shores, partly inaccessible, and an abrupt profile of the lake bed.

As for the catch techniques, during the LLP the materials and equipment used gradually improved, as well as the way of using them, achieving greater effectiveness that probably accelerated operations. In fact, we believe that the deadlines for the achievement of possible new ambitious targets for the eradication of fish in lakes, through intensive fishing campaigns, can be considerably reduced as a result of the practical knowledge obtained, especially in the case of the fight against minnow. At the same time, this practical knowledge will also allow the possibility of dealing with more important milestones, such as the eradication of minnow from major lakes, such as Naorte, or the eradication of two or more species present in the same lake of significant dimensions.

In any case, it is still necessary to improve the effectiveness of the available catch techniques, or to develop new techniques, especially again in the case of minnow. For example, it is probably feasible to improve the efficiency of electrofishing in high mountain waters, which is still very low, although it would be necessary to involve the manufacturers of this equipment. There is also a long way to go in the design of new types of effective traps in locations where it has not been possible to achieve positive results, such as an efficient trap in medium and large depth substrates. The introduction of other methods, such as the mass application of ichtyiocides, also remains a pending challenge, especially to address this problem, perhaps with a lower economic cost, in situations of high complexity, such as interconnected systems of various lakes, rivers or mires.

Proper campaign planning is also critical. Initially, a key first aspect is the allocation of an appropriate catch effort in form and quantity. In addition, it is essential to keep track of progressively obtained results, to introduce the necessary variations in time, if appropriate. For example, in this project the fishing intensity initially planned in one of the lakes proved to be insufficient, specifically in the lakes of Naorte and Cap de Port, a situation to which several other problems were added, such as poor durability of the nets, not initially foreseen, among others. It was necessary to respond to these situations with the allocation of more and better material resources, without being able to avoid a delay in achieving objectives in these lakes.

The eradication of introduced fish should be a priority operational objective in the Pyrenean lakes to be ecologically restored. But while this is not feasible for any lake, it is not necessary to rule out the intensive demographic control of the fish as an alternative, at least in the case of minnow. The results of the LLP project, and in particular the experiment conducted in the lake at Naorte, show that a sufficient reduction in the density of this fish species can be achieved, with a good response from the ecosystem (Buchaca et al., 2019). In order to maintain these results in the medium term, we must provide for constant maintenance actions, which we now know how to execute efficiently in terms of cost.

CONCLUSIONS

The eradication of fish introduced into Pyrenean lakes through intensive and continuous catching is feasible, but only when it is based on an adequate allocation and planning of human and material resources.

In the case of salmonids, their complete elimination can be achieved through the use of only gillnets, although in certain situations the complementary use of electrofishing may be essential.

In contrast, in the case of minnow, an effective combination of at least three techniques (nets, traps and electrofishing) is necessary. In addition, a continuous review of the work plan must be carried out, in order to adjust to the response of each population and lake.

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~ Lake Cabana ~

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.

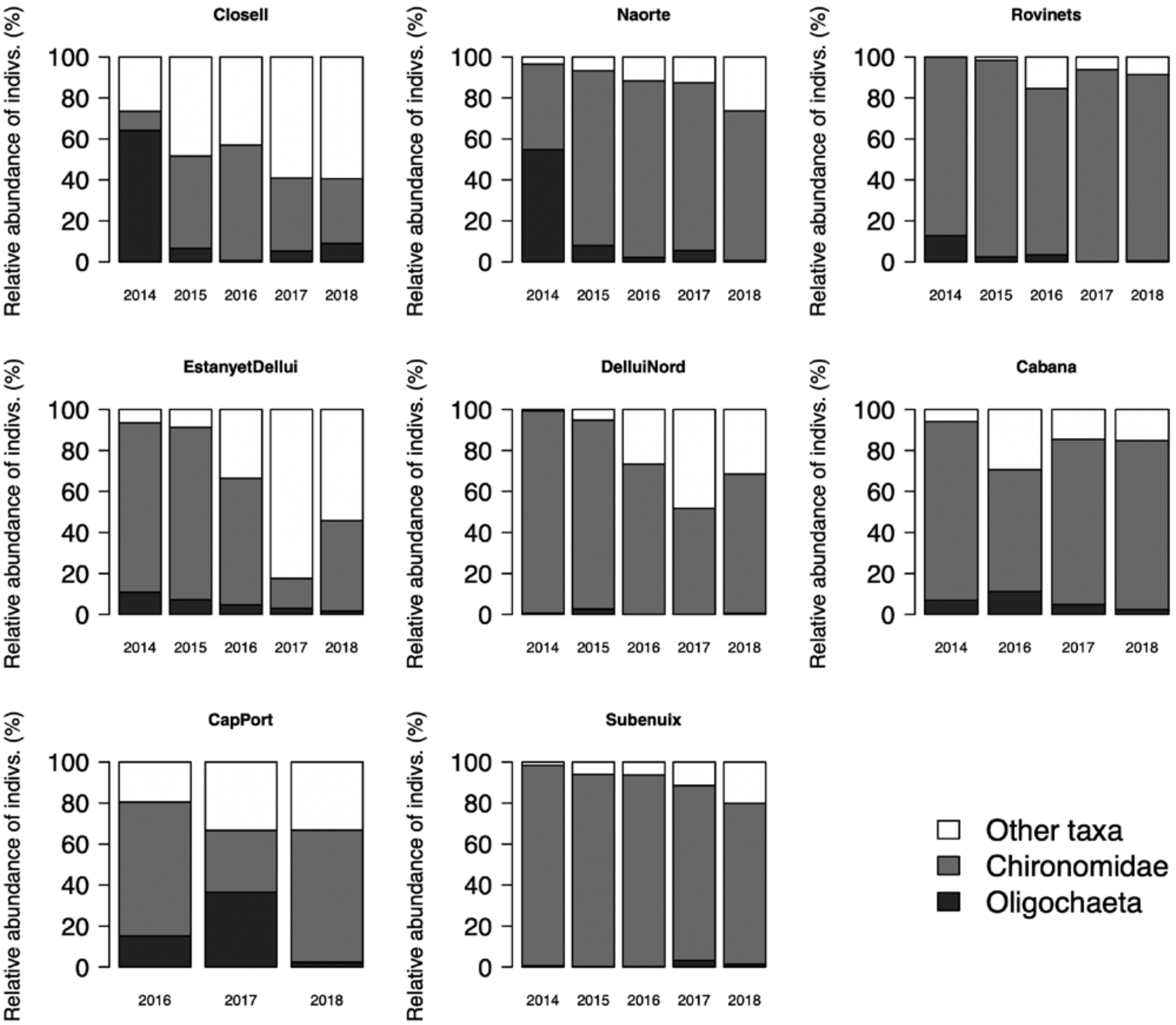


Figure 1. The evolution over time of the relative abundance of three main fractions in macroinvertebrate samples: oligochaetes, chironomids and other groups.

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.

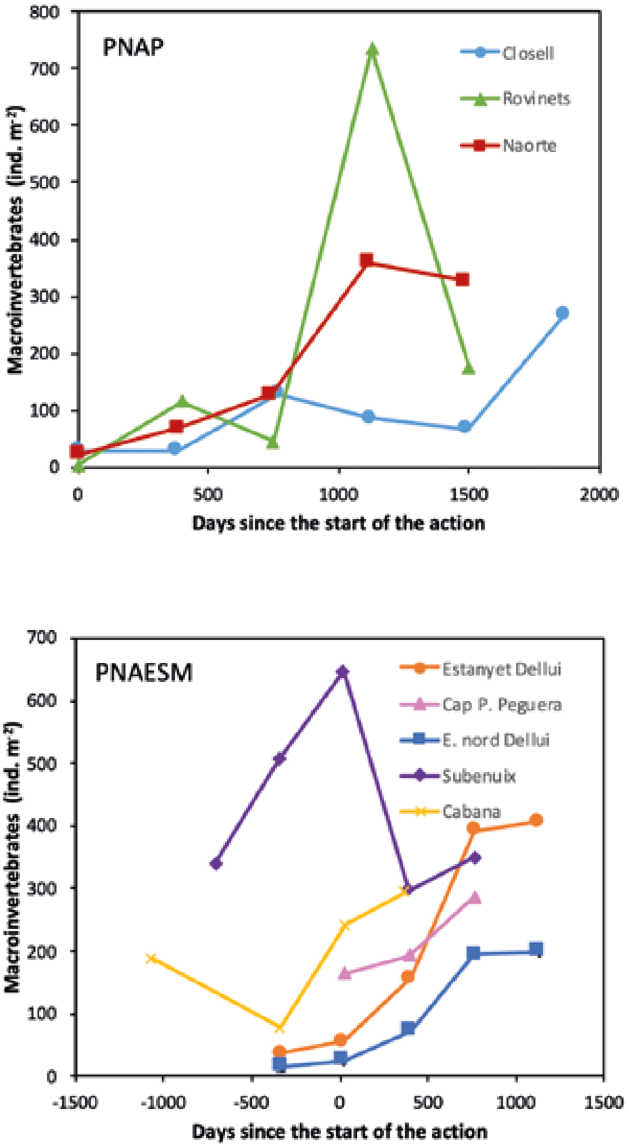
The fraction that includes oligochaetes tends to reduce in significance over time, although the pattern of evolution of this fraction compared to that of chironomids is more erratic and is not as directly influenced by predator fish.

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).

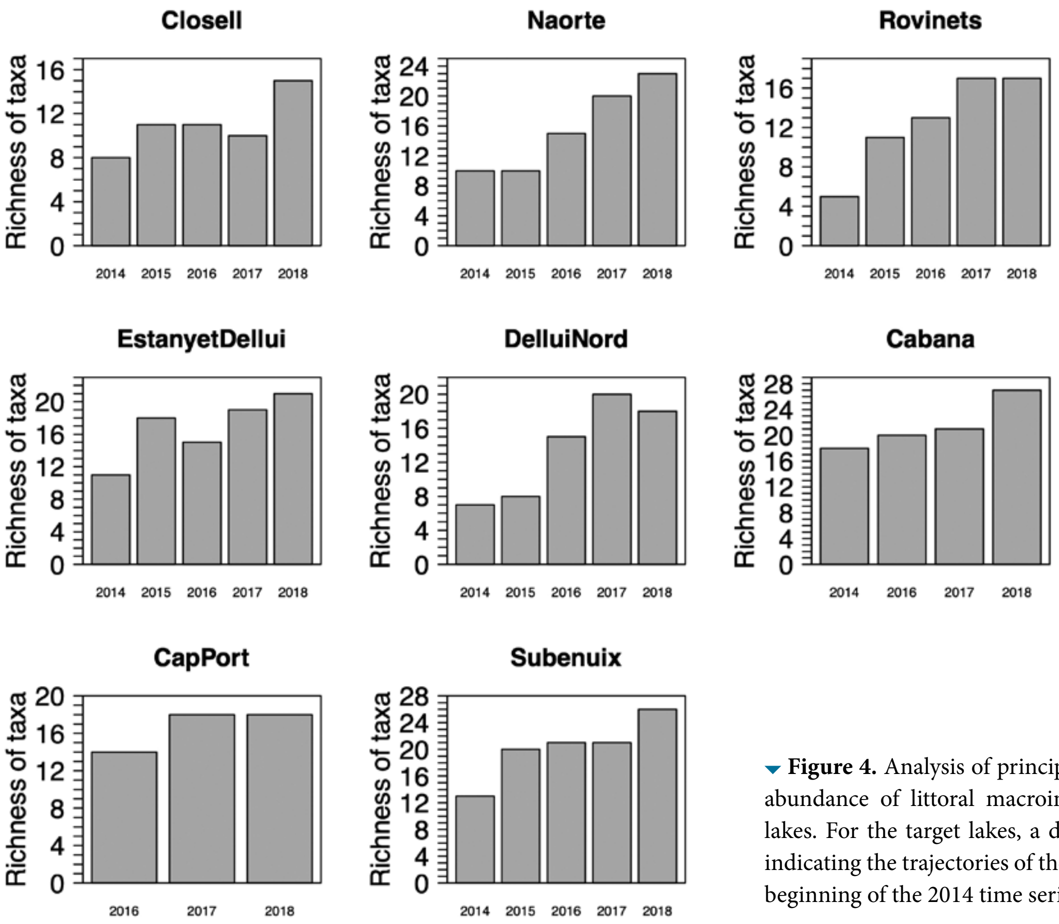
This increase in the number of taxa is mainly due to the catching of new organisms vulnerable to the presence of introduced fish that have colonised the system or have increased their abundance once the pressure of predation on them has decreased.

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*.

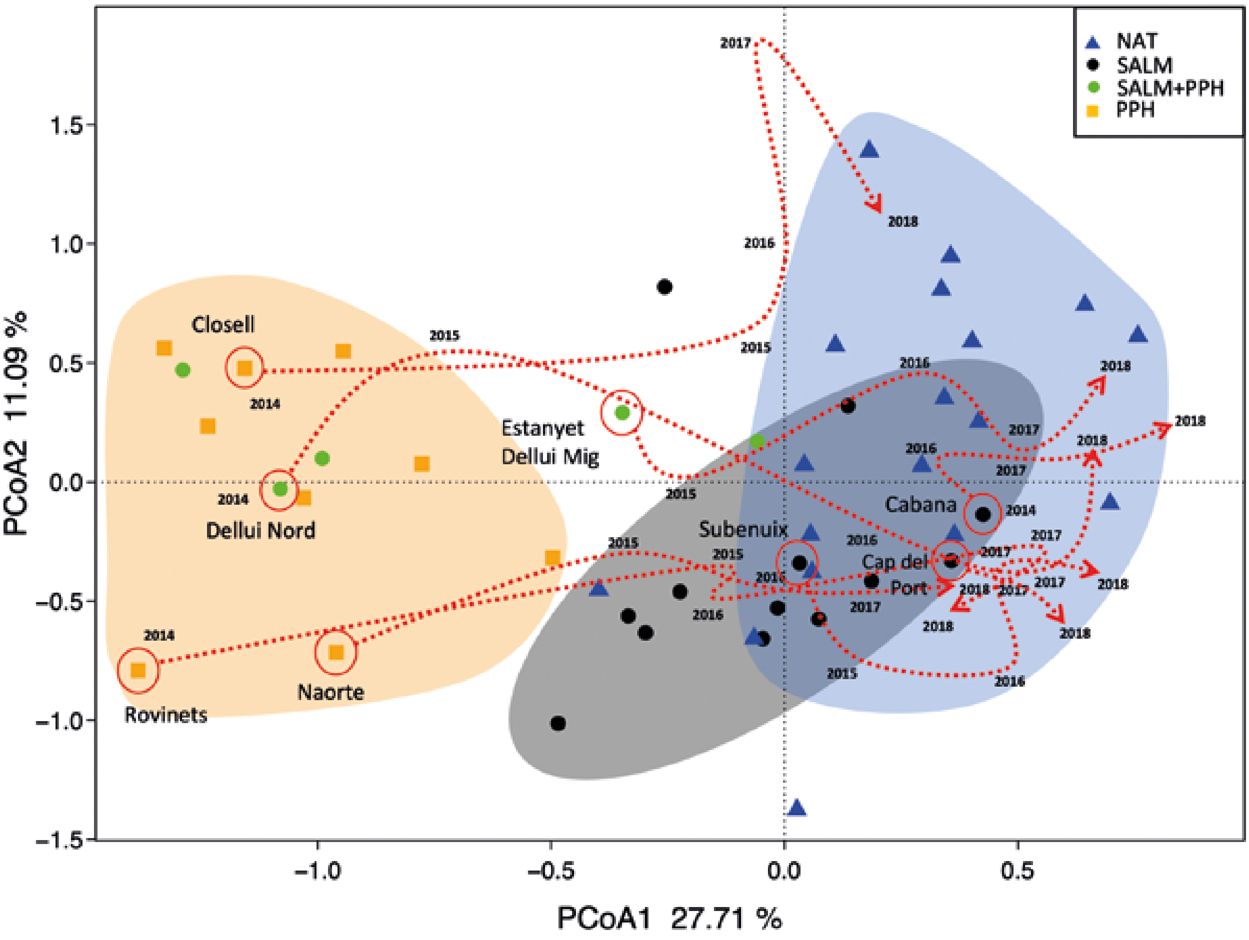


▲ **Figure 2.** Absolute abundance of macroinvertebrates (ind. m^{-2}) according to the days since the start of the action.



◂ **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.

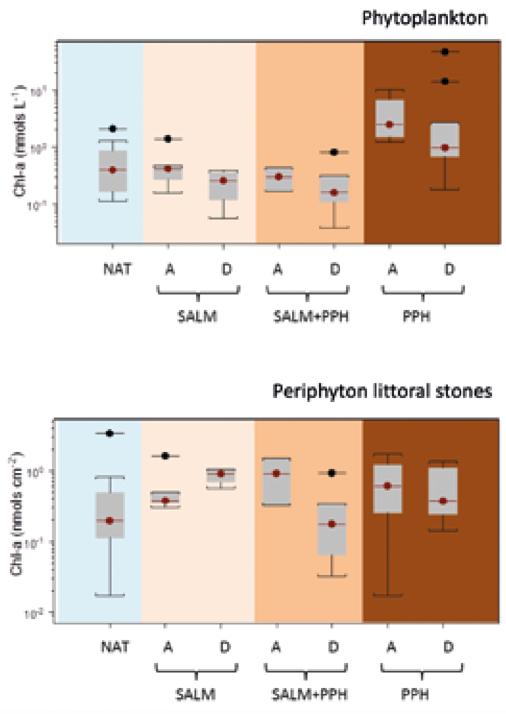
Macrozooplankton

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.

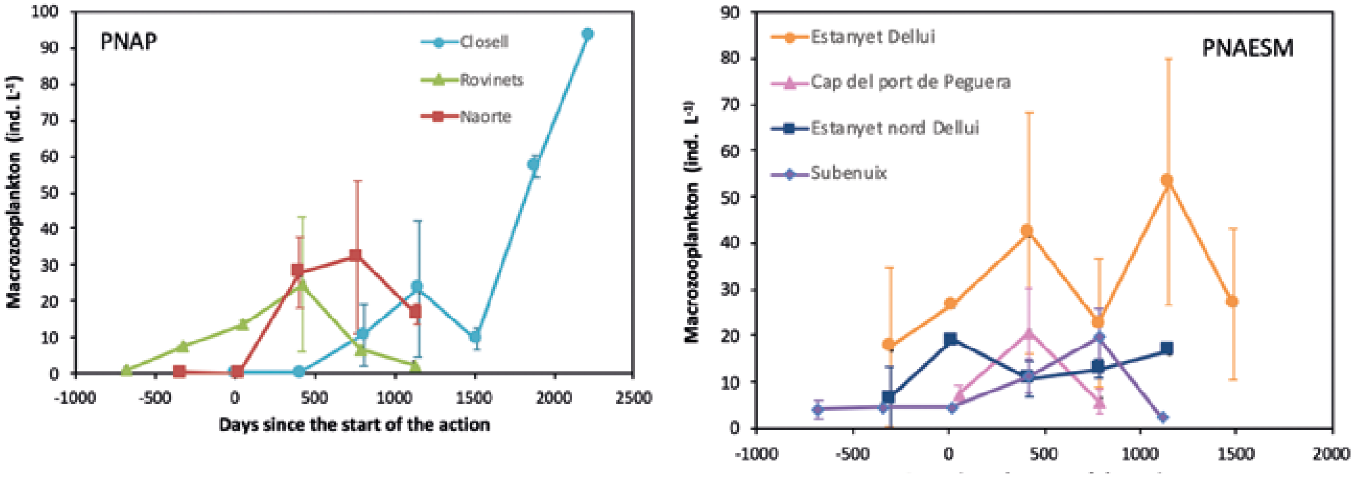
After starting the actions, the crustacean abundances increased very clearly in the lakes where the minnow had been present as the only species (Closell, Naorte and Rovinets). The crustacean populations of the lakes with salmonids also began to respond (Subenuix and Cap del Port de Peguera). In contrast,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

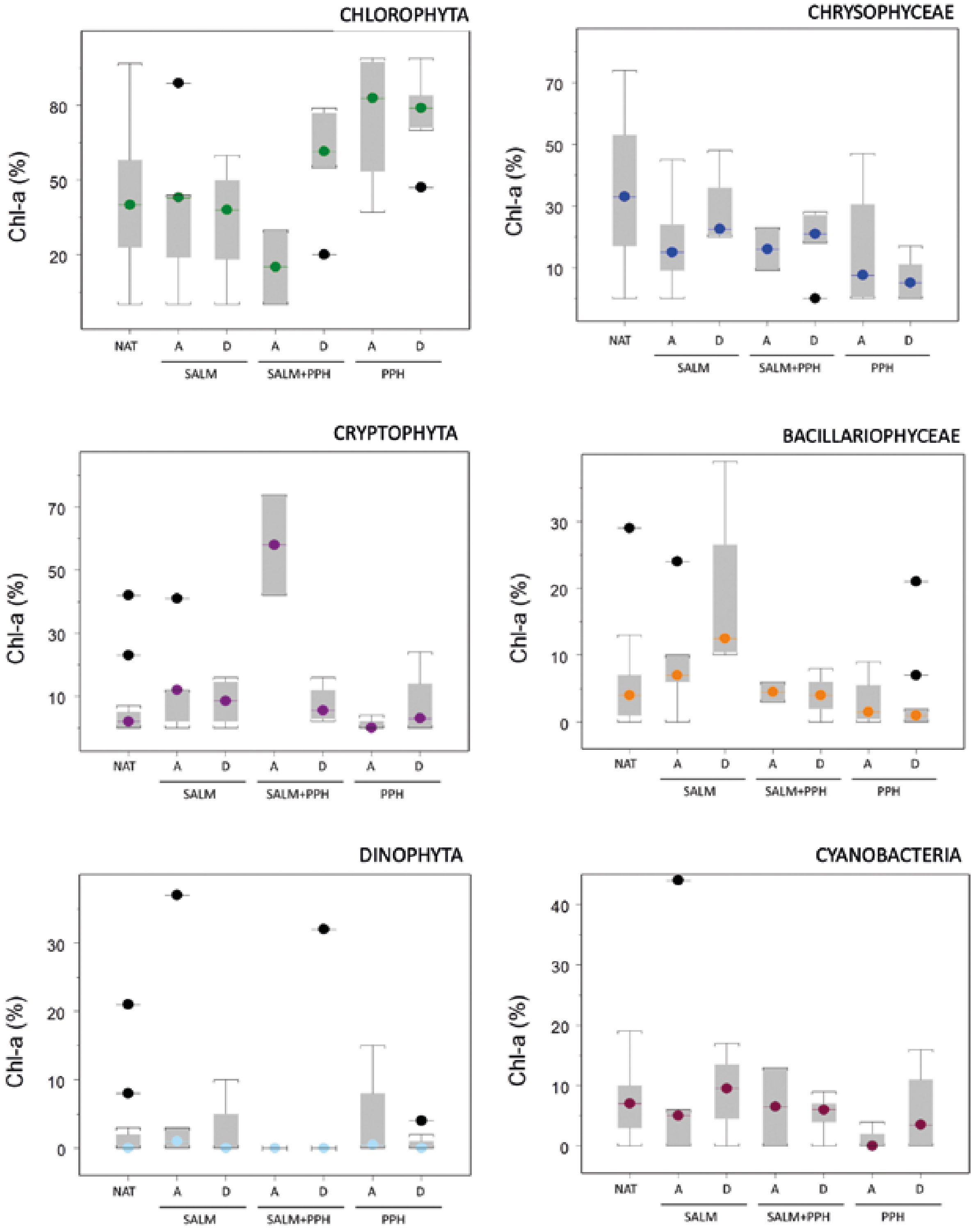
Chlorophyll-a (Chl-a) in the water column, used as an estimate of phytoplankton biomass, shows a slight decrease after starting actions (Figure 6, upper graph).



▲ **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.

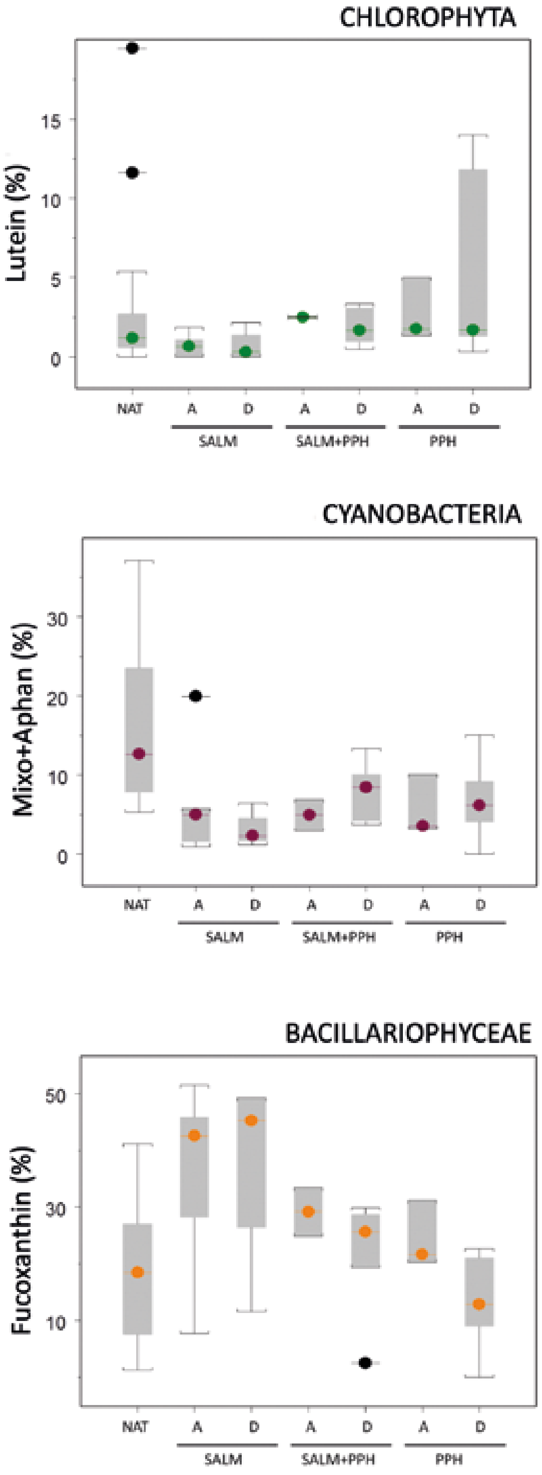


▲ **Figure 7.** The box diagrams showing the frequency distribution of the percentage of Chl-a of each algal group and of phytoplankton cyanobacteria of the target lakes and for the different scenarios. Abbreviations the same as in Figure 6.

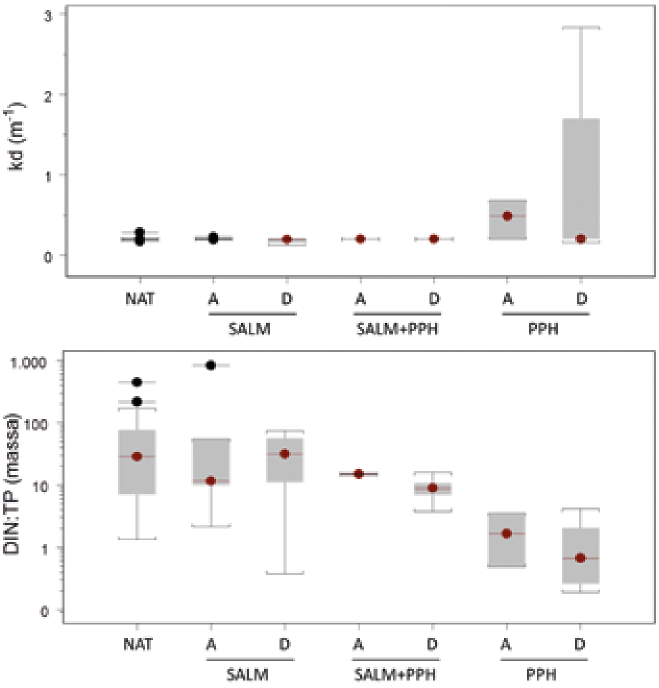
The decrease was more pronounced in the lakes where there was only minnow before action started, although it can be seen that the dispersion of the data is vast. Regarding the periphyton of the stones, the dispersion of the data is also vast, but the trend is the same as in the case of phytoplankton 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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~ Cap del Port de Peguera ~

~ Common frog (*Rana temporaria*) ~

RAPID RECOVERY OF AMPHIBIAN POPULATIONS IN EIGHT HIGH MOUNTAIN LAKES LINKED TO THE ERADICATION OF EXOTIC FISH

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ABSTRACT

The LIFE+ LimnoPirineus project (2014-2019) includes the recovery of amphibian populations in eight lakes of the Aigüestortes i Estany de Sant Maurici National Park and the Alt Pirineu Natural Park, by eradicating or control non-native fish. During the summers from 2014-2018 practically all of the fish present in the lakes have been removed. We found that most of the autochthonous amphibian species present in each valley have naturally colonised all lakes during or following fish removal. This shows the high resilience of the amphibian fauna of high mountain lakes, following reduction or removal of pressure from non-native fish.

INTRODUCTION

As in many other aquatic ecosystems, the amphibian species that live in the high mountain lakes of the Pyrenees are part of different food compartments, and are involved in complex trophic relationships that make them key species (Wells, 2007). Firstly, tadpoles are fundamentally herbivorous and they live on algae and other microorganisms (mainly bacteria and archaea) that grow on the stones or sediment of the lake bed, thus maintaining the natural structure and functionality of the benthic community (Altig et al., 2007; Nyström et al., 2001). At the same time, tadpoles represent a source of food for natural aquatic predators, such as beetle larvae and adults, dragonfly larvae, adult water striders and backswimmers, and adult newts (McDiarmid & Altig, 2000). In addition, adult amphibians are part of the super-predator level in natural lakes. They are at the top of the trophic chain and feed on different invertebrates, among which are insects that prey on tadpoles (Wells, 2007). Finally, amphibians in general are preyed upon by birds, reptiles and land mammals that may visit the lake more or less frequently, which is an entry of matter and energy to the surrounding ecosystems (McDiarmid & Altig, 2000; Wells, 2007).

Despite their ecological importance, amphibians of high mountain lakes are in decline worldwide due to different threats that reduce or eradicate entire populations (Whittaker et al., 2013). In this regard, different studies have shown the negative effect of pesticides, emerging infectious diseases and the global increase in temperature (Bradford et al., 2011; Maxwell & Knapp, 2018; Smith et al., 2017). However, the most documented threat in many mountain ranges, including the Pyrenees, is the presence of exotic fish (Miró et al., 2018; Ventura et al., 2017).

High mountain lakes are isolated from rivers in the lower part of the basins by hydrographic barriers that prevent natural colonization by fish (Pechlaner, 1984; Pister, 2001). However, especially during recent centuries and in order to promote sport fishing, a global process of introducing fish has been carried out, which has meant a significant presence in these bodies of water throughout the world (e.g. Miró, 2011; Reissig et al., 2006; Wiley, 2003). In the Pyrenees, the introductions were carried out in historical times in some cases, but also in recent times in many others, and have involved the presence of different species of trout or minnow in 35-85% of the lakes, depending on the valley (Miró & Ventura, 2013; 2015). The impact caused by exotic fish in lakes is that, in these ecosystems, fish become a higher trothic level that did not previously exist. Fish (both trout and minnow) prey directly on amphibian larvae, juveniles and adults until they are eliminated in most cases (Knapp, 2005; Miró et al., 2018; Tiberti and von Hardenberg, 2012), but also indirectly harm them by depleting insect larvae and other invertebrates that are shared prey (Maxwell et al., 2011).

During recent decades, different conservation projects combined with scientific studies have shown that the impacts described can be reversed (Knapp et al., 2016). In the 1990s, the first experience of eradicating exotic trout from high mountain lakes in Sierra Nevada, California (Knapp & Matthews, 1998) was carried out. This study, together with others in the same field, documented the effectiveness of gillnetting to eradicate populations of exotic trout from high mountain lakes and, thus, facilitate the recovery of native amphibian populations within a period of between one and three years after the eradication begins. Those studies showed the recovery of the populations of a threatened native frog in Sierra Nevada, the *Rana muscosa* (Knapp et al., 2007; Vredenburg, 2004). Subsequently, more recent work has shown the recovery of two other frog species, the *Rana cascadae* in the Klamath mountains, in Northern California, and the *Rana temporaria* in the Italian Alps; in both cases within a period of between two and five years after eradication projects began (Pope, 2008; Tiberti et al., 2018). The aforementioned studies have also documented the difficulty that some high mountain amphibians have to colonise new locations, such as *R. muscosa*, which is conditioned by a maximum dispersion distance of 1 km (Pope et al., 2001).

Recent experiences in California and the Alps demonstrate the rapid recovery of a frog species in each case, after eradicating exotic trout. However, native amphibian communities in high mountain lakes can have up to four or five species (Knapp, 2005; Miró et al., 2018), and may be threatened by the presence of minnow as well as the presence of trout (Miró and Ventura, 2015; Miró et al., 2018). Therefore, it is of general interest to study to what extent it is possible to recover the entire amphibian community of a lake by eradicating exotic fish, which may include trout, but also minnow. Answering these questions is especially important for the conservation of high mountain amphibians, since there are other known additional negative factors that endanger them and add uncertainty to the chances of recovery. We are talking about pesticides, emerging infectious diseases and the global increase in temperature (Bradford et al., 2011; Maxwell and Knapp, 2018; Smith et al., 2017).

The main objective of this study has been to investigate the recovery of the amphibian community in eight high mountain lakes in the Pyrenees after initiating actions to eradicate exotic trout and minnow. Specifically, we wanted to answer four questions: (i) Is the natural recovery of the entire amphibian community possible as a result of the eradication of exotic fish? (ii) Can recovery be achieved in just a few years as we know happens with some anuran species? (iii) Would individuals and colonizing species come from nearby or remote locations? (iv) Would there be any difference in the recovery of amphibians depending on whether the exotic fish present in the lake are trout or minnow? The conclusions of the study may be useful to design, evaluate and execute future conservation projects with similar objectives.

METHODS

Study Area

We have carried out the study in the eight lakes subject to the LIFE+ LimnoPirineus project (2014-2019), where we have carried out actions to eradicate and control exotic trout and minnow (Buchaca et al., 2016). The eight lakes are located within protected areas included in the Natura 2000 Network, five of them in the Aigüestortes and Estany de Sant Maurici National Park (Dellui Mig, Dellui Nord, Subenuix, La Cabana and Cap del Port) and the other

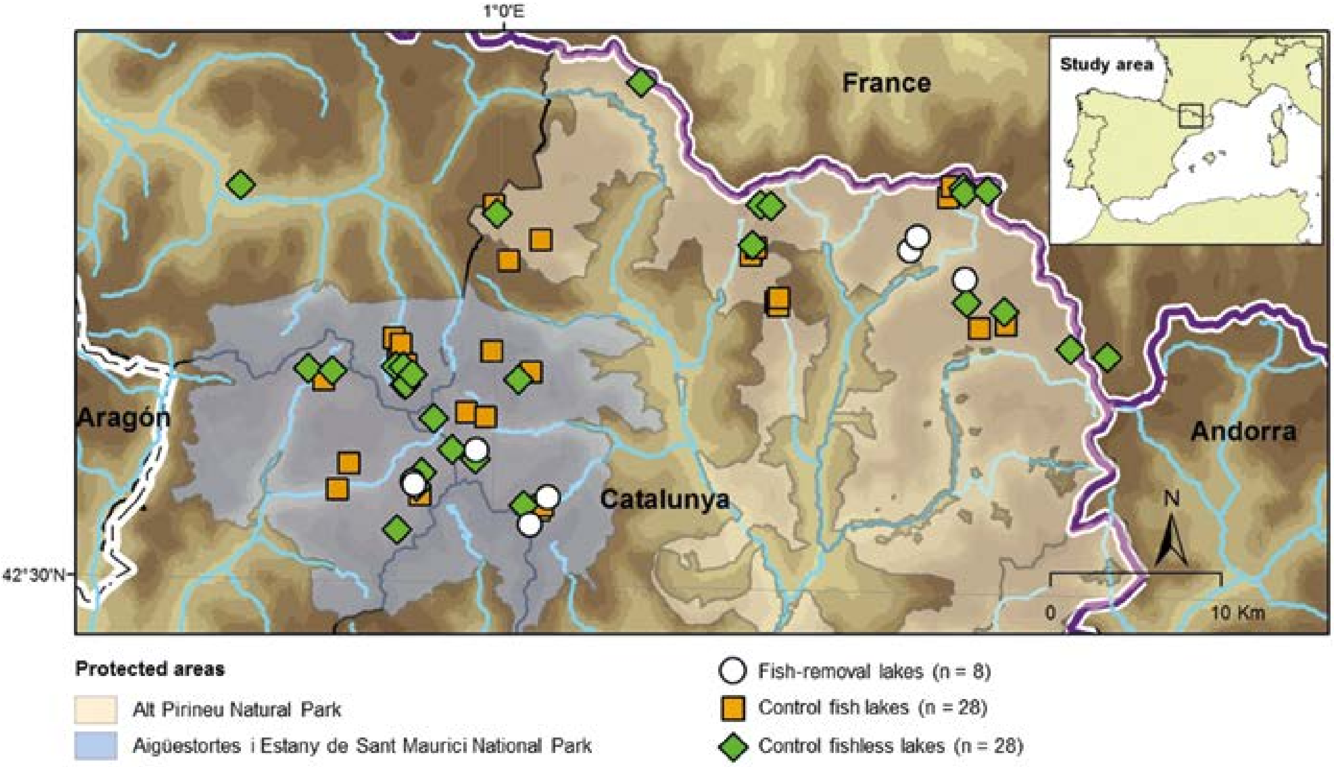
Name (code)	Protect area[a]	Maximum depth (m)	Surface area (ha	Altitude (M.A.S.L.)	Fish species 2012[b]	Year of commencement of fish eradication	Amphibian species 2012[c]	Amphibian species 2018[c]
Dellui Nord (1831)	AESMNP	6.7	0.35	2306	STR, PPH	2015		Casp
Dellui Mig (1838)	AESMNP	6.2	1.09	2314	STR, PPH	2015		Rtem, Bspi, Casp
Subenuix (2066)	AESMNP	11	2.64	2194	SFO	2015		Rtem, Casp
Cap del Port (2213)	AESMNP	31.7	7.35	2521	STR	2016		Rtem
Cabana (2259)	AESMNP	11.7	2.33	2376	OMY	2017		Rtem, Casp
Closell (2468)	APNP	3.7	0.75	2074	PPH	2013	Bspi	Rtem, Bspi, Lhel
Naorte (2479)	APNP	14	3.94	2150	PPH	2015		Rtem, Lhel
Rovinets (2654)	APNP	5.4	0.37	2223	PPH	2016	Lhel	Rtem, Lhel

three in the Alt Pirineu Natural Park (Closell, Naorte and Rovinets). Table 1 shows the descriptive topographic features of the eight lakes and the fish and amphibian species present. For comparative reference data, we chose 56 control lakes with similar characteristics, located in the same study area (Figure 1). Half of the control lakes had trout or minnow or both, and the other half had no fish. Most of the control lakes in this study coincided with the control lakes chosen for the limnological monitoring program of the LimnoPirineus project (Buchaca et al., 2016), to which we added other lakes with the presence of specific amphibian species. In all cases, we knew the previous presence / absence of amphibians by censuses conducted in previous studies (Miró et al., 2018).

Eradication of exotic fish

As of 2015, we have carried out the eradication of exotic trout and minnow from the eight target lakes, as planned in the LimnoPirineus project. Three of the target lakes only had minnow populations, three others had a different species of trout each, and the last two had both groups of fish (Table 1). Since most of the fish are caught at the beginning of eradication and a large volume of work is created, extraction operations cannot be initiated at all locations at the same time. Therefore, we started in the first lakes in 2015 and, over the following years, we progressively began to work on the others. (Table 1; first chapter this volume). By way of exception, in the case of Closell, we began the eradication work in 2013, under the context of a specific experimental project to evaluate the possibilities and most appropriate methods for the eradication of minnow in high mountain lakes (Table 1).

Three methods have been used in the campaigns for the eradication of exotic fish: gillnetting (5 to 43 mm mesh size), electric fishing along the shore, and small mesh (4 mm) creels. The first two methods had already been previously tested in high mountain lakes in California and the Alps, and we knew their efficacy for catching trout (Knapp & Matthews, 1998; Tiberti et al., 2018).



▼ **Figure 1.** Map locating the target and control lakes for the LIFE+ LimnoPirineus project amphibian monitoring plan.

◀ **Table 1.** Descriptive features of the eight lakes in the LIFE+ LimnoPirineus project and fish and amphibian species present.

a) AESMNP: Aigüestortes i Estany de Sant Maurici National Park. APNP: Alt Pirineu Nature Park.

b) STR: Brown trout (*Salmo trutta*). OMY: Rainbow trout (*Oncorhynchus mykiss*). SFO: Brook trout (*Salvelinus fontinalis*). PPH: Common minnow (*Phoxinus* sp.).

c) Rtem: Common frog (*Rana temporaria*). Bspi: Spiny toad (*Bufo spinosus*). Casp: Pyrenean brook salamander (*Calotriton asper*). Lhel: Palmate newt (*Lissotriton helveticus*).

The tubular creels, almost submerged and resting on the rocky or muddy bed of the shore, had been quite effective in catching minnow in a previous project conducted at Closell. 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.

Monitoring amphibians

Whilst in the process of eradicating the fish, we documented the changes in the amphibian populations of the target lakes, both in terms of presence and abundance. Censuses from previous studies had shown the presence of five amphibian species in the study area (Miró et al., 2018). Three were anurans or amphibians without a tail: the common frog (*Rana temporaria*), the spiny toad (*Bufo spinosus*) and the common midwife toad (*Alytes obstetricans*). The other two were urodeles or amphibians with a tail: the Pyrenean brook salamander (*Calotriton asper*) and the palmate newt (Lissotriton helveticus). These are the five amphibian species that we included in this study. However, it should be noted that, one of them, the spiny toad, like the other species of the Bufo genus, is not usually negatively affected by exotic fish (Knapp, 2005; Miró et al., 2018), due to the toxins present in its skin, both in the larval and adult phase, (Benard & Fordyce, 2003). At the beginning of the fish eradication work, there were only two target lakes that had amphibians: the spiny toad inhabiting Closell and the palmate newt in Rovinets (Table 1).

Data on the presence and abundance of amphibians in the eight target lakes come from two different sources: (i) the catches of accompanying fauna during fish eradication and, (ii) specific amphibian censuses. Regarding the first information source, once fish densities were considerably reduced, catches of amphibian adult and larvae specimens increased, which allowed us to quickly document colonization events. The catches were found mainly in the creels installed on the shore of the lakes, and showed no damage to the amphibians captured, which were released again in the same area of the lake once recorded and measured.

Regarding the second data source, we have conducted annual censuses of presence and abundance of amphibians in the eight target lakes during the summers from 2012 to 2018. We have carried out the censuses in the control lakes over the same study period (2012-2018), visiting each one of them once following the pre-established schedule of limnological monitoring of the LimnoPirineus project (Buchaca et al., 2016a; Buchaca et al., 2019).

Amphibian censuses are conducted using the visual encounter methodology (Crump & Scott Jr., 1994). Preferably during the hours of greatest sunshine, we walked all along the lake shore visually looking for amphibian larvae and adults, estimating the abundance per metre of the corresponding shore. In the case of the Pyrenean brook salamander, since they are basically active during the hours of darkness, we conducted additional nocturnal censuses. The nocturnal methodology was also visual encounter, in this case while we illuminated the lake shore with headlamps, as indicated by the British protocol specialised in this task (ARG-UK 2013). In the case of the palmate newt, since they are very active in the water, where they frequently swim to feed, we used creel catches. In all cases, for each species, we have used the same census methodology at all the lakes studied. The differences in abundance that could be related to the specific methods and the diverse reproduction strategies in terms of the number and size of larvae produced, we have homogenised by re-scaling abundance by species (see detailed explanation in the following paragraphs).

Statistical analysis

Once the data was obtained, we performed different specific statistical analyses to answer each of the four questions that we proposed at the end of the introduction. Previously and in order to correctly assess the temporal changes in amphibian communities, we standardised all the data according to the year in which the fish eradication tasks began. Thus, for each target lake, we assign the value -1 to the year prior to the start of eradication work, the value 0 to the year in which we the eradication tasks began, and the values +1, +2, +3, +4 and +5 to the following successive years. The standardised +4 and +5 years are not used in some statistical analyses because, in these two years, we had only included one lake, Closell.

The abundance of extracted fish was calculated from the trout catch in the nets and the minnow catch in the creels. We transform the data into catch per unit of effort (CPUE). Specifically, in the case of trout, we transform them into "individuals / hm of net × day", and in the case of minnow "individuals / trap × day". For the abundance of minnow, we have data up to the standardised year +5, while for trout, we have up to year +3 since we started work two years later.

The recovery of the amphibian community in the target lakes and the timeframe within which they could be reached are simultaneously quantified using two different methods. First, we calculate the specific abundance of each lake, and second, the abundance of each species we find. Then we compare the data for the different standardised years (from -1 to +3) between them and with the two control lake groups, with and without fish, by means of the Kruscal-Wallis non-parametric group homogeneity test (Hollander et al., 2014).

The proximity of the colonising populations is investigated by building a contingency table with all the amphibian species studied which are potential colonisers of the target lakes. This gives us a total of 38 potential colonisations: 5 species × 8 target lakes, but excluding the two cases of amphibians already present in the target lakes at the start of eradication. Then we apply a Chi-squared test (Agresti, 2007) on the combined factors "colonisation event during the entire period studied (yes / no)" and "presence of the species in the same valley of the target lake (yes / no)".

Additionally, we have investigated the importance of nearby colonisations by calculating the Mantel correlation (Legendre and Legendre 2012), for the different standardised years separately (from -1 to +2), between the geographic distance matrix for the target lakes, and the distance matrix extracted from their amphibian communities. The distance matrix of amphibian communities is constructed by calculating the Euclidean distances between lakes from the abundance tables for the different species, for each standardised year. Previously, and to reduce the difference in the range of abundances and balance the weight of the different species, we transformed abundances into logarithm + 1, and we re-scaled them between 0 and 1 for each species separately (Legendre & Legendre, 2012). Finally, we normalise abundances, using the Hellinger transformation (Legendre & Gallagher, 2001). In order to have the values with absence of amphibians as reference, we also kept the locations without any species in the data tables. The Mantel tests were calculated by applying the Pearson correlation and 9999 permutations (Legendre & Legendre, 2012).

We investigated the possibility that the type of exotic fish present in each target lake (trout or minnow) could have some influence on the recovery of the amphibian community, using a permutational multivariate analysis of variance (PERMANOVA, Anderson & Gorley, 2008). The analysis was applied to the same matrix of Euclidean distances based on the amphibian community and explained in the previous paragraph. In this case, to avoid casual correlations, we joined the two species of newts studied (*C. asper* and *L. helveticus*) into a single taxon, called tritons, since their distribution in the valleys of the target lakes was basically discordant and concordant, respectively, with the presence / absence of minnow (Miró et al., 2018). In this way, we were able to test the relationship of the amphibian community, in each standardised year (from -1 to +2), with the two factors "presence of trout in the lake (yes / no)" and "presence of minnow in the lake (yes / no)".

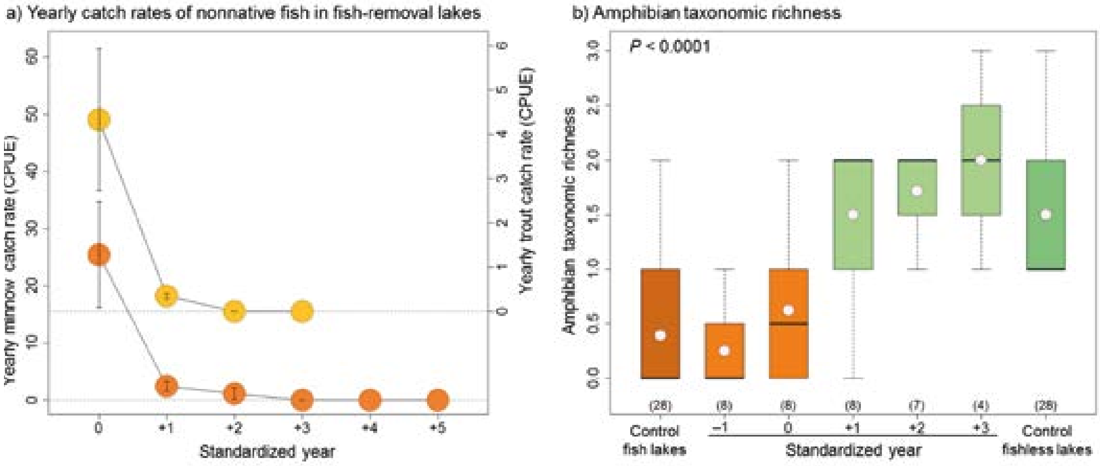
Finally, in order to identify possible biases in the PERMANOVA analyses, we tested the homogeneity of multivariate variance of the amphibian data matrices, for the two positions (yes / no) of the factors presence of trout and presence of minnow, for standardised years from -1 to +2. We investigated this by applying an ANOVA to test the differences between the distances of the members of each category to the centroid (spatial median) in a main coordinate space (PCoA; Anderson, 2006).

We performed the statistical analyses with the R program, using the basic functions (R Core Team, 2018) and the *vegan* package (Oksanen et al., 2018), except the PERMANOVA analysis, which we carried out with the PERMANOVA + program for PRIMER (Anderson & Gorley, 2008). The significance level we used for all analyses was α = 0.05.

RESULTS

The eradication actions allowed the catching of most of the exotic fish during the year in which they started (standardised year 0), and zero catch values, or very close values, were reached two years after the work began in the case of trout and three years later in the case of minnow (standardised years +2 and +3 respectively; Figure 2a). The summer of 2018, the last one that provided data for this study, no trout were caught in three of the target lakes, Subenuix, Dellui Mig and Dellui Nord. For the rest, we obtained reductions in populations of more than 98%.

The amphibian community of the eight target lakes responded quickly to the decline in exotic fish populations. Specific abundance was recovered to match the levels of the control lakes without fish, only one year after the eradication work began (standardised year +1; Figure 2b). On the other hand, the abundance of the



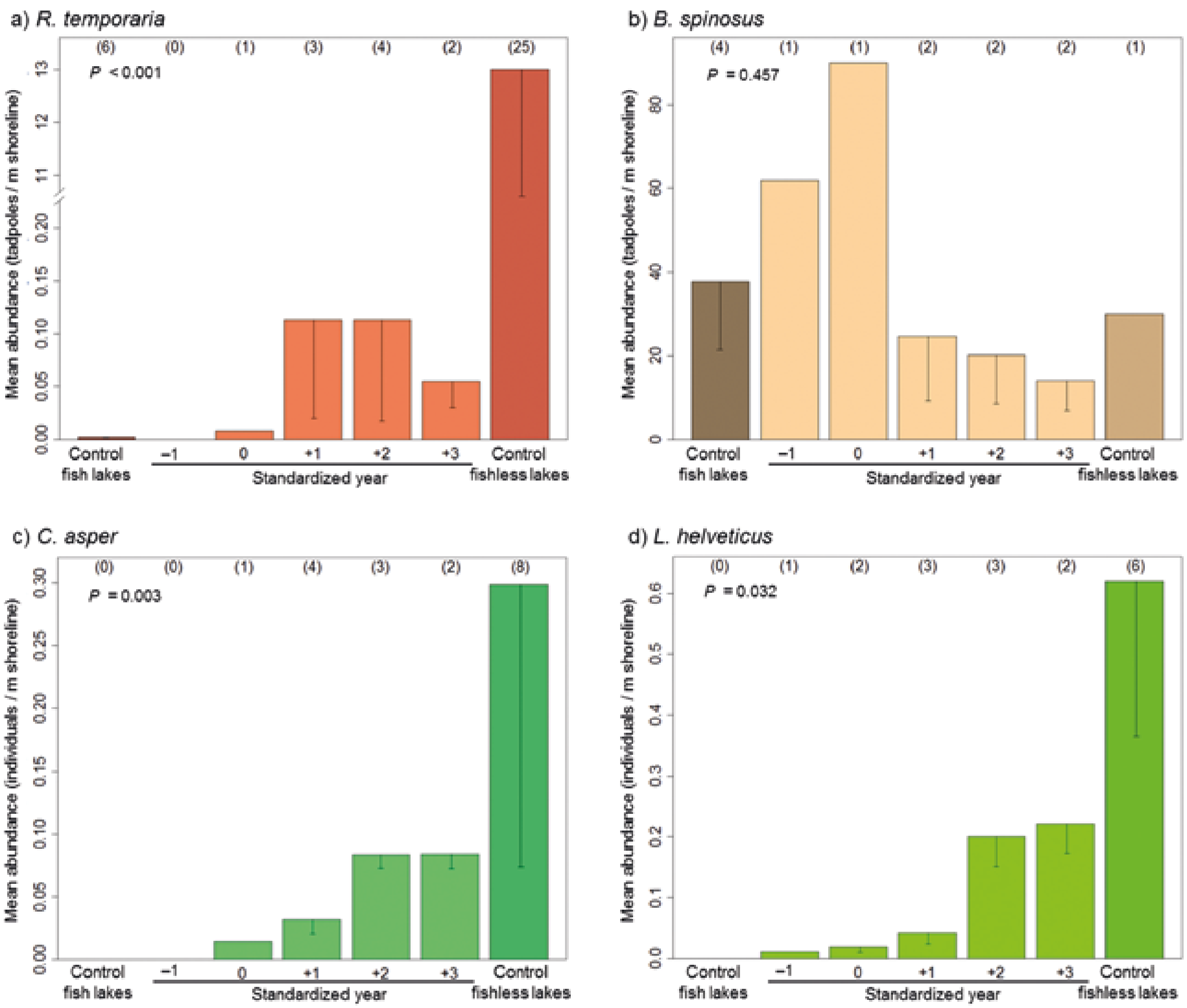
▲ **Figure 2.** Mean and ±SE of annual catches per unit of effort (CPUE, individuals / trap × day for minnow and individuals / hm of net × day for trout) of exotic fish (a), and boxplots that show the taxonomic abundance of amphibian species for standardised years in the eight target lakes (b). At the ends of graph b we have added the abundance and the standard error in the control lakes with and without fish. The lower part of the graph shows the number of lakes included in each category and the upper part shows the p-value of the Kruscal-Wallis homogeneity test between all categories. The brown boxplots show significant differences compared to the green ones in the post-hoc test. The white dot in the boxplots indicates the arithmetic mean.

different species sensitive to the presence of fish (common frog and Pyrenean brook salamander and palmate newt) did not reach the level of the reference lakes without fish during the entire study period (Figure 3). In the case of the common frog especially, the abundance of the target lakes in standardised year +2 were an order lower than those of the control lakes without fish (Figure 3a).

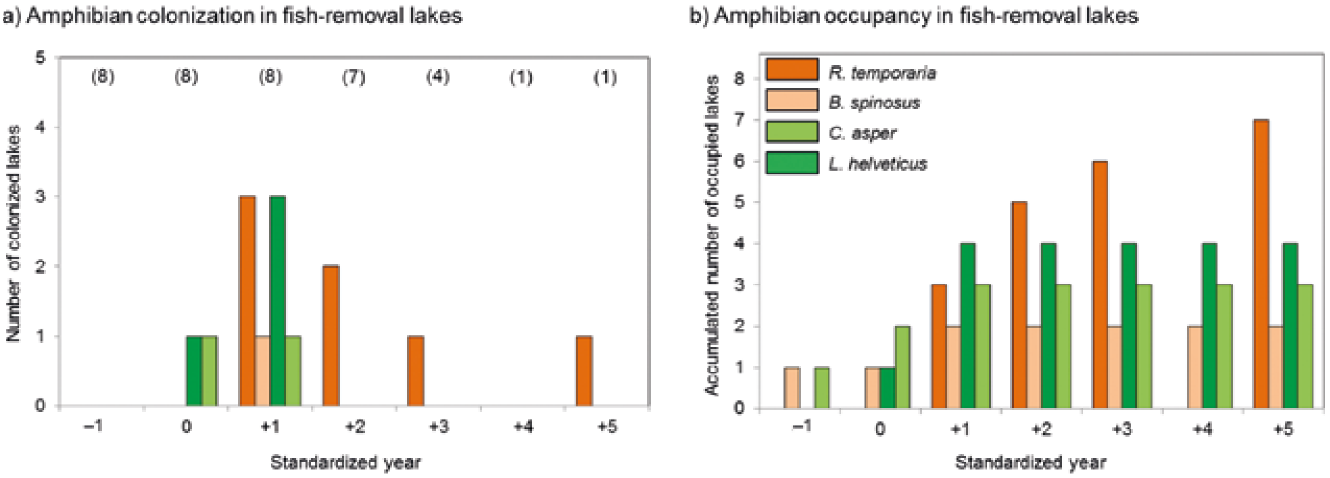
The nearby populations that continued to take refuge in the different valleys were crucial in promoting the rapid recovery of the amphibian community. Of the 38 potential colonisations that could occur, we documented 14 during the study period, two of them during the year in which eradication work began and eight during the following year (Table 1 and Figure 4a). In all cases, colonisations were carried out by species present in the same valley of the target lake (Chi-squared = 19.95, p value <0.0001). Six potential colonisations of species that were in the same valley have not yet occurred. Similarly, none of the 18 potential colonisations of species that were not in the same valley have occurred.

Colonisations starring the two species of newts were the earliest and all occurred in the same year as or the year following the start of eradication work (standardised years 0 and +1 respectively; Table 1 and Figure 4a). On the other hand, the common frog has a slower but more prolonged colonisation pattern, since, apart from starring in some colonisations a year after the start of eradication, it continued colonising over the rest of the years until it became established in seven of the eight target lakes (Figures 4a and 4b). The high rate of colonisation and establishment of the new populations has meant that, in the summer of 2018, there were already amphibians in all eight target lakes, with the combined presence of four of the five species that could potentially colonise them (Figure 4b).

Mantel tests confirmed the importance of nearby localities to explain the composition of amphibian communities of the target lakes. This analysis illustrated



▲ **Figure 3.** Abundància i SE per anys estandarditzats de les espècies d'amfibis trobades als vuit estanys objectiu, *Rana temporaria* (a), *Bufo spinosus* (b), *Calotriton asper* (c) i *Lissotriton helveticus* (d). Als extrems de cada gràfica hem afegit l'abundància i l'error estàndard dels estanys control, amb peixos i sense peixos. A la part superior de la gràfica es pot veure el nombre d'estanys inclosos en cada categoria i el *p*-valor del test Kruscal-Wallis d'homogeneïtat entre totes les categories. Els segments SE indiquen només el valor negatiu.



▲ **Figure 4.** Esdeveniments de colonització (a) i presència acumulada (b) d'espècies d'amfibis als vuit estanys objectiu. A la part superior del gràfic (a) s'indica el nombre d'estanys recollit dins de cada any estandarditzat. El codi de color per a cada espècie es mostra a la llegenda de la gràfica (b).

that, the year before the start of eradication, there was no correlation between amphibian communities and geographical distances between lakes (Mantel r = 0.354 and p-value = 0.071, standardised year -1). On the other hand, the year in which the eradication work began and the subsequent years, amphibian communities showed a positive correlation with geographical distances, with the closest lakes most similar to each other (Mantel r = 0.454 and p-value = 0.012 for standardised year 0, Mantel r = 0.644 and p-value = 0.004 for the year +1, and Mantel r = 0.617 and p-value = 0.043 for the year +2).

We have not found differences in the recovery of the amphibian community of the target lakes depending on whether they were occupied by different types of exotic fish, i.e. trout or minnow. PERMANOVA analyses showed that the two factors "presence of trout (yes / no)" or "presence of minnow (yes / no)" were not significant in explaining the composition and abundance of the amphibian community for the different years studied, from the year prior to eradication work starting up to two years after starting. The p-values obtained for the presence of trout and presence of minnow factors were, respectively: 0.440 and 1 for standardised year -1, 0.640 and 0.637 for the year 0, 0.517 and 0.600 for the year +1, and 0.540 and 0.616 for the year +2. In the only year where there was enough data to analyse the interaction between the two factors (standardised year +2), this was also insignificant (p-value = 0.404).

The PERMANOVA analyses described for the four standardised years that we investigated have proved insignificant, although, in some cases, there was no homogeneity of multivariate variance in the amphibian data for the two categories (yes / no) of the "presence of trout" or "presence of minnow" factors. The p-values obtained in the multivariate variance homogeneity analysis for the two factors were, respectively: 0.002 and 0.266 for standardised year -1, 0.073 and 0.004 for the year 0, 0.974 and 0.475 for the year +1, and 0.951 and 0.332 for the year +2.

DISCUSSION

The data we have collected has allowed us to answer all the questions we had asked ourselves. (i) The recovery of the entire amphibian community in high mountain lakes can be achieved by taking action to eradicate exotic fish. (ii) Recovery at the level of specific abundance is achieved the year after the eradication action begins. The recovery of abundance for the different species needs a longer period of time than we had in this study. (iii) The nearby refuge locations are crucial for restoration, since all 14 colonisations of amphibian species documented during the study period come from nearby populations located in the same valley as the receiving target lake. (iv) The available data did not show any influence regarding the type of exotic fish present in the target lake (trout or minnow) in the recovery of the amphibian community.

The data shows that amphibian populations have recovered by their own means, reaching the specific abundance levels of the natural control lakes, as we have progressed in the eradication of fish. This shows the high resilience of the amphibian fauna of high mountain lakes, following reduction or removal of pressure that kept them absent. Our results are in line with those obtained in studies conducted with species of the Frog genus in California and in the Alps, which showed rapid recoveries, within periods of only a few years (Knapp et al., 2007; Pope, 2008; Tiberti et al., 2018; Vredenburg, 2004). Other groups of organisms, such as the more conspicuous macroinvertebrates or planktonic crustaceans, had also shown very high resilience, in high mountain lakes in California, after eradicating exotic fish (Knapp et al., 2001). In the Pyrenees, results obtained in a parallel study (Buchaca et al., 2019), show that, after amphibians, the group that presents the clearest response to the eradication of fish are littoral macroinvertebrates, which increase the abundance of taxa progressively, at the same time as their composition converges with that of natural lakes. The change in the biomass of peripheral algae is less pronounced and only occurs where minnow was the only fish species or was accompanied by some salmonid. In the pelagic ecosystem there was only an increase in the abundance of crustaceans and a decrease in the phytoplankton biomass where minnow the only species (Buchaca et al., 2019).

However, the most remarkable fact in our case is the combined recovery of the entire amphibian community after eradicating or controlling exotic fish. Until now, only the recovery of a single frog species had been described in different cases (Knapp et al., 2007; Pope, 2008; Tiberti et al., 2018; Vredenburg, 2004), but not the entire community.

We have verified that the different species of native amphibians present in each valley have colonised the lakes quickly and progressively. We have observed the first natural colonisation of amphibians, in all cases, from the first or second year of the eradication work, although there is still a certain percentage of fish in the lakes. In this sense, the conservation of isolated amphibian populations sheltered in some valleys is of great importance. These marginal populations have a direct capital significance, since they maintain the possibility of local survival of the species. At the same time, they also have great indirect importance, as a source population to provide colonising individuals in the context of possible similar conservation actions.

As for the speed of colonisation of the different species, it seems that, in general, the Pyrenean brook salamander and palmate newt colonise and reproduce in the lakes quicker than the common frog. This suggests that newts are less sensitive to the presence of fish and discriminate less among the lakes where they are present. On the other hand, the delayed colonisation of the common frog, suggests that, for reproduction, adults of this species refuse lakes where there have been fish or where fish remain, even, with a certain inertia after the risk of predators disappears. This could be explained by a greater sensitivity in detecting the presence of chemical substances excreted by fish, which would lead this species to prioritise safer locations. In the case of the Pyrenean brook salamander and the palmate newt, the dynamics we have observed leads us to think that they take part in a certain number of migrations to the lakes regardless of whether or not there are fish. A large number of these migrant newts would usually be preyed upon when they reach a lake with fish.

We have observed the recovery of amphibian populations, with the same speed and elasticity in the eight lakes, regardless of the species of fish introduced. This is more remarkable news that our study provides, since, until now, all eradication action in high mountain lakes had been carried out only by working with exotic trout. Our data shows that the eradication of minnow requires more time than that of trout, but finally, in both cases, the same result is obtained, completely satisfactory in terms of the recovery of native amphibians.

However, the recovery of amphibians we have observed remains at an obvious risk if, in some cases, all the fish present cannot be eradicated or, if control actions are stopped before completing eradication (in some cases, during the LimnoPirineus project, the objective for lakes with minnow was to control but not eradicate exotic fish). In this case, only a few fish that survived the eradication or control tasks would be sufficient to restore entire populations in a few years. With the aim of reducing this risk, from its inception, the LimnoPirineus project provided a post-project plan that aimed to facilitate the continuation of eradication or control actions, when necessary, beyond its completion, in May 2019. Future actions considered in this plan should ensure the control of exotic fish populations in lakes that have not been freed of fish. Only in this way will it be possible to ensure that the recovery of native amphibians can be consolidated and sustained in the medium and long term.

CONCLUSIONS

We must conclude that the recovery of the entire amphibian community in high mountain lakes is possible after eradicating or drastically reducing exotic fish populations. In addition, it is clear that, at the level of amphibian occurrence, recovery can be achieved just two years after initiating eradication action. These conclusions can be clearly applied to the design or execution of future conservation projects in high mountain lakes. However, if we want to go beyond conservation objectives, we need to actively encourage the abandonment of fish introductions in high mountain lakes, regardless of whether or not they are within a protected area. This would be the best way to prevent the suitable and preferable amphibian habitat from decreasing further in mountainous areas.

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~ Pyrenean brook newt (*Calotriton asper*). ~

~ Estanho de Vilac ~

ALTERATIONS AND CHANGES THAT AFFECT THE CONSERVATION OF THE BIODIVERSITY OF ESTANHO DE VILAC (ARAN VALLEY)

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ABSTRACT

Estanho de Vilac is located in a small endorheic basin in the Aran Valley (Catalonia) at an altitude of 1640 m. Originally, it was a small pond of fluctuating level, but due to livestock interests it was recreated with an external water supply. In recent years, with the modification of the bed and the continuous increase in the volume of water accumulated, it has also been used for trout fishing. As a result of these modifications, there has been a notable alteration of the system jeopardising the conservation of various amphibian species, especially *Alytes obstetricans* and *Rana temporaria*, as well as *Luronium natans*, an aquatic plant that throughout the Pyrenees can only be found in this lake. All three are species protected at European level by the Habitats Directive. The aim of this work is to improve the conservation status of these species and the ecosystem as a whole. For this, a study of the water cycle has been conducted, calculating the water balance of the basin and the changes in level caused by altering the inlet of artificial water have been monitored; the variability of the nutrient cycle and of the aquatic biodiversity and especially of the indicator organisms has been studied. The results show that Estanho is a temporary pond that could remain dry for some time during the summer, but that a water supply that exceeds the natural loss values can continuously increase the volume of water that it would have without external alterations. It is also noted that the presence of trout has significantly altered the diversity of fauna in the body of water and this also affects amphibian populations. In addition, the presence of a large number of equine cattle causes eutrophication of the water, as well as the disturbance of the population of *Luronium natans* and the integrity of the substrate where it grows. This population is also affected by the entry of *Potamogeton berchtoldii*, a water-based macrophyte common to alpine lakes. The actions carried out to reverse the ecosystem towards a more natural state, include the extraction of all trout and the installation of a new watering hole with regulated water inlet. The elimination of trout has led to a rapid recovery of littoral macroinvertebrates and amphibians. Meanwhile, the decrease in the water level, caused by the decrease in the flow of the external contribution, has modified the extension of the *Luronium natans* population. This has been monitored in the two areas it occupies: in the area where the plant grows rooted to the floor, submerged with leaves floating on the surface of the water, and in the marginal area of the lake where the plant grows with amphibious forms that are exposed to the air. The decrease in water level has caused an increase in the density of the amphibious subpopulation, but in turn has been altered by the presence of livestock that deteriorates the edges of the lake. The aquatic subpopulation has been maintained, although it has low vitality. Plants have always been observed in a vegetative state and simultaneous flowering of multiple individuals was only seen in 2019. Based on these results, we propose that the existence of Habitats of Community Interest (HCI) 3150 and 3130 related to the water body and the two subpopulations of *Luronium natans* be recognized in the Estanho.

INTRODUCTION

Estanho de Vilac is a small lake of the Aran Valley corresponding to an old endorheic pond of glacial origin, whose ecological function should be conditioned by rainfall and probably maintained significant water fluctuation throughout of the year (Figure 1).

▲ **Figure 1.** Estanho de Vilac.

This lake is included in the list of Special Areas of Conservation (SAC code ES5130006) as a Site of Community Interest for its biodiversity. The greatest interest in terms of biodiversity lies on a stable population of *Luronium natans* (L.) Raf., an endangered macrophyte according to the Spanish Catalogue of Endangered Species (RDL 139/2011, of 4 February). In addition, several amphibians nominally protected in Catalonia (LD 2/2008, of 15 April) have been observed in the area, such as *Bufo spinosus* Daudin, 1803, *Lissotriton helveticus* (Razoumowsky, 1789), *Alytes obstetricans* (Laurenti, 1768) and *Rana temporaria* L., 1758. The last three species (Figure 2) also appear in the list of wild species under special protection of the National Catalogue (Directive 92/43/EEC), and *Luronium natans* and the last two amphibians in Annexes II, IV and V of the Habitats Directive, respectively.

▲ **Figure 2.** Species of the Habitats Directive present in Estanho: *Luronium natans*, submerged form (left) and amphibious form (right); *Alytes obstetricans* (lower left) and *Rana temporaria* (lower right).

However, this biodiversity could currently be compromised by the concurrence of a series of stressors that influence the variability of the ecosystem function.

Estanho de Vilac has historically been used as a water supply point for livestock (cattle and horses). Its fluctuating nature was an inconvenience for this use in the driest season, so a watering hole was incorporated with an artificial inlet of water from a ravine outside the basin. The excess water was guided to the pond in order to maintain its level. These modifications are old (probably from the end of the 19th century) and over time they have been renewed and others have been incorporated, such as the excavation of the pond bed that was carried out around the 90s to expand the storage capacity. More recently, and facilitated by the hydrological changes derived from the watering hole, common trout, *Salmo trutta* L., 1758, was also introduced for sport fishing. Currently, the lake environment is still used for grazing of horse and cattle (Figure 3).

▲ **Figure 3.** Estanho de Vilac. Artificial water inlet and equine livestock.

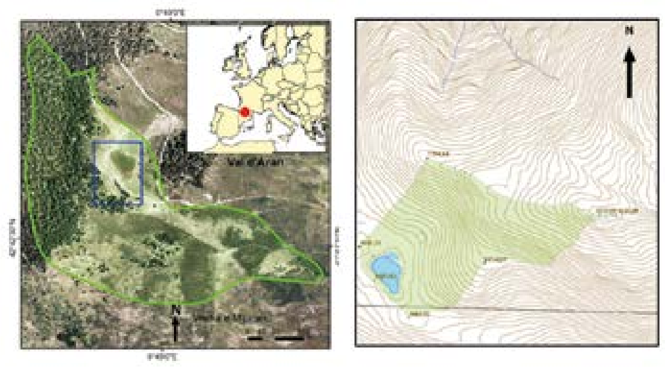
We hypothesise that the artificial water inlet and the extra supply of nutrients may compromise the survival of *Luronium natans,* and that the introduction of fish may affect some groups of organisms, especially the amphibians.

The importance of biodiversity at Estanho de Vilac together with the presence of *L. natans* (the only location of this aquatic plant in the Pyrenees) led to it being declared a Site of Community Importance (SCI) and Special Area of Conservation (SAC) specifically for this ecosystem, and to including it within the conservation objectives of the LIFE+ LimnoPirineus project (http://www.lifelimnopirineus.eu).

One of the actions of this project, which is reported in this work, is the improvement of the conservation status of *L. natans*, of two of the four amphibian species present at Estanho de Vilac, and of the ecosystem as a whole. This includes the study of the effect of hydrological changes and the elimination of introduced fish fauna.

GENERAL DESCRIPTION OF THE STUDY AREA

The area under study is Estanho de Vilac located in the Aran Valley, near Vielha, at an altitude of 1,640 m, on the western slope of Mont dera Solana, with coordinates 0° 48’ 50.18” N and 42° 42’ 37.43” E. The endorheic basin where it lies accounts for an area of 11.37 ha, and is mainly used by livestock (grazing by horses and cows) during the summer and occasional recreational use, given the easy access and relative proximity to populated areas (Figure 4).



▲ **Figure 4.** Location of Estanho de Vilac and SCI ES5130006 Estanho de Vielha (left) and Estanho basin (right).

In the Aran Valley the climate is mountain Atlantic, with relatively high rainfall distributed evenly throughout the year, and with mild temperatures in summer and cold temperatures in winter (in Vielha, 939.7 mm/year and 9.6 °C on average). Given the clear altitudinal gradient, both of precipitation and temperature, rainfall at Estanho is higher and temperatures are lower than the average values of the nearby Vielha station. The relatively high number of days without sunlight is notable, largely due to the presence of fog, which causes a decrease in evapotranspiration.

Estanho de Vilac is located in the syncline sector of the Aran Valley, dominated by Devonian outcrops that are mainly formed by schistose materials in this area. The natural floor of Estanho, of holocenic origin, should be flat and formed by lacustrine deposits of clays and silts rich in organic matter, which alternate with sandy levels (Colomer *et al.*, 2014). These deposits can be several metres strong, and fill and waterproof the bottom of lakes, both of glacial origin and lateral distension, as is surely the case here (Bordonau *et al.*, 1989). Estanho de Vilac has been transformed into a conical base structure, with a maximum depth in the centre of 2.7 m and a perimeter that has been enlarged to about 290 m in 2015. In 2015, it could already be considered a small-sized, elliptically shaped (approximately 100 m by 60 m) lake with the modified floor, probably formed by a mixture of the primitive materials present in the area, and with unknown permeability.

Hydrologically, Estanho is located in a small endorheic basin located above the aquifer of the Devonian metamorphic limestone of the Aran Valley, which can be up to 400 m in depth (ACA, 2015). It naturally receives rain and runoff water from the basin, which gives an irregular periodic regime of oscillation of level, both annual and interannual, determined by the meteorological conditions of the year (precipitation and temperature). In a period with low rainfall and high temperatures it would probably have been almost dry. The interest of a water point at medium altitude (1,640 m) for cattle had to be important at the time, as evidenced by the presence of old buildings (barn and cabin) in the meadows that surround it. To remedy the lack of water in the dry years, a fountain-watering hole with water channelled from a spring outside the basin was arranged. Over the years this guidance has been renewed, each time with better materials and more efficiently. Since the last renovation (approximately in 2003), the surplus water from the watering hole has become a notable additional contribution to Estanho, causing its continued growth. Alongside this collection work, the floor of the pond was excavated to increase its capacity, without anticipating the possible alteration of the impermeability of the bed. During the LIFE+ LimnoPirineus project, a new watering hole has been built and the water inlet has been modified to control the flow, with the aim of reversing the expansion of the Estanho.

The soil dominating the basin is of dortent lithic type, which is characterised by having a low organic matter content and a depth limited to a maximum of 30 cm. It includes some rock outcrops and may be mixed with other types of soils similar to this one, either deeper or with more organic matter (Colomer *et al.*, 2014). Vegetation currently comprises some 80% of broom scrub (formed by *Genista balansae* (Boiss.) Rouy subsp. *europaea* (G. López & CE Jarvis) O. Bolòs & Vigo, HCI 5120), 10% of natural forest of *Pinus sylvestris* L. (HCat 42.5B11), 9% of mesophilic and acidophilic grasslands (HCat 35.122) and 1% of outcrops of schistose rocks (HCI 8220). The present dominance of *Genista balansae* subsp. *europaea* comes from its expansion over the space formerly occupied by pasture.

Inside Estanho we find a homogeneous environment with absence of stones and large amounts of silt deposits. In the centre, in the deepest part, there are only sediments. Around this area there is a macrophyte community consisting of three species: *Luronium natans* (L.) Raf., *Potamogeton berchtoldii* Fieber. and *Eleocharis palustris* (L.) Roem. &. Schult. In addition to the macrophytes, filamentous algae have been observed of the *Spirogyra* Link. and *Mougeotia* C. Agardh genera.

Luronium natans is an aquatic species, with some phenotypic plasticity as it grows in permanently flooded or temporarily exposed beds. It has a rosette of linear submerged leaves and elliptical floating leaves. It produces rhizomes and long stolons that often break, so that the separated rosettes, pushed by the wind act as propagules and recolonise the shore. It blooms from June to August, although throughout the years of study it has practically not done so. The population of Estanho is the only one known in the Pyrenees and is probably a clonal population.

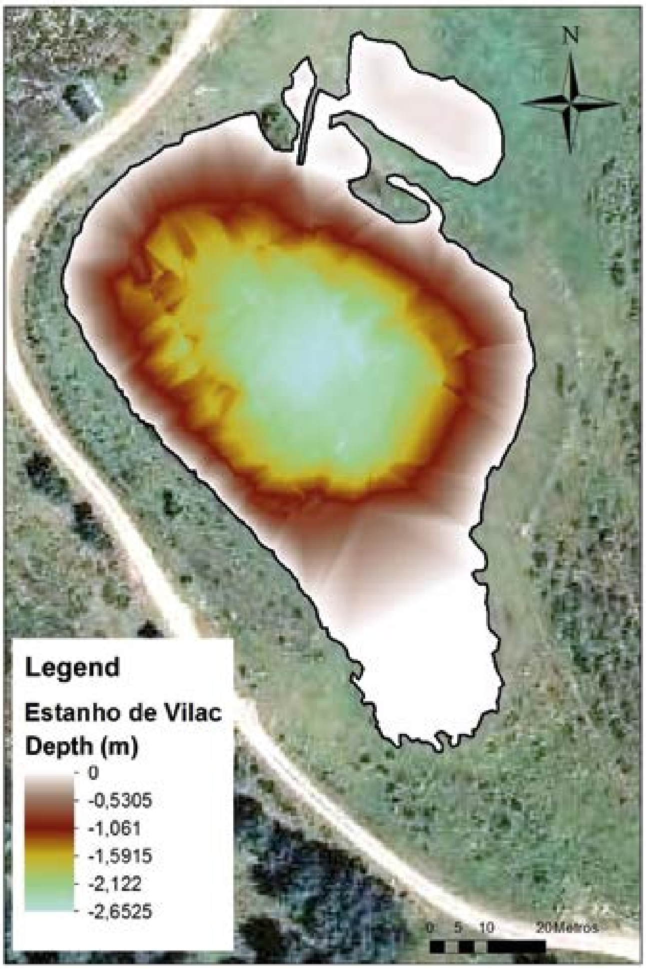
In the 80s, when *L. natans* was discovered in Estanho de Vilac, it covered practically the entire surface of the pond and was in bloom from June to September (Perdigó, 1983). At the beginning of the project, in the summer of 2014, *L. natans* covered almost half of Estanho's surface, about 2,462 m^2, with varying, but generally low densities depending on the areas. The most notable presence was in an inner strip that corresponded to the old pond shore. According to Lansdown & Wade (2003), in lacustrine environments *L. natans* tends to occupy the bank areas. Apparently, the species has been colonising the flooded parts more recently thanks to its ability to produce new stolons and fragments.

As for the other species, *Potamogeton berchtoldii* occupied 12% of the surface, about 588 m^2, and *Eleocharis* *palustris* occupied just 0.3%, 15 m^2 in 2014. In fact, *Eleocharis palustris* formed a small stand that was originally on the shore of the lake, and as it increased in size, has ended up in the middle, surrounded by water. *Potamogeton berchtoldii*, more typical of lakes than of temporary ponds, appeared recently, and occupies the same areas as *Luronium natans*, except for the shore and the shallowest area. Filamentous algae grow as epiphytes on petioles and macrophyte leaves, so that they interfere with them in the occupation of space and the use of light. They form extensive floating carpets that were found in variable quantities throughout the study, mainly accumulated towards the leeward shore.

VARIABILITY STUDY OF THE HYDROLOGICAL CYCLE

Historical changes that affect the physical structure of the Vilac lake

Using the bathymetry of Estanho de Vilac, carried out in autumn 2014 with Sonda Echomap 50DV (Figure 5) and following a standardised protocol (Cooke et al., 1993), the area and volume have been calculated every 5 cm with the "Surface-Volume" tool of the ARC MAP 10 program and the Estanho area-volume ratio has been established.



▲ **Figure 5.** Bathymetry of Estanho de Vilac conducted in October 2014.

Historic aerial images of the ICGC (Institut Cartogràfic i Geològic de Catalunya [Cartographic and Geological Institute of Catalonia]) (http://www.icc.cat/vissir3) have been compiled from 1956 to 2014 and the perimeter of the Estanho has been digitised in different years. Actual data have been obtained from the lake area and the corresponding volumes have been determined based on the area-volume ratio established. This has led to historical real volume increases.

Water balance

Water balances have been carried out between 2003-2005, 2005-2007, 2007-2008, 2008-2009, 2009-2011, 2011-2012, 2012-2013 and 2013-2014 according to the available data on the increase of real volume of water in Estanho, in order to create a system operating model. This period has been chosen because it is assumed that bathymetry has not undergone changes.

Each water balance has been conducted matching the equation of inlets and outlets to the system:

$$P + EA = ED + ETR + ESC_s + GW \pm \Delta V$$

where P is the precipitation on the basin, EA is the artificial water inlet, ED is the direct evaporation from the surface of the lake, ETR is the real evapotranspiration of the basin vegetation, $ESCs$ is the surface runoff towards other basins, GW is groundwater and ΔV is the volume of increase or decrease in storage in the lake.

The baseline data for the calculation of water balances were: measurements *in situ* of artificial water inlet (with flowmeter) and climatological data of daily cumulative precipitation (p) and average daily temperature (t) at the Vielha-Mijaran station. Data from the stations at Sasseuva and Bonaigua have been used in cases of missing data. The p and t data were corrected by the difference in altitude at each of the stations with that at Vilac Estanho. The correction factor for annual precipitation is +30.1 mm every 100 m, applied by Del Valle (1997) for the basin of the Esera river in Benasque, and -0.49 °C every 100 m for the daily temperature, according to the recommendations of Lampre (2001).

The annual direct evaporation of water from the surface of the lake has been calculated using the Visentini equation (1963). In order to calculate the actual evapotranspiration, the monthly and annual evapotranspiration potential (ETP) has been calculated according to the Thornthwaite (1984) equation, which takes into account the average monthly temperature (T) and the latitude from the annual heat index (I), a monthly factor (a) that depends on this and a factor f that depends on the location.

Using the ETP, the ETR that is finally used in the balance of the basin has been calculated, providing the monthly hydrometeorological balance according to Thornthwaite for the water requirements of the vegetation. This method (Doorenbros & Kassam, 1979) takes into account a 42 mm water retention capacity (CAD), calculated according to the soil type in the basin, starting at a *mean CAD* = 1.4 mm cm^{-1} for medium texture soils, and considering a maximum depth of the root system (*Zr*) of 30 cm.

For the hydrogeological balance of the basin, ETR and P were applied to the entire surface of the Estanh de Vilac micro-basin, which has 11.37 ha, and the lake surface ED in each period. The balance in the micro-basin has been closed calculating the volume of groundwater as the only unknown, since the basin is endorheic and there are no surface water outlets. Water that supposedly goes underground is treated as surplus.

Determination of flood level

To control the water level variations of the lake, a metallic rod was placed in the bed at a distance of 2 metres from the watering hole inlet channel. In the different campaigns, the distance from the end of the rod to the surface of the water was measured, taking the first as the zero point to assess the level changes. The same type of observation was conducted by taking another reference element (upper margin of the watering hole drainage channel) to corroborate the data. Data was collected until 2018.

Study of the nutrients cycle

During the summer and autumn of 2014, four samples of the water from Estanho de Vilac and three from the artificial water inlet were taken. Sampling include, among others, the collection of physicochemical data (temperature, turbidity, conductivity, pH and alkalinity). The water temperature was measured in a depth profile every 0.5 m. In addition, a data-logger was placed in the lake that measures continuous temperature to know the exact dates of freezing and defrosting. Turbidity was measured with Secchi's disc. The conductivity and pH were determined in the field, with manual multi-parameter probe, and in the laboratory. Alkalinity was determined by Gran's automatic potentiometric titration.

The water collection from both the inlet and the pond was carried out following standard protocols (ACA, 2005, 2006). Sampling of the pond was always conducted in the deepest place, taking representative samples of the entire water column.

In all samples, the main nutrients were analysed: nitrate (NO_3^-), ammonium (NH_4^+), phosphate (PO_4^{3-}), total phosphorus (TP) and total nitrogen (TN), following the methods recommended by Ventura *et al.* (2000). NO_3^- was determined by electrophoresis with CIA-4000; NH_4^+ by spectrophotometry with the phenol-hypochlorite method; PO_4^{3-} with the malachite green stain method; TN was determined by persulfate digestion followed by ultraviolet spectrophotometry; and TP was oxidised to phosphate by persulfate digestion.

Lake water samples were taken and chorophyll *a*, *b* and *c* were analysed as a surrogate of phytoplankton biomass. Filtering with 47 mm GF/F filter was conducted to saturation, noting the volume (minimum volume of 2.5 l). Chlorophyll was extracted by sonication with 5 ml at 90% acetone and measured with spectrophotometer. Chlorophyll concentrations were calculated with the equations of Jeffrey & Humphrey (1975).

We estimated the DIN/TP molar ratio (DIN is the dissolved inorganic nitrogen) recommended by Bergstrom (2010) as one of the factors that can be related to phytoplankton biomass.

The main entries of nitrogen and phosphorus were studied, considering the contribution of direct rainfall on the pond and on the basin, the contributions that arrive through the artificial water inlet and the contributions derived from the presence of equine livestock in the basin.

The concentration of DIN, in the form of NO_3^- and NH_4^+, that arrive through the rain, was taken from studies that include the area of Vielha (Catalan & Camarero, 1994). The TP in the rain was taken from Camarero & Catalan (2012). The amount of nitrogen and phosphorus that can be derived from equine livestock droppings was taken from agricultural studies (Iglesias, 1995; Boixadera *et al.*, 2000). Lastly, the DIN, TN, TON, TOP and TP means of the samples taken at the artificial water inlet for the pond were used.

To calculate the annual entry of nitrogen and phosphorus at Estanho de Vilac from contributions to the basin (not direct to water), it was taken into account that not all nutrients that enter the basin go to the pond, but instead a part thereof is retained by the vegetation. This occurs in percentages that depend on the vegetative period, and for nitrogen has also been seen to depend on the state in which it is found (organic or inorganic). The percentages for these calculations were taken from Iglesias (1995) and were applied to the estimated contributions of equine livestock (Iglesias, 1995; Boixadera *et al.*, 2000) and the amount of inorganic nitrogen and phosphorus from the average precipitation on the basin (Catalan & Camarero, 1994; Camarero & Catalan, 2012).

VARIABILITY STUDY OF THE BIODIVERSITY OF ESTANHO DE VILAC

To assess the variability in the biodiversity of Estanho de Vilac and the significance of the anthropogenic factors that affect it, analyses were conducted from two points of view. First, the trout community introduced in the lake and the stomach content thereof has been studied in order to assess the composition of its diet, since from the results obtained in other studies (Knapp, 2001; Jones & Sayer, 2003) it was considered that the predation by *Salmo trutta* of certain groups may be one of the determining factors in the loss of biodiversity at the Vilac lake. Second, a temporal analysis of the samples carried out with the LimnoPirineus project was conducted on several indicator groups: littoral macroinvertebrates, amphibians and crustaceans. These data were analysed in reference to the presence of trout introduced and in the case of crustaceans, the community has also been analysed with respect to the biomass of phytoplankton, using chlorophyll *a* as a surrogate of biomass.

Actions with the introduced fish

The trout detected during the study period were most likely introduced into the lake in 2011. One of the objectives of the LIFE+ LimnoPirineus project to eradicate introduced fish from high mountain lakes, was conducted in this regard at Estanho, by the company Sorelló S.L., project partner, through the installation of sampling nets (multi-mesh net; Nordic fishing standard). In July 2014, initial fishing had been carried out at Estanho, from where six specimens were extracted. Second fishing in October of the same year, estimated that almost all of the specimens had been eliminated, and in July 2015, the last specimen was recovered with a new net.

The 18 specimens caught were weighed, measured, and gutted. The stomach and intestines of each trout were preserved, first by freezing and then in ethanol for analysis. To assess the effect of *Salmo trutta* on the biodiversity of the lake, the stomach content of 12 individuals was studied. In the laboratory, cleaning and separation of these stomach contents was performed, and the identification and counting of organisms was conducted using a stereomicroscope with 6 to 40 magnifications. For the identification of macroinvertebrates, both in trout stomachs and benthos, the Puig (1999) illustrated identification guide was used.

Sampling and analysis of the variability in indicator groups

The temporal analysis of the abundances for each of the indicator groups was carried out based on data collected as of June 2014, and in the case of amphibians, also with information prior to the introduction of fish in Estanho (2008-2013). The data available and the type of sampling and analysis conducted for each group were the following:

Amphibians: Data from 16 censuses carried out over the different years of the project have been analysed. In all cases, the sampling consisted of counting the number of individuals in five 2 m transects, representative of the habitats on the pond shore. When different censuses were available for the same year, the highest data was used.

Benthic macroinvertebrates: Four sampling campaigns were performed (July, August, September and October 2014). Sampling of this group was carried out following a manual scanning methodology from the shore (littoral strip-sampling) in order to avoid damage to the *Luronium natans* macrophyte (20 sweeps of 1 m x 0.4 m). Samples of benthos collected were passed through a 1 mm sieve and the counts were carried out using a stereomicroscope with 6 to 40 magnifications.

Crustaceans: Six sampling campaigns were done (July, August, September and October 2014 and June 2015). For this, representative samples of the entire water column were filtered using a 200 µm conical net. Subsequently, the samples were separated under a microscope.

Sampling and analysis of the *Luronium natans* population

The *Luronium natans* population was differentiated into three subpopulations according to rooting depth. The one that occupies the internal part of the lake (subpopulation C), the one that develops in the flooded area with depths between 10 and 100 cm (subpopulation B), and the one established at the perimeter edge that may emerge temporarily (subpopulation A).

For subpopulations A and B, sampling was carried out to determine its size at the beginning of the project (2014) and in the years after the modification of the water inlet flow (2016-2019). For this, 10 to 14 samples were considered in each zone, each corresponding to a surface area of 50 x 50 cm using a metal square, and the number of *Luronium natans* branches included in the square were counted. The samples were regularly and randomly distributed, following the edge of the lake. For subpopulation C, an estimate was made of the area covered by the plant, based on its abundance in 50 x 50 cm squares arranged on the water surface (values from 1 to 4 according to the occupation of the 4 quadrants) These samples were randomly distributed 10 times throughout zone C. An initial estimate (2014) was also made for biomass.

Phenology was monitored using the same number of samples and with the same arrangement as those used to assess the population size. Throughout the vegetative period, from May to September (October), there were monthly visits in 2014, 2015 and 2016, and more distanced visits from 2017 to 2019. The different forms of vegetative growth (linear basal leaves, spatulate basal leaves and floating elliptical leaves) and reproductive growth (flower, fruit) have been differentiated. No permanent samples were established due to the rapid change of the environment when uncovered by water. Good or bad state of growth and direct disturbances on the population have also been considered (livestock, tourism and occasional events). The spread of *Potamogeton berchtoldii*, which competes directly with *L. natans*, was simultaneously evaluated.

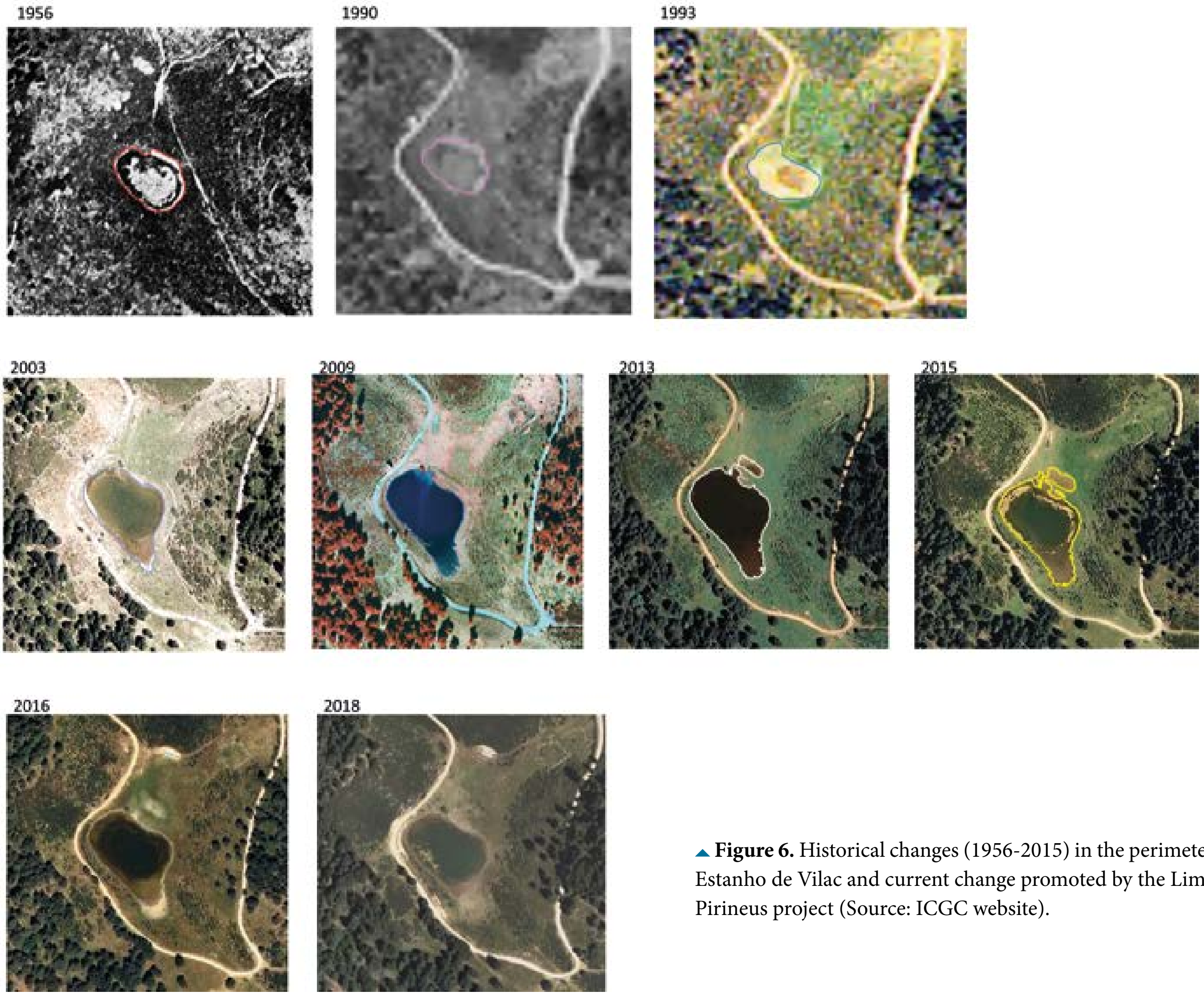
In order to verify the persistence of fruits and seeds in the lake sediment, in autumn 2014, (10/10/2014) a sampling was carried out of the sediment of the flooded area. A total of 16 samples were collected (10 cm from the top of a sediment cylinder extracted with a 7.5 cm diameter cylindrical probe) distributed at different depth points. The littoral zone was not sampled because it was a space of new colonisation where there has been no flowering or fruiting of the species. From the collected sample, the superficial 10 cm were selected and stored in the freezer until their study. Each sample was washed and screened to retain sediment components that were similar in size to fruits or seeds (*Luronium natans* produces monosperma achenes). Once this fraction was dried, the seeds were separated under a stereomicroscope and counted.

RESULTS

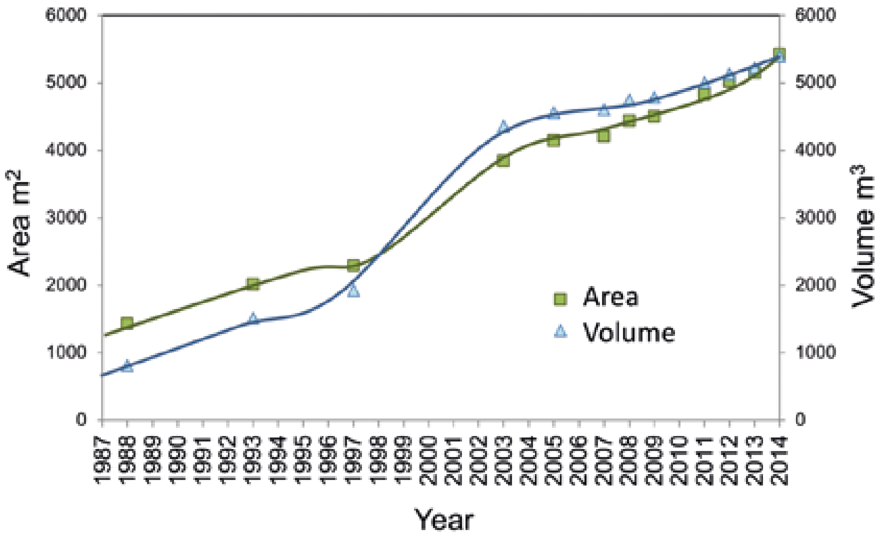
Hydrological variability: water balance

Estanho de Vilac has undergone very clear hydrological changes (Figure 6) that have been determined with the analysis of the historical aerial photographs that the ICGC (http://www.icc.cat/vissir3) has compiled. Mapping of the variation of the perimeter of the pond, together with the water balance, allows the representation of the historical evolution of the estimated surface area and volume (Figure 7).

Hydrometeorological balances revealed an average real evapotranspiration (ETR) of 395.3 ± 65.1 mm/year. This represents 78% of the ETP (504.38 mm/year), which is consistent with climatological studies carried out in the

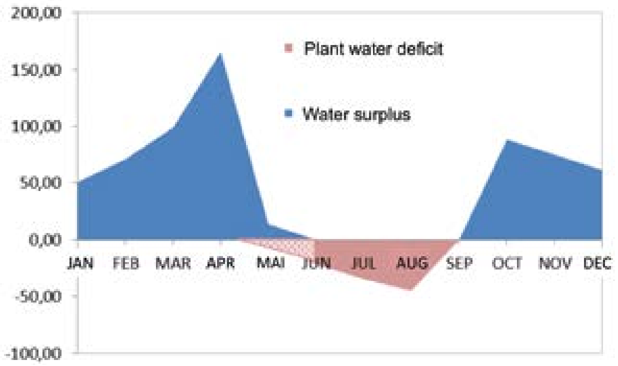


▲ **Figure 6.** Historical changes (1956-2015) in the perimeter of Estanho de Vilac and current change promoted by the Limno-Pirineus project (Source: ICGC website).



◀ **Figure 7.** Historical evolution of the surface and volume at Estanho de Vilac.

Aragonese Pyrenees (Lampre, 2001). The average precipitation (P) between 2003 and 2014 was 1112.68 ± 184 mm/year and the average direct evaporation from the lake surface was (ED) 842.26 ± 64.3 mm/year. Vegetation in the basin suffers water stress for 2 to 3 months a year, normally between June and August, and from September-October there is a water surplus (Figure 8).



▲ **Figure 8.** Hydrometeorological balance in an average year (balance 2011-2012) with indication of periods of surplus and plant water stress.

Applying the results of the hydrometeorological balances to the Estanho de Vilac basin and taking the artificial water inlet for the lake into account, we obtain that *P* is the main input of the balance in the basin (129.75 ± 21.01 hm^3 per year) and *GW* the main output (98.55 ± 17.09 hm^3 per year). *EA* is a constant value that represents 12% of the inlets (17.86 hm^3 per year). It has a flow rate of 0.89 ± 0.127 l/s for 232 days when the water is not frozen. Taking *P* as the reference value, *EA* represents 14.06% of *P*. *ETR* represents 35% of *P* and 30.7% of the balance output (45.08 ± 7.32 hm^3 per year). Figure 9 shows the distribution of balance components in percentages of total input and output.

The average storage of lake surface water (ΔV) is a very small value of the balance (0.1%), but it is always positive, which results in a constant increase in its volume. *ED* represents only 2.6% of the balance output but is 78.6 ± 4.24% of the volume of the lake (3.88 ± 0.44 hm^3 per year). The excess (*EXC*) represents 66.6% of the balance output and 75.9% of P, and it is argued that its destination is mainly to recharge aquifers (*GW*).

Chemical variability. Input of nutrients

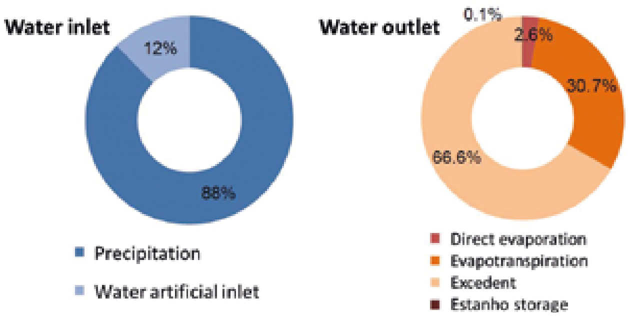
According to the data collected, the Estanho de Vilac water has an average conductivity of 113.2 µS/cm and an average pH of 8.59. Water temperature varies a lot throughout the year. It remains below zero from mid-November to the end of March and a maximum value was measured during the sampling of 22.3 °C. The average is 15.98 °C. The artificial water inlet to Estanho freezes at the same time as the lake, but the rest of the year it remains at a constant temperature of 12.5 °C and has conductivity and pH values similar to those of Estanho (168.2 µS/cm, pH 8.6). Thermally, the lake is free of ice 7.74 months per year (232.25 days), freezing approximately in mid-November and defrosting at the end of March. The maximum temperature does not usually exceed 25 °C. In the four samples taken, the Secchi disk indicated that light penetrates to the bottom of the lake (2.7 m).

The average content in the main compounds of nitrogen and phosphorus, both in the Estanho water and the artificial inlet can be seen in Table 1, where there are notable differences between the composition of both, especially in terms of nitrate content.

		TON µg N/l	NO_3^- µg N/l	NH_4^+ µg N/l	TN µg N/l	DIN µg N/l	TOP µg P/l	PO_4^{-3} µg P/l	TP µg P/l
Artificial inlet	Average	0.00	378.62	1.51	313.35	380.13	0.65	0.78	1.42
	St. Dev.	0.00	105.48	2.10	138.43	105.77	0.77	0.03	0.79
Estanho	Average	397.08	5.98	5.13	406.54	11.11	15.86	2.79	18.65
	St. Dev.	130.83	3.24	5.32	132.16	8.56	8.34	1.89	9.96

▲ **Table 1.**
Average concentration and standard deviation of nitrogen and phosphorus compounds in the artificial water inlet to the lake and in the lake itself.

The inlet water through the tributary has an average NO_3^- concentration of 379 µg/l, while that of Estanho is only 5.98 µg/l. As for P, the differences are less pronounced, but the concentration is greater in the lake water, both for PO_4^{3-} and above all TOP (total organic phosphorus). There are also differences in TON (total organic nitrogen). While the water inlet does not have organic nitrogen, the lake water has an average concentration of 379.08 µg/l.



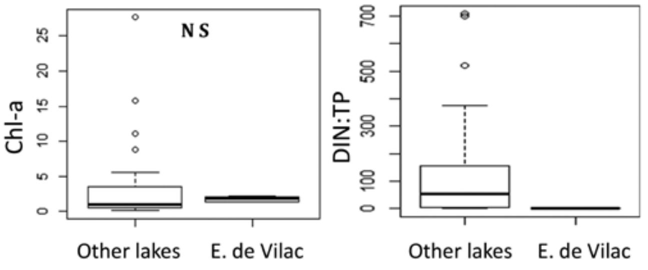
◀ **Figure 9.** Distribution in percentages of the water inlet and outlet balance in the system.

The ratio DIN/TP may indicate a limitation in Estanho primary production due to any of the nutrients (nitrogen or phosphorus) (Bergstrom, 2010). The values found in Estanho are shown in Table 2.

Date	DIN:TP	Limiting factor
16/07/2014	2.2	P
07/08/2014	1.1	N
15/09/2014	1.5	limit
10/10/2014	2.1	P
17/06/2015	0.6	N

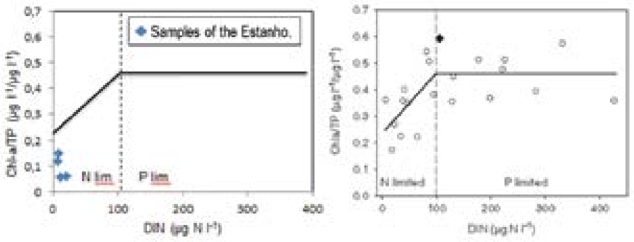
▲ **Table 2.** Limiting factor of phytoplankton production according to DIN: TP ratio (Bergstrom, 2010) in Estanho water samples

The *chlorophyll a* concentration was found at values between 1.19 µg/l and 2.18 µg/l, not being significantly different (P = 0.262; Mann-Whitney-Wilcoxon test) from the rest of the LIFE+ LimnoPirineus project lakes (40 lakes of different characteristics), although they are relatively low compared to these (Figure 10 left). If we compare the ratio DIN/TP of Estanho de Vilac with that of the rest of the lakes in the project, we find that the values in this case are significantly different from those of the whole (P = 0.0218; Mann-Whitney-Wilcoxon test), and lower compared to most of them (Figure 10 right).



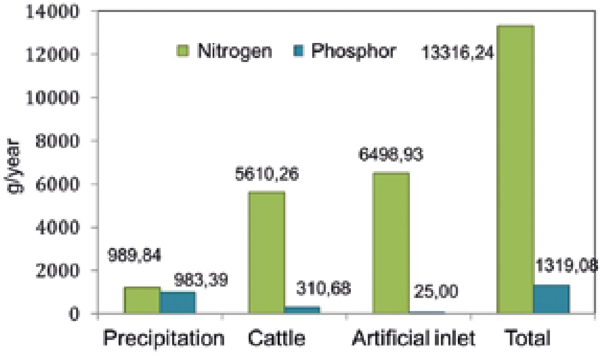
▲ **Figure 10.** Comparison between Estanho de Vilac and other 40 lakes in terms of chlorophyll *a* and DIN/TP, by means of the Mann-Whitney-Wilcoxon test for unpaired samples. NS= not significant; * P<0.05; ** P<0.005; ***P<0.001.

If we compare the relationship between phytoplankton biomass, TP and DIN with other studies carried out in the Pyrenees (Camarero & Catalan, 2012), the samples from Estanho de Vilac would be placed in a condition limited by N, with very little Chl *a* and DIN in relation to TP (Figure 11).



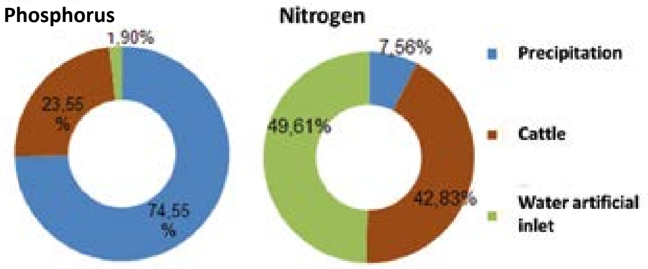
▲ **Figure 11.** Relationship between phytoplankton biomass, TP and DIN for the Estanh de Vilac samples (left) compared to the studies by Camarero & Catalan (2012).

In addition to the entry of artificial water, other sources of nitrogen and phosphorus entry into Estanho may be precipitation and the presence of equine livestock. Input for the entire Estanho microbasin have been calculated, taking a year as a reference period, and the results obtained can be seen in figure 12.



▲ **Figure 12.** Estimated amount of inorganic nitrogen and phosphorus that enters Estanho de Vilac in one year by different routes.

The origin of nutrient input as a percentage of the total input can be seen in figure 13. The main input of phosphorus, 75%, is produced through rain, while that of nitrogen is 92%, of local anthropic origin. 49.6% comes from the artificial water inlet and 42.8% from faeces of livestock present in the basin not retained by vegetation. It is necessary to consider that for the livestock, a conservative position has been proposed: 30 horses were counted grazing in a single month in summer in the area, that only half of the faeces are in the Estanho microbasin and that a large part is retained by the vegetation (Iglesias, 1995).



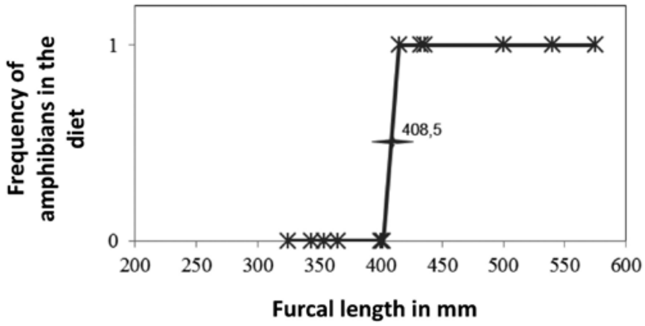
▲ **Figure 13.** Source of the nutrients that enter Estanho de Vilac according to percentage of entries.

Variability of the biodiversity

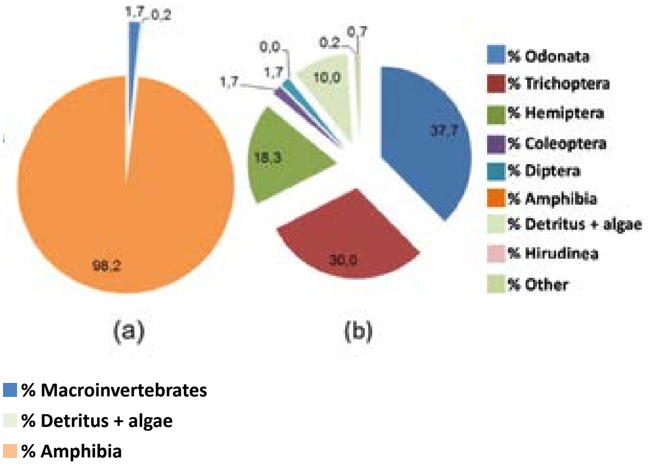
A. *Stomach content of introduced fish*

It is known that the trout at Estanho de Vilac were probably introduced in 2011. The age of the trout was determined by the otoliths of their inner ear (they were 3 years old in 2014) and the weight-length ratio of many individuals agreed with the same, which is consistent with having specimens of the same age. Despite their young age, some of the trout caught reached a considerable size and weight (> 50 cm and > 3 kg).

The analysis of the stomach composition of the 12 trout caught in the October 2014 campaign revealed that their main diet consisted of macroinvertebrates and amphibians. A vast difference was observed between the diet of some trout and others. Some individuals seem to feed only on macroinvertebrates and others practically only on amphibians and this seems to be related to their size. Figure 14 shows how the logistic function indicates that small-sized *S. trutta* specimens feed almost exclusively on macroinvertebrates until they reach an average critical measurement of 408.5 mm. As soon as they exceed that length, amphibians become their preferred prey, representing almost 100% of their diet (Figure 15a). Up to 4 amphibians at once were counted in the stomach of a single trout. The analysis found a greater number of individuals (or parts of them) that belong to the Anura order than those that may belong to the *Caudata* order.



▲ **Figure 14.** Logistic function for the furcal length in mm of *Salmo trutta* after which amphibians appear in the stomach contents.



▲ **Figure 15.** (a) Average diet composition of large *Salmo trutta* specimens (FL> 408 mm) introduced in Estanho de Vilac. Macroinvertebrates included odonata, trichoptera and coleoptera. (b) Average diet composition of small *Salmo trutta* specimens (FL< 408 mm) introduced in Estanho de Vilac.

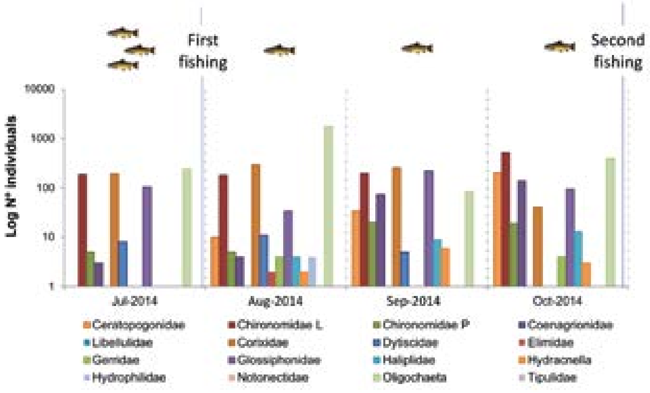
As for the macroinvertebrates found in the stomachs of small trout (Figure 15b), we can say that most stomach contents consist of *Odonata* (37.7%), *Trichoptera* (30%) and *Hemiptera* (18.3%) orders. Other orders that appeared in smaller proportions are *Diptera, Coleoptera* and *Hirudinea*. Crustaceans and some terrestrial invertebrates were also found. This type of content was counted as "Others". Algae and sediment appeared in both large and small fish, sometimes in considerable quantity, although it is believed that they were collected involuntarily by trapping other prey.

Identification of the samples, in the case of amphibians, was only possible at the order level, since most were in an advanced state of digestion. In the identification of macroinvertebrates, however, it was possible to reach the level of families and subfamilies in some cases. We know that most *Hemiptera* in the trout diet are *Heteroptera* of the *Corixidae* family. And that all the *Diptera* found were from the *Chironomidae* family. From this family, both pupae and larvae were found and were counted separately in order to compare the results with benthos sampling. The larvae were mostly from the *Orthocladiinae* subfamily, while the *Tanypodinae* subfamily dominated for pupae.

B. ***Temporal analysis of macroinvertebrates***

In most cases of littoral macroinvertebrate counts sampled in the benthos of E. de Vilac, it was possible to identify the family level, with the exception of mites and *Oligochaeta* that were counted at subclass level.

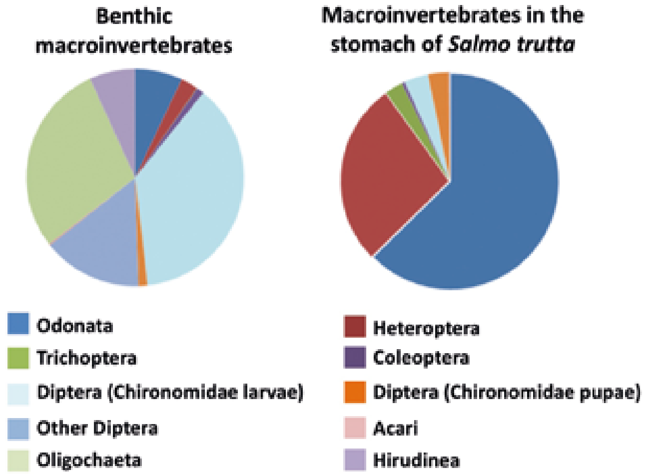
Figure 16 shows the evolution of the abundance of individuals in the families/subclasses over time using bar diagrams and reference is made to the *Salmo trutta* fishing carried out. The abundances are represented in a logarithmic scale so that all values are visible. In August there was an explosion of *Oligochaeta* that later disappeared.



▲ **Figure 16.** Evolution of the composition of the macroinvertebrate community from July to October 2014 expressed in abundance (in 8 m^2 of sampling).

All macroinvertebrate samples were collected from an environment with trout, although in July 2014 the pressure from these should be greater as no fishing was carried out. In this case, there are no samples after the second fishing, which makes it difficult to assess the changes in the composition with respect to the presence of trout. However, we can compare the October sampling of macroinvertebrates with the stomach contents of the small trout that feed on them, since the sampling was done just a few days before the second trout fishing.

Figure 17, where the frequency of the macroinvertebrate groups in the benthos samples is compared to those from trout stomachs, reveals a vast difference between them that shows us the selection of prey by trout.



▲ **Figure 17.** Relative frequencies compared between the benthic macroinvertebrates sampled in the lake and in the stomachs of 6 trout.

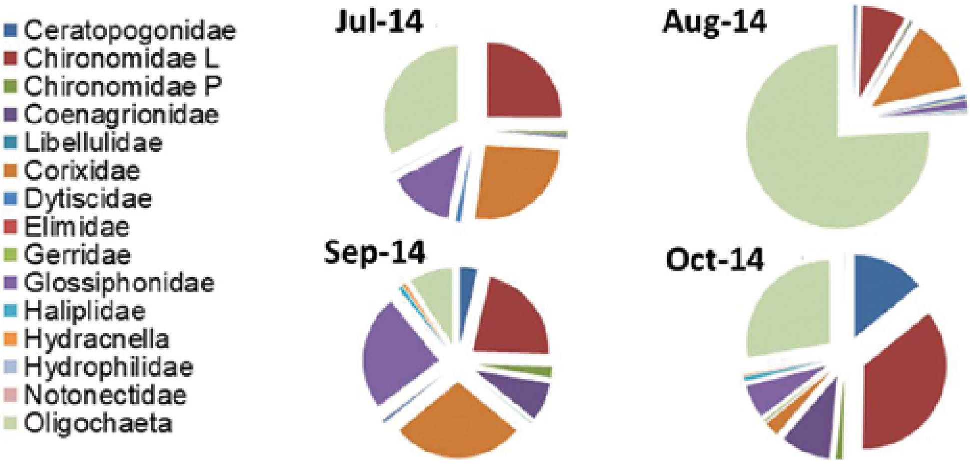
Returning to the composition in the benthos, Figure 18 shows the evolution of the relative abundance of each family/subclass in the samples in percentages. Clear differences in time can be seen, but these may be due to seasonal changes in taxonomic groups.

Some families of macroinvertebrates have specific increases in abundance, which may be due to seasonal hatching, as would be the case of *Oligochaeta* that have a very noticeable increase in August and a subsequent marked decrease. Other similar cases would be the *Corixidae* family of *Heteroptera* in August-September, and the *Glossiphoniidae* family of *Hirudinea* in September.

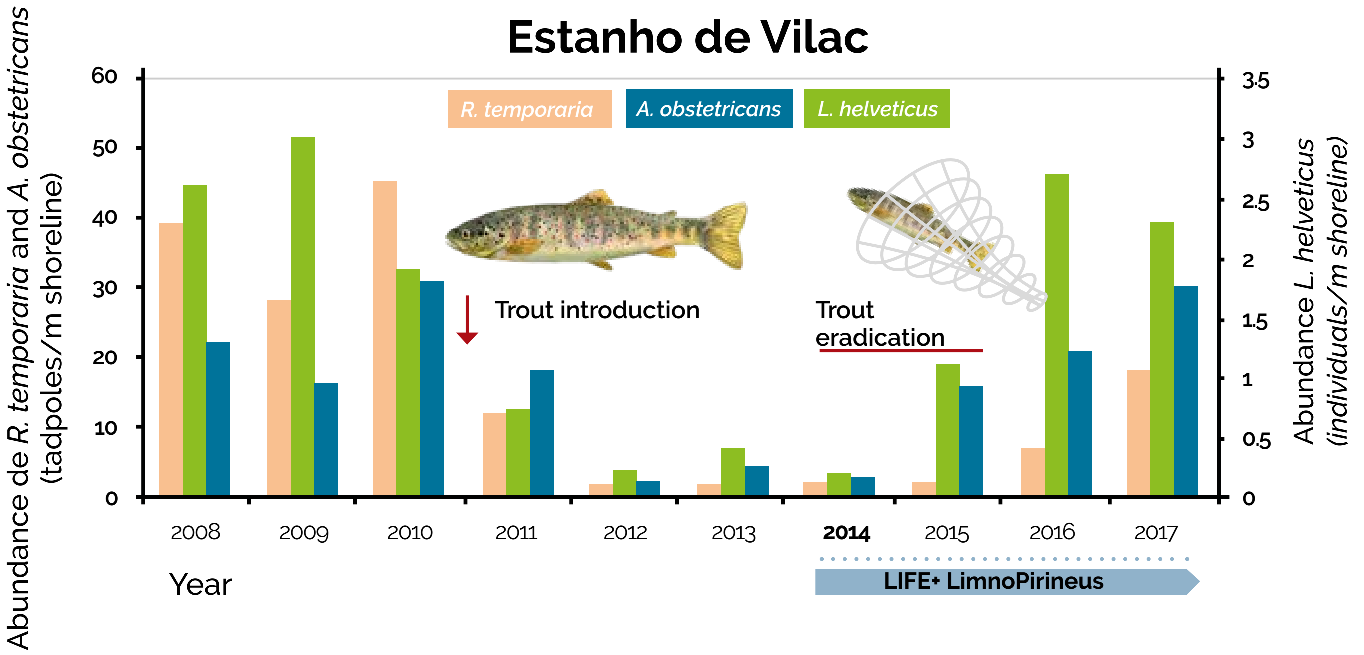
There are four families in which there is a progressive increase in relative abundance not attributable to seasonality. These are the *Diptera Ceratopogonidae* (from 0% in the sample in July to 14% in October) and *Chironomidae*, especially those in larval state, which go from 25% in the sample in July to 36% in October. The *Coenagrionidae* family of *Odonata*, go from 0.4% in July to 9.6% in October and the *Haliplidae* beetle family, which increases slightly but progressively from 0% to 0.9%. In the trout stomachs, only *Odonata* and *Chironomidae* were found in abundant proportions, so that not all of these changes can be explained by a lower pressure on behalf of the trout. A fact that attracts attention in this regard is the almost total absence of *Trichoptera* in benthos samples, when in trout it is a common prey.

C. *Temporal analysis of the abundance of amphibians*

The incorporation of data collected in previous projects allows us to verify that the trout introduced caused a significant decrease in densities, of 90-95% depending on the species, of amphibians present in the lake, which include the *Anura* of European interest *R. temporaria* and *A. obstetricans*, as well as the urodel *L. helveticus* (Figure 19). Once these trout were caught, amphibian populations recovered quickly and have been maintained at excellent levels throughout the project development.



◀ **Figure 18.** Evolution of the composition of the macroinvertebrate community from July to October 2014 in terms of relative abundance of families/subclasses in the sample.



▲ **Figure 19.** Evolution in the composition of the amphibian community at Estanho de Vilac. The estimated abundance of larvae or adults is represented per unit of coastline length.

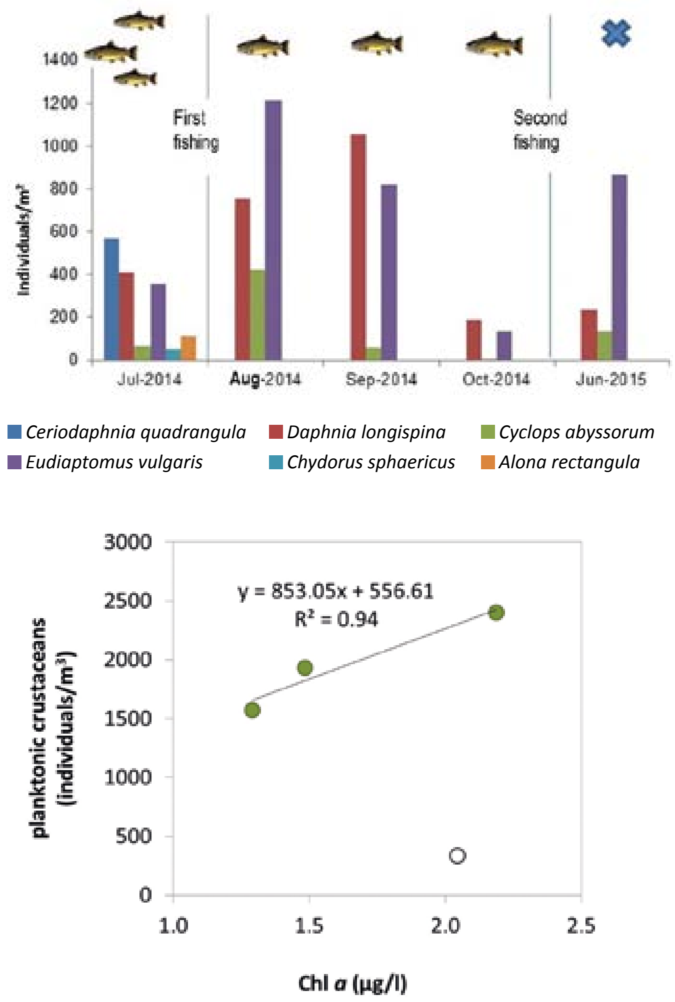
In the stomach of the trout caught in the second fishing, the two orders (*Anura* and *Urodela*) were identified, although it was not possible to reach the species level given the degree of decomposition of the amphibians.

D. *Temporal analysis of the abundance of zooplankton*

In the case of crustaceans, there is a sample of the crustacean community prior to fishing (July 2014), one after having eliminated all the fish, (June 2015) and two intermediate samples of the status with fish after the first fishing.

As shown in Figure 20a, which shows the evolution in the crustacean community, the first sample showed individuals of three species that have not been detected again in subsequent samples (*Ceriodaphnia quadrangula* OF Müller, 1785; *Chydorus sphaericus*, *O.F. Müller, 1776* and *Alona rectangula Sars*, *1861*). The three species not detected after trout fishing, have preference for eutrophic waters.

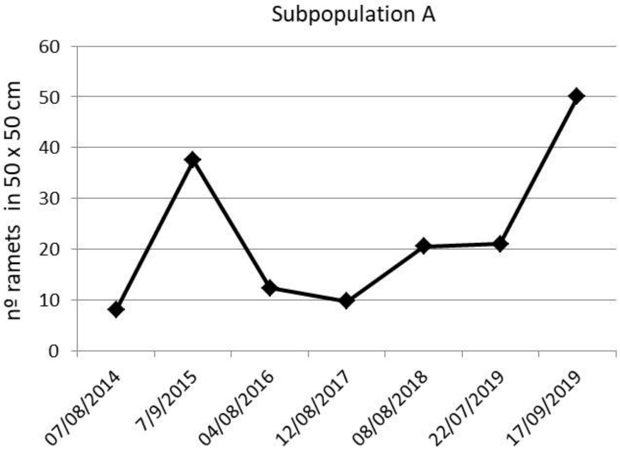
One of the factors that may be more related to the abundance of crustaceans is algal biomass. Figure 20 b shows the concentration of chlorophyll *a* (subrogating the algal biomass) against the abundance of crustaceans. The results indicate that between the months of July and September there is a positive relationship between algae biomass and the abundance of crustaceans, indicating that the abundance of crustaceans is related to the availability of food. In October, the abundance of crustaceans drops drastically, probably due to the phenology of these species, since they spend the winter in forms of resistance.

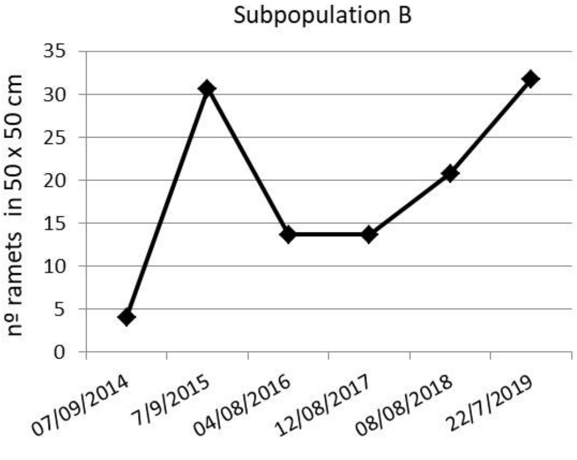


▲ **Figure 20.** Evolution in the composition of the crustacean community at E. de Vilac. (a) The number of individuals of each species is represented per estimated cubic metre. (b) Ratio of phytoplankton biomass estimated from chlorophyll a to the total abundance of planktonic crustaceans between July and September (green dots). In October the phytoplankton relationship is lost due to the seasonality of crustaceans.

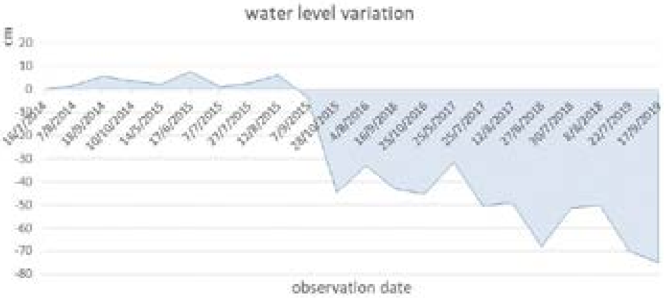
E. *Temporal analysis of the Luronium natans population*

The *Luronium* population was monitored from July 2014 to September 2019. Of the three differentiated subpopulations, we were only able to establish continuous monitoring of subpopulations A (perimeter edge of the lake and temporarily exposed areas) and B (flooded area of low depth). The samples taken reflect the location status at each sampling moment (zone A or B) despite not having the same site. The withdrawal of the lake from the moment the artificial water supply is closed modifies the status of the sampling squares. Figure 21 shows how there is an increase in the number of individuals in the population as a whole at the beginning of September 2015, 11 days after the closure of the artificial water supply to carry out the installation works of a new watering hole. The water level has already dropped a few centimetres (Figure 22) and exposes an area with a large population of amphibious forms of *Luronium* and also improves the density of the flooded subpopulation. The 2016 sampling shows a sharp decrease in density, which slowly recovers in successive years.





▲ **Figure 21.** Evolution of the *Luronium natans* population according to the sampling carried out between July 2014 and July 2019 in subpopulations A and B, evaluated according to the number of branches per 0.25 m²).



▲ **Figure 22.** Evolution of the water level between July 2014 and July 2019.

Regarding the development of the population's phenological cycle, since it is not possible to mark individuals, the data refer to observations based on both the density assessment samples and the set of different subpopulations.

Subpopulation A, scarcely developed in 2014 and widely extended in subsequent years, has always remained vegetative. No flower or fruit has ever been observed. Its growth status is variable according to soil moisture, showing larger sizes on silt with water at surface level or high water tables (saturated soils) and less developed forms in drier soils. The status of *Luronium natans* on these substrates recently emerged from the water is very vulnerable to trampling and disturbances caused by livestock, especially horses, both because of their weight and tendency to wallow and splash. Since the flood control started in 2016, subpopulation B has been moving towards the centre of the lake as its level decreased. In September 2019, it formed a dense crown of leaf rosettes that covered a wide area of the bottom of the Estanho edges.

Subpopulation B showed floating leaves and some rosettes with linear basal leaves during the first sampling years (2014-2016). In areas with less flooding, a remarkable expansive activity of the plant was observed, producing stolons and new rosettes. In deeper areas, shoots with long-petiolate leaves remained, with a tendency to decrease in size and vitality. This area is affected by occasional disturbances from livestock (they enter to drink water and cool off) and by direct competition from *Potamogeton berchtoldii* (macrophyte typical of mountain lakes that appeared in Estanho on an unspecified date). *Potamogeton* has expanded from the deepest waters of the centre of the lake (2014) to the edges (2018). Masses of filamentous algae that grow epiphytes on the plants detaching and floating agglomerates on the surface, also affect the growth of *L. natans.* Although the vegetative development is not good, the subpopulation of zone B has shown remarkable reproductive activity in terms of the formation of vegetative propagules (fragments of stolons with leaf rosettes) that the wind relocates within the lake; flower formation has been low. During 2014 and 2015, no flowering was observed. In 2016, 2017 and 2018, a few isolated flowers were seen, and in 2019, there was abundant flowering (number of flowers already uncountable overall).

sample no.	depth (m)	location	n. of seeds
1	2.3	zone C	84
2	2.3	zone C	60
3	1.8	zone C	5
4	1.9	zone C	0
5	1.6	zone C	0
6	1.4	zone C	29
7	1.4	zone C	0
8	0.8	zone B	1
9	0.8	zone B	1
10	0.6	zone B	0
11	0.6	zone B	7
12	0.8	zone B	3
13	<0.4	zone B marginal	2
14	<0.4	zone B marginal	0
15	<0.4	zone B marginal	5
16	<0.4	zone B marginal	0

▲ **Table 3.** Evaluation of the *Luronium natans* seed bank in Estanho de Vilac (10/10/2014), based on the number of seeds detected in a total of 16 sediment samples, extracted from different depths.

Subpopulation C has remained more or less stable in status, although there is apparently a decrease in vitality (less leaf density and smaller leaves). In 2014, a central area in the shape of a crown was well identified in the lake, which in the years of monitoring has gradually disappeared.

The seed bank sampled in 2014 showed very variable densities according to the sample (Table 3). The results show that there were seeds in 62% of the samples, but these were only abundant in the samples obtained from the deepest part of the lake.

DISCUSSION

Hydrological variability

The hydrometeorological balance of the area indicates water stress for two months in summer. The balance in the Estanho basin gives an evapotranspiration of 35% P and a groundwater recharge of 75.9% P. This means that in the current climatic conditions and in a year without significant summer precipitation, the lake would dry within the period of plant water stress if there was no artificial water inlet, recovering quickly towards the month of September. Despite being only 12% of the water entering the water balance, the entry of artificial water into Estanho is an imbalance that results in a continuous increase in the volume of the lake.

The changes in the hydrological regime of Estanho could affect *L. natans* compromising its survival. It has been observed that individuals in the deepest areas of the lake have a weaker appearance than those closer to the shore. Lansdown & Wade (2003), point out that this species has an ecological plasticity to adapt its way of life and reproduction to the environment in which it lives, so that there are three different adaptive typologies. The populations that grow in temporary water zones and in the area where permanent waters fluctuate, are annual and bloom abundantly. Plants that live in permanent waters are perennial and can give two other types. Those that live in bodies of water more than 2 m deep with little seasonal water variation are typically vegetative and only reproduce clonally from their rhizomes and stolons production. The last type presents the two types of reproduction, both sexual by flowering and clonal by stolons, and occurs in permanent water bodies with small level fluctuations.

The population of Estanho de Vilac would have evolved in conditions of significant water fluctuation (Perdigó, 1984) and since the modifications in the lake's artificial water inlet, the macrophyte tries to adapt to the new conditions, decreasing its sexual reproduction and increasing that of clonal type. This would explain the reduced flowering observed in recent years, and the current absence of this, in addition to the weakened appearance of individuals in the deepest areas. There is a very active clonal expansion towards the shore from the detachment of stolons and rosettes of individuals from the slightly flooded area. It is believed that some natural fluctuation of the water level would be adequate in this case, since sexual reproduction would help maintain genetic diversity, which would be fundamental in the Vilac population, which is isolated. Lansdown & Wade (2003) also point out that *L. natans* has more chance of survival in environments with permanent water than in seasonal water regime environments, so the best thing for the species seems to be to keep Estanho with permanent water at a level similar to the original (Perdigo, 1984) that allows a slight summer fluctuation of the level and exposes the flat bottom colonisable by the amphibious forms of *L. natans*.

Variability in the nutrients cycle

The calculation of nutrient input in the Estanho de Vilac ecosystem indicates a very high local anthropic input source, especially inorganic nitrogen (91%). It has been estimated that the annual amount of nitrogen entering Estanho is 10 times higher than P entering. With these data we would expect to find a clear limitation by phosphorus in the lake water and yet the DIN:TP ratio, Chl *a* and TP (Figure 11) shows that Estanho de Vilac is limited by nitrogen, with low phytoplankton biomass values compared to other lakes studied in the area (Camarero & Catalan, 2001). According to the DIN:TP ratio (Bergstrom, 2010) the situation would change from one limiting factor to another over time (between nitrogen and phosphorus). These changes could be due to phosphorus input in the system due to atmospheric depositions that, as Camarero & Catalan (2001) point out, may become specific events that are heavily charged with phosphorus. In their article, the findings of Camarero & Catalan (2001) on recent anthropogenic changes in the depositions of atmospheric nitrogen and phosphorus and their consequences in the TN/TP ratio, lead them to argue in favour of the idea presented by Goldman (1988) that, contrary to what is classically thought, the limitation by phosphorus in mountain lakes is not natural but induced by the anthropogenic increase in nitrogen depositions.

One of the factors that may be limiting the growth of phytoplankton could be competition for light and nutrients with the community of macrophytes and epiphytic algae (Ventura *et al.*, 2008). It should be said that Estanho has significant seasonal differences in the state of development of floating algae, which could condition the entry of light and competition. In addition, both *L. natans* and *P. berchtoldii* are tolerant to high levels of nutrients, and in competitive circumstances this type of macrophyte usually has an advantage over the rest (Ventura *et al.*, 2008). With regard to the latter, it should be noted that *L. natans* has been described as intolerant of competition (Lansdown & Wade, 2003). The same authors point out that, in the event of eutrophication the flowering perennial forms of *L. natans* tend to reduce their coverage in favour of monocots and algae. During the project, considerable expansion of *P. berchtoldii* was observed from year to year, so it is possible that it is gaining ground against *L. natans* by competition.

Regarding zooplanktonic, there is a community of heleoplanktonic crustaceans in Estanho that may be helping to control phytoplankton biomass. It has been proven that in other target lakes, where there are no crustaceans, chlorophyll *a* content is much higher than that found in the Estanho. Phytoplankton biomass remains relatively low despite high nutrient input, as seen in previous sections, and crustaceans increase their abundance with phytoplankton biomass (Figure 20b). However, the amount of crustaceans present does not seem enough to explain low values of chlorophyll *a*, but they can serve as an indicator of a series of more complex processes in which other zooplanktonic and most likely heterotrophic organisms would also participate, which would help to keep phytoplankton biomass at low values despite large continuous nutrient input. Phytoplankton biomass could be limited by the combination of these factors (competition with macrophytes and feeding of zooplanktonic organisms) and most likely also by heterotrophic bacterial activity.

The high concentration of total organic nitrogen in the lake water, not being phytoplankton, may be organic matter in suspension. This TON would be scarcely available for the microbial loop as the lake is in conditions limited by nitrogen.

Variability of the biodiversity. Effects of the introduction of *Salmo trutta* in indicator groups

Trout are very selective predatory fish and, whenever possible, will prefer large sized prey that is easy to detect. This data has been confirmed by the study, since a critical size of around 40 cm has been found, after which trout go from basing their diet on macroinvertebrates to basing it on amphibians, since they acquire the ability to catch them when they grow larger. The high selectivity in catches made by the trout at E. de Vilac is not only seen among large and small trout, but in small trout that basically feed on macroinvertebrates, where a selection of some orders and families compared to others is clearly observed, since the differences between the composition of the macroinvertebrate community in the benthos and that found in the trout stomach is very different. In the stomach content of small trout, crustaceans also appeared, but to a much lesser extent than macroinvertebrates, it is assumed that with the same efficiency motivation. Crustaceans are likely to be more attractive to trout in case of population explosions.

Regarding the effect of salmonid predation on the indicator communities, a very significant effect on amphibian abundance has been proven, with the total abundance measured in Estanho decreasing by 90-95% in periods with trout versus periods without trout. The species most affected by the presence of trout were the common frog (*R. temporaria*), the common midwife toad (*A. obstetricans*) and the palmate newt (*L. helveticus*). The fourth amphibious species present in Estanho, the spiny toad (*B. spinosus*), is not predated by fish, possibly due to the toxicity of its skin (Miró *et al.*, 2018). However, the amphibian community seems to have recovered very quickly by eliminating trout and the four species that were found before the introduction of salmonids have reappeared in the usual abundance only a few months after their elimination. With the analysis of trout stomachs, it has been proven that they can be very voracious in terms of amphibians by concentrating on them as prey. The rapid recovery of amphibians has been undoubtedly favoured by the fact that the introduction of *Salmo trutta* had been recent and not all trout had yet acquired the ability to prey on amphibians, so the lake still had sufficient reserves of individuals to recover the community. The rapid and timely action of eradicating recently introduced trout in Estanho has prevented the disappearance of some amphibian species from the area in the medium term, as has been shown to happen in high mountain lakes where salmonids have been introduced, both in the Pyrenees and in other mountain ranges (Knapp 2005; Miró *et al.*, 2018).

As for the effect on macroinvertebrates, it is difficult to specify with the data available. It can be argued that the pressure exerted by salmonids has been greater for *Odonata, Heteroptera, Trichoptera* and *Diptera* of the *Chironomidae* family, because they are the ones found in trout stomachs, but we cannot confirm these results in the environment. It is possible that a recent introduction of *Salmo trutta* makes these effects still not noticeable, since they mainly prey on amphibians. Over a longer period of time, it would be expected that by completely eradicating amphibians, as happens in all high mountain lakes with fish (Ventura *et al*., 2008), the pressure would pass to the most conspicuous macroinvertebrate groups.

As for crustaceans, there are three species that disappear after the first trout fishing. One could argue from this, that trout exert pressure on larger crustaceans, since by decreasing this pressure these species prevail. However, trying to establish a relationship between changes in the crustacean community and the presence of salmonids is very complicated, since there may be many other influential factors such as food availability or environmental factors. It should be taken into account that some species such as *A. rectangula* and *C. sphaericus* have been reported as not very abundant and difficult to be continuously detected in the environment (Armengol, 1978).

Variability of the *Luronium natans* population

The population of *L. natans* is very conditioned by the morphology of the Estanho lake and the changes in its hydrological balance. The modification of the bed and the continuous growth of the volume of water from the end of the 90s until 2015 caused a change in the living conditions of *L. natans*, making deep water forms prevalent over amphibian forms. The geographical location of Estanho, expressed by altitude and other variables, is not favourable to the growth of this type of forms (Bardin *et al*., 2012). The limitation of the artificial water inlet has caused the level to decrease returning the Estanho to its pond status. The amphibious forms of *L. natans* become dominant again, as when Perdigó (1983) discovered the species. From the conservation point of view, the artificial flow of water must be controlled to ensure the maintenance of the pond and also the passage and permanence of livestock. *Luronium natans* has a large ecological range within the aquatic environment, but it is limited by high concentrations (greater than 20 µg/l) of phosphorus (Bardin *et al*., 2012), and due to its pioneer character, it is only competitive in habitats poor in nutrients (Willby & Eaton, 1993). Currently, Estanho de Vilac is an alkaline alpine pond according to the parameters evaluated (> 200 µeq l^{-1}). Although the concentration of chlorophyll in late summer is low (ca. 2.3 µg l^{-1}) as well as total nitrogen values (TN ca. 526 µg l^{-1}), it has high total phosphorus values (TP ca. 22.7 µg l^{-1}) that place it in the mesotrophic domain (9.3 µg l^{-1} <TP <31 µg l^{-1}).

The change of the environmental conditions promoted by the LIFE+ LimnoPirineus project at Estanho de Vilac and better knowledge of the habitat in which *Luronium natans* develops, leads us to propose the modification of its inclusion within the CORINE Habitat 22.433, as this has oligotrophic bodies of water. According to the characteristics of the water, the permanently flooded population corresponds to Habitat 22.431 (with a particular code, 22.431n) and should be included in HCI 3150. However, the temporarily flooded population should be treated with the perennial amphibious communities of the Eurosiberian and Alpine region (22.31) with their own code that identifies them (22.31n amphibious populations of *Luronium natans*) included in HCI 3130. Similar formations of *Luronium natans* have been described from the Czech Republic as an association (*Luronietum natantis* Szankowski) within the *Littorelletea* class and the *Eleocharition acicularis* alliance (Chytrý, 2011).

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~ Lake and nire system of Trescuro ~

THE TRESCURO MIRE SYSTEM: HOW SHARP ENVIRONMENTAL GRADIENTS AND CLIMATIC EVENTS CONSTRAIN THE LOCAL DISTRIBUTION OF PLANT COMMUNITY TYPES

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RESUM

Mire ecosystems found in Alpine mountains are of great ecological value, since their distribution is very limited by rough physiography and by Mediterranean climatic influence. Understanding how environmental factors together with varying water regime drive the vegetation patterns is a key step for the management and conservation of vulnerable mire systems. Here, we analyse the relationship between these abiotic drivers and the distribution of different plant community types in the Trescuro mire system. We set up 30 sampling points across the vegetation mosaic where pH, electrical conductivity, water table depth and ion concentration were recorded during the 2014 and 2015 summers. The results evidenced that variation in the water table level is the main driver for small-scale vegetation distribution, followed by the ion concentration. Soligenous communities, which are highly dependent on water runoff, were the most affected by drought events, and thus the most sensitive to eventual invasion by trees.

INTRODUCTION

Mires are fairly common ecosystems in Europe, especially in boreal regions which contain more than 60% of the 187,000 km^2 of European peatlands (Jones-Walters & Summary 2010; Raeymaekers 2000). At southern latitudes, these habitats are far less widespread, being chiefly restricted to special topographic Alpine positions. The Pyrenees represent the south-western distribution limit of these ecosystems, facing the Mediterranean region.

Mires have a wide range of ecosystem functions, such as carbon storage, acting as energy and water buffers, providing specific habitats for flora and fauna, etc. (Joosten *et al.* 2017). They host plant species and form habitats that are of great interest (Council Directive 92/43/EEC), especially within the Pyrenees and similar ranges, due to their regional scarcity. The loss and degradation of mires that has occurred over recent decades, mainly due to human activity, has led to a decrease of 70% in the total mire surface in Europe (Raeymaekers 2000), as a result, mires and bogs are the most threatened groups of habitats in the European Union (Janssen *et al.* 2016). All these characteristics make mire conservation and restoration a major priority on European and national agendas.

The shortage of nutrients and extreme hydrological conditions (flooding, water flushing or overflow) in mires act as strong limiting factors for many species (Bridgham *et al.* 1996). For this reason, the biotic communities of these areas are highly specialized and often include a high rate of unique and rare species that deserve high conservation status (Jimenez-Alfaro *et al.* 2014). Moreover, due to this specialization, mire species are very sensitive to environmental changes that may lead to disturbance of the system. Particularly, they are sensitive to oscillations in the water regime associated with climate variations, such as decreasing precipitation and rising temperatures, or due to anthropogenic activity, such as water management by hydroelectric facilities. These disturbances could lead to a loss of mire habitats (Essl *et al.* 2012) in the lower mountain areas and restrict their distribution more to higher alpine areas. Thus, understanding the response of mire vegetation to hydroecological conditions, especially during extreme weather events, is a key step in setting mire management and conservation within a context of global climate change.

In the Pyrenees, most mires are situated in basins that are exploited through hydroelectric power stations, which have directly destroyed some of these ecosystems in the past and are still a threat and a source of artificial disturbance. Nevertheless, the presence of dams and the possibility to ecologically regulating the hydrological regime could provide an opportunity to improve the state of conservation of these habitats.

The mire vegetation communities in southern European massifs have been studied over the last few decades (Aldasoro *et al.* 1996; Bragazza & Gerdol 1999; Carrillo *et al.* 2008; Pérez-Haase *et al.* 2012; Pérez-Haase & Ninot 2017). Nevertheless, we are still far from the level of knowledge of Atlantic and boreal mires, which have been studied in depth and monitored for a long time. The vegetation of Pyrenean mires is mainly dominated by sedge-moss fen communities, in places including small ombrotrophic *Sphagnum* hummocks, and horsetail or tall-herb stands. Due to the physiographic and bioclimatic conditions, their optimal development is in the subalpine zone (Pérez-Haase *et al.* 2012).

Distribution patterns of mire communities along ecological gradients have been studied in different areas around the world (Bridgham *et al.*, 1996; Castelli *et al.* 2000; Sjörs 2002). The main gradients that have been found to determine vegetation are water table depth (Malmer 1986; Sekulová *et al.* 2013; Pérez-Haase & Ninot 2017), richness of nutrients and fertility (Gredol 1995; Okland 1989), and water acidity (Sekulová *et al.* 2013; Wheeler & Proctor 2000). These gradients act together in mires and determine the composition and characteristics of the vegetation found at each point. Apart from isolated cases (Pérez-Haase & Ninot 2017), Pyrenean mires have been poorly studied in terms of the relationship between environmental gradients and the distribution of plant communities. This study focuses on the Trescuro mires, which constitute a unique system, because of both its hydrological structure and the high degree of diversity of rare species and valuable wetland habitats (Carrillo *et al.* 2008). Moreover, the Trescuro lakes and mires are included in an integral reserve within the Aigüestortes i Estany de Sant Maurici National Park, which has helped to keep it remarkably untouched and in a good state of conservation, despite the presence of hydroelectric dams in the basin and also pressures from human activity and cattle grazing (Figure 1).

This paper has two main objectives: (1) to evaluate the main ecological gradients that determine the distribution of different types of vegetation in the Trescuro mires, and (2) to analyse how different vegetation community types respond to variations in environmental conditions, paying special attention to a drought event occurred in summer, 2015.

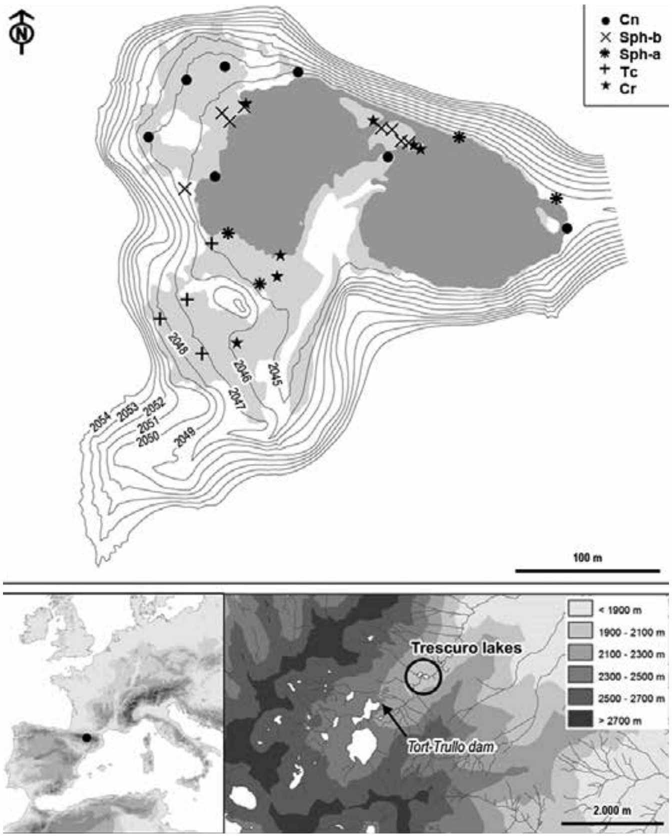
Figure 1. General view of the Trescuro site. The mire system develops as a complex vegetation mosaic on the gentle areas surrounding the two small lakes.

MATERIALS AND METHODS

Study area

The Trescuro lakes and their surrounding mire complex, located in the central Pyrenees, are situated at 2,045 m a.s.l., and experience a high mountain climate The mean annual temperature is 5.3 ºC and the average annual precipitation is 1,150 mm with spring and autumn peaks (SMC 2016).

The lentic complex consists of two small lakes, 5.7 and 5.4 m deep with surface areas of 0.8 and 0.7 ha, and a total volume of 21,139 and 17,709 m^3 located on granitic substrate (Generalitat de Catalunya 2007). The two water bodies are well connected (Figure 2) and the main water inlet comes from the main river in the valley, which flows into the right lake; water is mainly supplied to the left lake through small rivulets. The topographic representation of the area (Figure 2) shows gentle relief surrounding the left lake, which promotes the development of mire communities. In contrast, the margins of the right lake are much steeper, and mostly covered by *Pinus uncinata* woodlands.



▲ **Figure 2.** Location of the study site and sampling points, classified according the vegetation type. In the main figure, dark grey represents lake surface and light grey represents the main mire area. Abbreviations stand for *Carex nigra* fens (Cn), *Trichophorum cespitosum* fens (Tc), *Carex rostrata* beds (Cr), Low *Sphagnum* hummocks (Sph-b) and High *Sphagnum* hummocks (Sph-a).

Although nowadays the area is protected, water flow was artificially altered in the mid-20th century by the construction of hydroelectric dams and several subterranean channels upstream. Hydroelectric regulation is still practiced and thus conditions the fluvial dynamics. As a whole, the lentic system is rich in plant biodiversity and habitats. Carrillo et al. (2008) reported up to 10 distinct CORINE Biotopes. Some rare and remarkable plant communities are found in the transition mires (*Sphagnum* quaking mires with *Carex rostrata* along the lake margins), bog woodland of mountain pine (*Pinus uncinata*), and the active raised bogs with *Sphagnum* and *Ericaceae*. The latter two units are classified as priority habitats of community interest (HCI) by the EC legislation (Council Directive 92/43/EEC). As a whole, it could be classified as a mixed mire, with both calcicolous and acidophilic vegetation. Regarding the hydrological conditions, a great part of the mire is situated on a gentle slope with soligenous conditions created by the several streams and the surface water circulation. But in the lower areas there are numerous *Sphagnum* ombrotrophic bogs, some of them raised more than 1m above the surface. The extension and density of the patches of bog woodland has changed over time, partly due to human activity. During the last decades, the density of the mountain pine population in the mire system has been clearly increasing in detriment of other mire units, which may be envisaged as a thread for mire conservation. The landscape surrounding the lentic system is dominated by forests of mountain pine (*Pinus uncinata*) with *Rhododendron ferrugineum*. Higher on the slopes of the basin, the forest gives way to a landscape of *Festuca eskia* and *Nardus stricta* grasslands and rocky areas (Carrillo *et al.* 2008).

Vegetation, groundwater and soil sampling

We first identified the main hydrological regimes of the mire and the main physiognomic vegetation types in it. We set up 30 sampling points spread across the study area, which covered all the hydrological conditions and all physiognomic vegetation types with at least three points each. At each sampling point, we installed a longitudinally perforated PVC pipe sunk vertically 1 m into the mire, in order to monitor the water table depth and to collect samples of groundwater for subsequent chemical analysis.

Around each PVC pipe, we carried out a vegetation relevé in an area of 2.5 × 2.5 m, namely an exhaustive floristic record of vascular plants and bryophytes. Each species was assigned a value on the Domin scale ranging from 1 to 10, according to its projected cover.

In the summer of 2014 we collected water from each pipe in order to measure pH and electrical conductivity by means of a portable instrument, and to perform further chemical analyses in the laboratory. After filtering these water samples (pore diameter, 20 µm), the concentrations of calcium, silicon, sodium, iron, aluminium, sulphur, zinc, copper, potassium, phosphorus, magnesium and manganese were analysed using inductively coupled plasma mass spectrometry (ICP-MS).

Water table depth (hereafter, WTD) was measured fortnightly during the 2014 and 2015 growing seasons (from 18th June to 9th October 2014, and from 2nd June to 30th October 2015). In parallel, we assessed the water level of the lakes by means of an automatic data logger installed in each lake.

In addition, we dug out a soil core by means of a steel bore at each sampling point for the purpose of characterize the upper soil layers (~20 cm) in terms of peat depth and other characteristics.

Data analysis

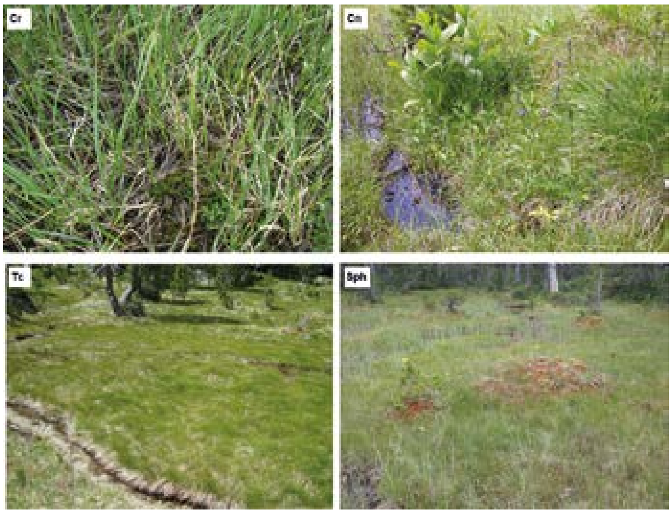
In order to classify the vegetation relevés numerically, we computed a distance matrix of the species data and finally produced a dendrogram to evidence the main vegetation groups or community types (for further details, see Colomer *et al.* 2019).

At the same time, we analysed the relationship between the environmental variables and the distribution of the vegetation through a canonical correspondence analysis (CCA), using the software CANOCO (Lepš & Smilauer 2003). The significant explanatory variables in the model (p-value<0.05 in a Monte Carlo permutation test) were selected by means of a stepwise selection procedure. The metric coordinates of each point were analysed as covariables in order to factor out the effect of spatial autocorrelation.

Before the analysis was run, we corrected the data of electrical conductivity when the pH was below 5, to eliminate the measured acidity corresponding to proton conductivity, following Sjörs (1950), since corrected conductivity data correlate better with vegetation distribution. Also, we calculated Pearson correlation coefficients between the explanatory variables. When the correlation between two variables was above 0.75, we discarded one of them.

Finally, the ecological data from different vegetation community types were compared using ANOVA for the normally distributed variables. The other variables were log-transformed, and those that still presented a non-normal distribution after the transformation were analysed with the Kruskal-Wallis test. ANOVA and Kruskal-Wallis analyses were performed with R (R Core Team 2013), using the package vegan (Oksanen *et al.* 2015).

The numerical classification of the vegetation samples yielded five vegetation types (Figure 3). The first one, *Carex nigra* fens, includes vegetation plots dominated by *Carex nigra* and a number of species that are characteristic of alkaline fens such as *Carex davalliana* or *Swertia perennis*. The *Trichophorum cespitosum* fens were mostly soligenous fens situated on gentle slopes with a constant surface or sub-surface water flow. The *Carex rostrata* beds were species-poor communities situated along the lake margins and frequently remained covered by the lake water. Low *Sphagnum* hummocks were only slightly raised above the surrounding fens. They were mainly formed by *Sphagnum* species, mainly *S. magellanicum*, *S. russowii* and *S. teres*, and included both some hygrophilous plants like *Viola palustris* and *Carex rostrata,* together with the most abundant *Calluna vulgaris* and *Potentilla erecta*. High *Sphagnum* hummocks included higher bogs, which resulted into a deeper water table position with respect to the hummock top. They were characterized by species that are unable to thrive in flooded substrata, like *Vaccinium myrtillus, Rhododendron ferrugineum* or *Pinus uncinata,* and exhibited high cover percentages of *Sphagnum capillifolium*.



▲ **Figure 3.** Examples of the vegetation types evidenced: *Carex rostrata* bed with *Sphagnum* (Cr), *Carex nigra* fen (Cn), *Trichophorum cespitosum* fen (Tc), and *Sphagnum* hummocks (Sph).

RESULTS

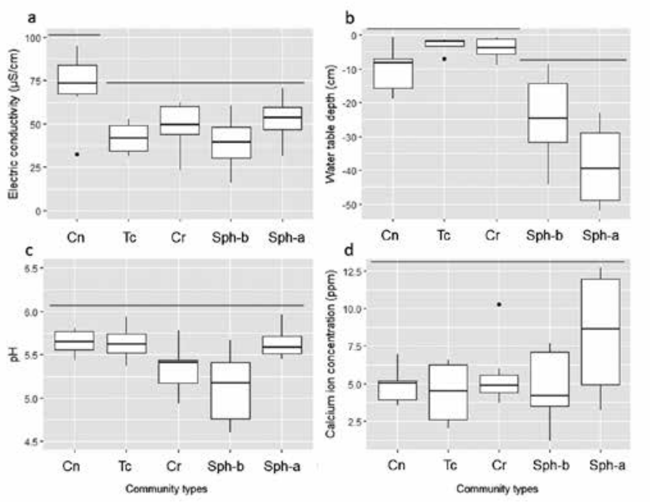
Vegetation

From the 30 sampling points, 108 species were recorded, of which 62 were vascular plants and 46 were bryophytes. Eight species were found in more than 50% of the 30 sampling points, although the cover of these species was highly variable. Only four species had a mean cover of more than 10%: two Cyperaceae (*Carex rostrata* and *Trichophorum cespitosum*), one Rosaceae (*Potentilla erecta*) and one sphagnum moss (*Sphagnum capillifolium*).

As for soil types, all sampling points had a hystic horizon of variable thickness between plant communities, situated above a sandy layer including coarse grains. Low and High Sphagnum hummocks were always peaty (i.e. hystic horizon or peat ≥30 cm), whereas other vegetation types were not necessarily peaty. Alkaline and soligenous fens showed the lowest mean peat thickness (33 and 41 cm, respectively). In relation to soil saturation, for all vegetation types the mean minimum WTD was higher than the mean peat depth.

Relationship between vegetation and environmental variables

The main environmental variables showed interesting trends within the vegetation studied, although clear differences between plant community-types were scarce (Figure 4). The electrical conductivity values in *Carex nigra* fens were significantly higher than in the rest of the communities (ANOVA; F=4.12; p=0.011) (Figure 4a). There were no significant differences in pH found between the vegetation types (ANOVA; F=2.75; p=0.108) (Figure 4b).

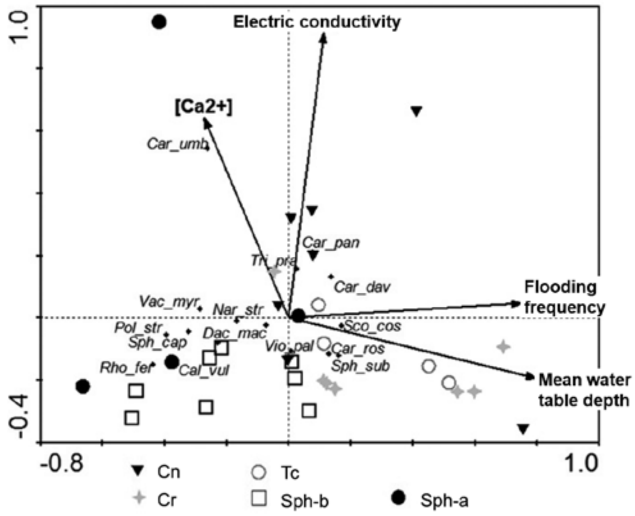


▲ **Figure 4.** Box plots of the main ecological variables: electric conductivity (a), pH (b), water table depth (c) and Ca$^+$ concentration (d) (measured from the 1 m deep PVC pipes). The plant community types are identified as in Fig. 2.

The mean WTD in the two *Sphagnum* communities (Low-Sph and High Sph) was significantly deeper than in the other communities (Kruskal-Wallis; K=2.02; p= 0.002), the High *Sphagnum* hummocks experiencing the lowest levels, which averaged 40 cm under the surface (Figure 4c).

Calcium (Ca^{2+}) was the most abundant ion; the mean value for the whole system was 5.56 ppm, with non significant differences between community types (ANOVA; F=3.54; p= 0.124) (Figure 4d). In most cases, copper, zinc and phosphorus had values below the detection threshold of the method used (0.2 ppm for P and 0.01 ppm for Cu and Zn), and thus their concentrations were not included in the statistical analysis.

The main environmental gradients describing the vegetation variability in the mire were evidenced through a step-wise CCA (Figure 5). The first two axes accounted for 17.2% of the total variance (10.0% on the first axis and 7.2% on the second axis). In the figure, the sampling points appeared mostly spread along the first axis of variation. This axis is strongly related to water table variables, such as mean WTD level and total number of flooding days, whereas the second axis is related to water mineral content and nutrient richness variables, such as electric conductivity and calcium concentration.



▲ **Figure 5.** Canonical correspondence analysis (CCA) ordination showing the sampling points and the most frequent species, with respect to the significant variables. The plant community types (symbols) are identified on the legend as in Fig. 2. The 15 species with best fit to the ordination are shown through their abbreviated names, and correspond to (in alphabetical order): *Calluna vulgaris, Carex davalliana, Carex panicea, Carex rostrata, Carex umbrosa* ssp. *huetiana, Dactylorhiza maculata, Nardus stricta, Polytrichum strictum, Rhododendron ferrugineum, Scorpidium cossonii, Sphagnum capillifolium, Sphagnum subsecundum, Trifolium pratense, Vaccinium myrtillus,* and *Viola palustris.*

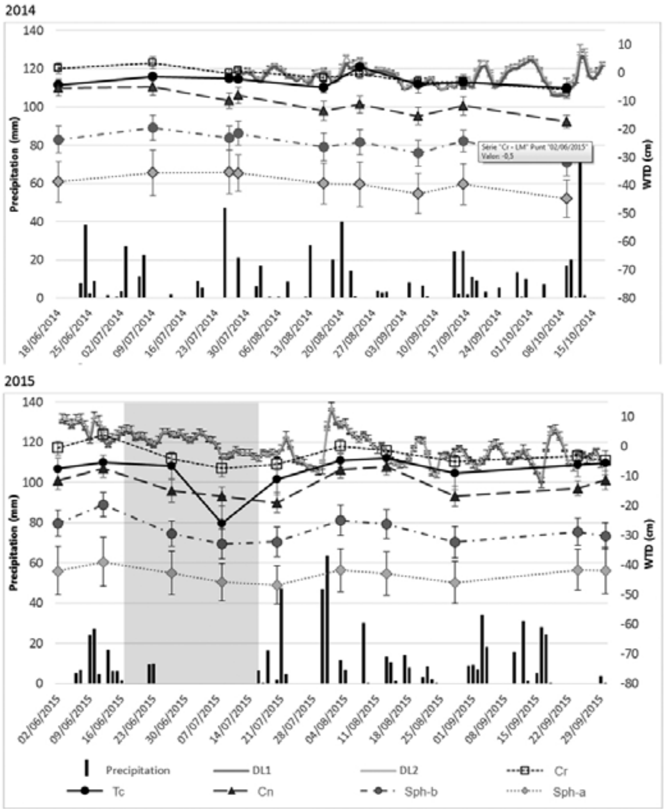
Hydrological regime

Water table depth –the most influencing variable on vegetation distribution– oscillated from −44 cm in the highest areas and in *Sphagnum* hummocks, to positive values (12 cm), which indicate flooding, along the lake and rivulet margins. Areas with a deeper mean WTD often corresponded to raised bogs produced by peat accumulation, and therefore do not necessarily correspond to marginal areas of the mire system.

There was a slightly lower mean WTD in 2015 than in 2014 at all the points sampled, although the differences were not significant (Kruskal-Wallis; K=3.02; p= 0.082) and the water level of the lakes (recorded by automatic data loggers) were also higher in 2014.

System response to precipitation

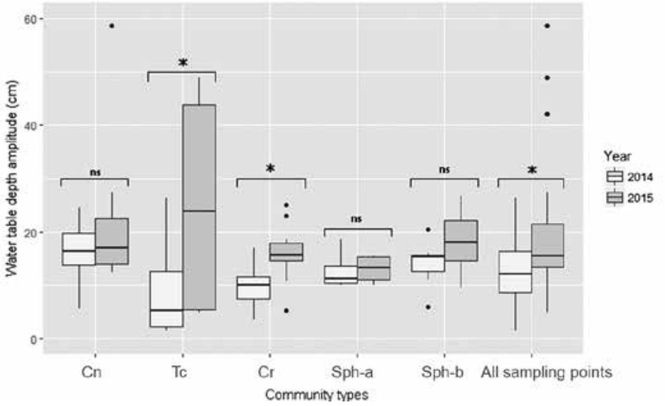
In order to determine the response of each type of vegetation to changes in water intake, we compared the oscillations of WTD during the growing period to precipitation data recorded at the closest weather station (two km away and about 500 m higher elevation) (Figure 6). During the 122 days of the 2014 study period there were rain events on 51 days, with a total precipitation of 602.4 mm. During the 121 days of the 2015 period there were 46 rainy days, with a total of 594.5 mm. In spite of the similarity of these raw data from one year to the next, the summer of 2015 included a noticeable dry period: between mid-June and mid-July, there were only two rain events, totalling just 19.1 mm. This drought episode, highlighted in grey in Figure 6, provided a great opportunity to evaluate the response of the different community types to extreme events.



▲ **Figure 6.** Water table depth variation in the communities studied (identified as in Fig. 2) and variation in the water surface level of the two lakes (DL1, left lake; DL2, right lake); and precipitation registered during the study period, in 2014 and 2015. The grey shading corresponds to the 2015 drought period.

During 2014, mean WTD was significantly different for all the vegetation groups (Kruskal-Wallis; K = 423.38; p = 0.00) except between the *Carex rostrata* beds and the *Trichophorum cespitosum* fens; while, in 2015, all the vegetation groups were significantly different in this aspect (Kruskal-Wallis; K = 220.46, p = 0.01). No significant differences were found between the two years of study within community types (Kruskal-Wallis; K = 312.31, p = 0.081). Nevertheless, the amplitude of WTD was significantly higher in 2015 that in 2014, indicating higher variability during 2015, when the drought period occurred (Figure 7). During the drought period, the community type that suffered the largest decrease in WTD was the *Trichophorum cespitosum* fen, which dropped from −6.5 cm to −25.9 cm (Figure 6).

Regarding the lake water level oscillation, a small delay in the response to the precipitation events of the left lake was detected, since it took longer there than in the right lake for the level to increase after a precipitation episode. In 2014, the amplitude of water level registered was of 13.8 cm in the left lake and 16.0 cm in the right lake, while in 2015 the amplitudes were 25.0 and 25.1 cm respectively.



▲ **Figure 7.** Amplitude of the water table depth in the community types sampled for 2014 and 2015. The plant community types are identified as in Fig. 2. Asterisks denote significantly different amplitudes between both years ($P < 0.05$) and *ns* not significant differences.

DISCUSSION

Vegetation and ecological gradients

The classification of the mire vegetation into five community types is consistent with the previous description of the vegetation in the area using the phytosociological method (Carrillo & Ninot 1992). Both classifications may be understood as the plant response to environmental gradients, since they are the result of the contrasting species composition found in the mire vegetation mosaic.

The WTD (mean WTD and number of flooding days) was the main ecological gradient explaining the variance in the vegetation. Similar results in previous studies (Bragazza *et al.* 2005; Castelli *et al.* 2000; McIlroy & Allen-Diaz 2012) also support the idea that, at detailed scales, mean WTD and WTD oscillation is the most important ecological gradient that determines vegetation composition and distribution, especially in high mountain mires (Pérez-Haase & Ninot 2017; Raulings *et al.* 2010).

As a secondary driver, water mineral content (evaluated through ion concentration) represented a relevant gradient, as in other alpine mires (Gredol 1995). The groundwater pH values gave also relevant differences between the community types. However, the pH gradient was not included as a determinant ecological gradient in the stepwise selection of variables, probably because its effects on vegetation could be masked by those of the mineral content.

The relevance of WTD and the water mineral content in the Trescuro system is in accordance with that found in other studies at local scales in mountain areas, while in regional-scale studies, climate variables and pH gradients were the most relevant drivers (Okland 1989; Sekulová *et al.* 2013).

Carex rostrata beds, situated along the lake margins, were characterized by their thriving at the highest water table level, with some days of flooding. *Trichophorum cespitosum* and *Carex nigra* fens presented much more variable WTD, depending on the location of each plot in relation to the streams and lake margins. Regarding both low and high *Sphagnum* hummocks, they have a permanent low water level as they are raised up from the rest of the mire. The values of WTD and pH, and the area covered by the communities, leads them to be included in 'bog hummocks' and 'fen carpets' respectively, following the terminology proposed in Wheeler & Proctor (2000) and Okland *et al.* (2001). Nevertheless, the great variability in the concentrations of calcium found in the high hummocks does not fit with either of these categories. This variability is most probably because the water in the surrounding fens influences the bottom parts of the hummocks, while the higher parts are almost exclusively influenced by rainwater. Therefore, hummocks have transitional conditions between minerotrophic and ombrotrophic conditions (Rydin & John 2006). Pyrenean *Sphagnum* bogs represent the southern-most location of this community type, so they are on the verge of extinction from a geographical perspective (Harrison *et al.* 2006; Joosten *et al.* 2017). The high dependence of these bogs on precipitation contributions means that the decrease in precipitation southwards in Europe could become a relevant threat for these valuable habitats (Hedwall *et al.* 2017; Strack 2008).

System response to water input variations

Over the last 50 years, there has been a rising frequency of periods of drought during the summer months. The summer temperature has risen 0.4°C per decade and precipitation has decreased by around 28 mm per decade (OPCC 2014; Serra *et al.* 2006). Moreover, these trends are very likely to increase throughout central Europe and the Mediterranean area in the coming decades (IPPC 2013).

Mires are highly sensitive to these climate variations, chiefly to the negative impact that summer drought periods have on the hydrological regime (Fenner & Freeman 2011; Romanowicz *et al.* 1993) and subsequently on vegetation depending on a shallow water table and surface runoff (Breeuwer *et al.* 2009). Moreover, when the WTD falls below the tipping point of -24 cm the peatland respiration increased significantly through increased fungal activity (Jassey *et al.* 2018). Nevertheless, the response of the different community types in a mire system to drought should vary according to the particular resilience of the plant species and the vegetation types (Breeuwer *et al.* 2009; Harrison *et al.* 2006).

The drought period that occurred in the summer of 2015, consisting of 22 days with no precipitation and with very low levels of atmospheric humidity, was a great opportunity to analyse the response of mire vegetation to such disturbance. Moreover, the average monthly temperature was 3.5°C higher than a previous 30-year average July temperature (period 1961 to 1990) (SMC 2015). This case study is very significant, since drought events represent a real threat to Pyrenean mires.

According to the hydrological response recorded at the 30 sampling points in the Trescuro mire, distinct plant community types responded in a similar way to the regular water intake and outlet. However, they showed noticeable differences after the extreme drought considered. *Carex rostrata* beds in the lake margins were highly correlated with lake water level oscillations, and thus WTD dropped a mean of 5 cm during the drought event.

Carex nigra fens, situated near streams or even near the lakeshore, showed greater water buffering capacity and resistance to drought, since they maintained a relatively constant WTD through the 2014 and 2015 summers. This may be partly due to the higher water retention capacity of these fen soils, which are finer-textured and peaty.

The *Sphagnum* hummocks, especially the highest ones, have a transition of hydrological characteristics from minerotrophic conditions in the lower parts to ombrotrophy at the top. This structure leads to precipitation water being retained (van Breemen, 1995) and taking more time to reach the underground water. Therefore, the water level depth remained locally more stable, with the lowest amplitude values even during 2015, when the drought event occurred (Figure 6).

Meanwhile, the *Trichophorum cespitosum* soligenous fens are highly related to flowing surface water, which is highly dependent on the precipitation regime. In the Trescuro system, they are mostly situated in the peripheral area of the mire, which is the most sensitive area to drought episodes. During the first days of the 2015 drought period, mean WTD of this community maintained similar levels as when it was receiving runoff water. Nevertheless, when a critical drought threshold was reached, the surface intake stopped and WTD dropped more than 20 cm on average. Some of the pipes became completely dry, indicating that the water level was below 100 cm. These characteristics make this community type the most sensitive to summer drought episodes.

On peaty soils, capillary fringe keeps soil water-saturated above the WTD (Wheeler 1999). This keeps the anoxic (or hypoxic) conditions in the main rooting zone and consequently it may have a determinant role on vegetation. However, in the most affected *Trichophorum* soligenous fen plots, capillary fringe is unlikely to influence the rooting zone since WTD was too deep. Therefore, ecological processes related to water table drawdown, such as peat depletion, would affect the areas showing extreme low values of WTD.

Moreover, sharp water table drawdown may lead to a decrease in stress by waterlogging on roots, which could be especially relevant to plants not adapted to saturated soils. This could potentially lead to colonization by plant species not adapted to waterlogging, such as ericaceous shrubs and other woody plants (Belyea & Baird 2006; Breeuwer *et al.* 2009; Dise 2009). Therefore, a higher frequency of drought periods will lead to greater soil oxygenation, thereby weakening fen species and allowing the settlement of woody species, such as *Pinus uncinata* and *Ericaceae* (Heijmans *et al.* 2013). Since the resulting forested peatland has higher evapotranspiration than open mires, positive feedback may enhance further encroachment by woody plants, and thus a reduction in the size of the mire (Hedwall *et al.* 2017; Holmgren *et al.* 2015). This could be operating in the Trescuro system, where over the last decades the tree cover has increased from 20% to almost 35% (Figure 8). In summary, our data indicate that mires are resilient to environmental changes up to a certain level of disturbance, but they may shift to new states when a critical point is reached, as described by Heijmans *et al.* (2013).

Although all the Trescuro mire system is interconnected, there were site-specific responses within the system following extreme events. Since the main river enters exclusively into the right lake, increases in flow occurred after strong rainfall events (Figure 6) or following generalized snow melt at the beginning of summer produces rapid rises in its water level. The effects of flash floods in the main river are far less noticeable on the left lake, which is mostly fed by small surface streams (Figure 2) and thus more influenced by current precipitation regime. This suggests that the mires related to the right lake would be more sensitive to significant variations in the river flow, whether caused by a downpour or by variations in the dam water management, whereas those situated in peripheral areas around the left lake would be more affected by drought events.

CONCLUSIONS

The hydrological regime, specifically variations in WTD, is the most significant ecological gradient for the mire vegetation distribution at the local scale. The comparative dynamics of the water level in the lakes and in the mire units presented here contribute to a better understanding of the functioning of alpine mires. The response to extreme events depends on the vegetation community types, but also on the situation of each community within fine scale mosaic of the mire, that suggests that the mire system partially resists extreme events such as those measured within this study.

▲ **Figure 8.** The Trescuro system includes small patches of bog woodland, which during the last decades densify and expand through the encroachment of juveniles of *Pinus uncinata* on *Sphagnum* hummocks.

Nevertheless, if the frequency of such events increases, as climate models predict, Pyrenean mires are likely to suffer severe consequences, and even end up disappearing. In Trescuro, we found that soligenous communities depending on flowing surface water, often situated at the edges of the mire, are the most sensitive units to drought events. Such events may enhance invasion by trees and subsequently the reduction of the mire area. Therefore, detailed monitoring of fens depending on runoff waters would be very valuable as an assessment of drought events effects on mires in southern Alpine ranges.

This case study is a science-based contribution to good approaches to mire management and conservation strategies in a context of global climate change. This is particularly important in south European mountain landscapes, where mountain mires are at their biogeographical limit, and subject to very restrictive conditions.

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~ Lake and nire system of Trescuro ~

~ Sphagnum mires associated to a small water course, in the locality of Liat ~

HABITATS OF COMMUNITY INTEREST IN THE MIRES OF AIGÜESTORTES AND ALT PALLARS: DISTRIBUTION, IMPACTS AND THREATS

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ABSTRACT

Here, we present the main results of a study of the wetland habitats found at the Sites of Community Interest (SCIs) Aigüestortes and Alt Pallars, aimed to create a solid basis for conservative management. This study includes three levels of approach: a general cartography of the wetland landscape units, larger than 1500 m^2; an extensive sampling of Habitats of Community Interest (HCIs) located among 15 main valleys distributed through the two SCIs; and an intensive sampling of 13 mire systems relevant in extent and complexity. Mostly from the results of the first two levels of approach, the mire HCIs are characterised in terms of geographic and ecological distribution, singularity and naturalistic interest, and affectations derived from anthropic activities.

The set of mires studied includes eight HCIs (as well as other water-related habitats) some of which are restricted to very few locations, hosting 19 plant species that are found in different threat categories or that are of high biogeographic and ecological interest. Among them, there are some species of *Carex* or *Sphagnum* found in very few Pyrenean locations. It should be noted that both rare HCIs and specialist species are often very scatered over the complex mosaic of Pyrenean ecosystems, which is a challenge to conservative management. In general, the conservation status of mires in these protected areas is good, although grazing represents a threat in some places where livestock are often concentrated. Frequenting by hikers and hydroelectric uses also cause damage to the mires, albeit in fewer locations.

INTRODUCTION

High mountain wetlands, also extensively called mires, are ecosystems of particular conditions because the soil is waterlogged at least during most of the vegetative period. This supposes significant limitations for the plants and other organisms that live in the soil, which is in a state of hypoxia or anoxia. The vascular plants of mires are almost always perennial grasses, especially rhizome-forming *Cyperaceae* and more or less dense turf, and are ecological specialists of quite restricted habitats. The plant communities of mires usually include bryophytes, which are not adversely affected by the fact that the soil is waterlogged. In fact, this may favour them over vascular plants, so that different species of mosses can take a leading role in mires (Vigo, 2009; Vitt, 2006).

Another common fact in mires is the low concentration of mineral nutrients present in their waters, which are in fact a somewhere between moderately and extremely oligotrophic and acid, a situation that translates into plant growth rates between moderate and low. Although primary production is relatively low, the poor oxygenation of the soil causes plant remains that are incorporated into the soil (roots and fallen parts) and other organic remains decompose very slowly, and therefore organic matter is accumulated in the form of peat. When this peat forms a layer of more than 30-40 cm thick, the ecosystem is correctly referred to as a mire (Rydin & Jeglum, 2006).

Within the mires, the small variations that occur in the flood regime, in the chemical properties of the water, or derived from the vegetation's own dynamics favour the coexistence of quite different environments, formed by sometimes contrasting plant communities (Pérez-Haase & Ninot, 2017). Thus, the areas dominated by phreatic water, or water that has generally passed through the soil, carry minerotrophic plant communities, since these waters contain substantial amounts of dissolved minerals. They are usually flat or low-slope areas, which lead to plant communities dominated by *Cyperaceae*. On other

▲ **Figure 1.** Most Pyrenean mires appear in the form of small and irregular surfaces, generally following small water courses and associated flat areas, as at Estanyeres.

occasions, however, mires are fed directly by rainwater, thus called ombrogenous mires. There, the mosses of the *Sphagnum* genus take centre stage and, with their vertical growth, form bulging plant communities, which are always above the water table. These are highly oligotrophic communities, almost always with a good peat thickness, and that often experience certain desiccation in the upper parts, where plants less adapted to waterlogged soil take root (Bragazza & Gerdol, 1999; Rydin & Jeglum, 2006).

In the Alpine mountains, mires are mostly restricted to the high mountain levels. In the Pyrenees, they appear at altitudes between about 1000 m and 2500 m, and so they are more frequent and extensive in the subalpine area. Both from the point of view of their biological content and their function, these high mountain ecosystems correspond to mires that occupy large areas in many lower altitude landscapes of temperate and cold biomes (Casanovas, 1991; Bragazza & Gerdol, 1999; Damman *et al*., 1987). But in the Alpine mountains, because the physiography is very complex and abrupt, these ecosystems occupy much smaller areas, linked to the landscape units where the groundwater remains close to the ground surface, such as lake shores, former glacial beds, or the edges of rivulets or springs (Figure 1). This limitation in extension is particularly evident in the Pyrenees, given their border position between the mid-European and the Mediterranean regions. Thus, if mires can still be quite large in much of the Alps, in the Pyrenees they almost always occupy smaller areas, often just a few square metres (Ninot *et al*., 2017).

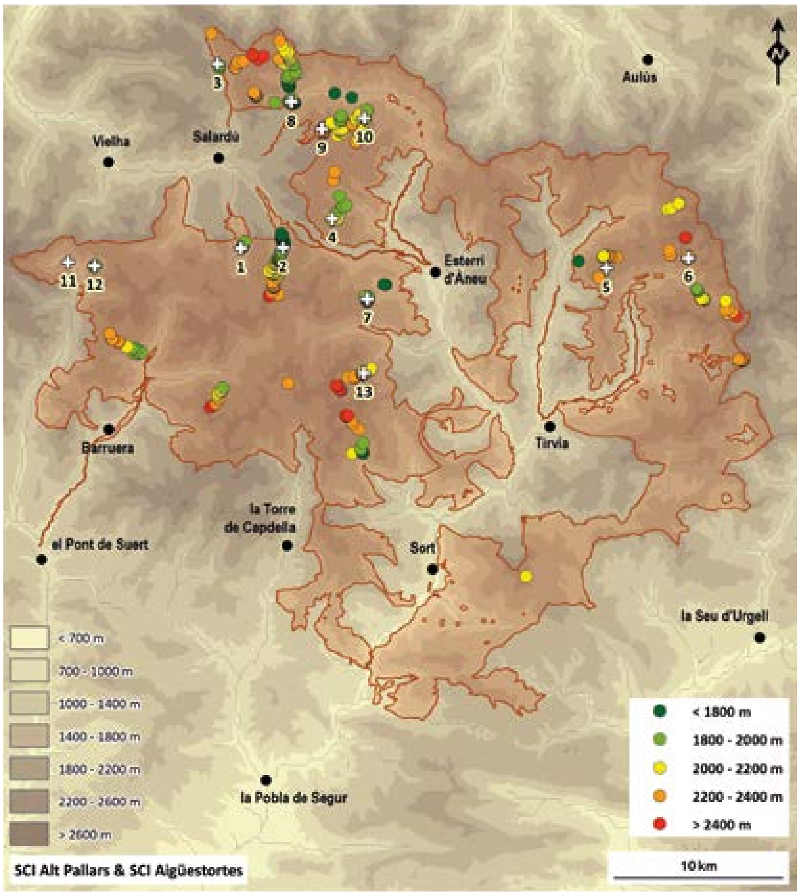
This reduction in the area that mires occupy in most of the Pyrenees, does not prevent the plant communities and other associated organisms from being completely comparable to those found in other Alpine landscapes and in more northern latitudes, with which they share a large part of the species (Jiménez-Alfaro *et al*., 2012). In this context, it is understood that mire ecosystems are susceptible to protection in the Alpine mountains, since a good part of the specialist species are found there in the form of very scattered populations, often in their geographical distribution limit towards the Mediterranean region. Mires also stand out for the important role they have as regulators of the water cycle in the mountains and as an organic carbon store.

Most mountain mires lay within protected natural areas. However, it should be considered that, even in protected areas, these unique ecosystems are subject to particular pressures and threats, such as hydroelectric exploitation (Catalan, 1997) which modifies surface watercourses and, therefore, ecological conditions of mires; grazing by domestic livestock, which is often particularly concentrated in mires; visitor frequenting, as mountain trails often run along streams and lakes; and also climate change, which manifests itself with increasingly frequent spring and summer droughts.

This is why, within the LIFE+ LimnoPirineus project, we approach a diagnostic study of the most unique mire habitats found at the Sites of Community Interest (hereinafter, SCIs) Aigüestortes and Alt Pallars, aimed to create a solid basis for conservative management..

OBJECTIVES

The purpose of this work is to prepare a diagnosis of the Habitats of Community Interest (hereinafter, HCIs) of mires found in the Aigüestortes and Alt Pallars SCIs, from the points of view of their geographical and ecological distribution, their naturalistic and heritage interest, the anthropic alterations that affect them, and the threats to which they are subjected. The corresponding study is based on a large sampling conducted at different levels, which includes a general mapping of mire ecosystems; extensive sampling of mire HCIs across different valleys; and intensive sampling of these HCIs in a dozen particularly complex locations in terms of hydrography and the mosaic of mires hosted.
Given the volume and complexity of the data obtained, in this paper we present the most global results of this study, and mainly those derived from general mapping and extensive sampling of HCIs, while only partially using those of the intensive study.



▾ **Figure 2.** Map of the study area (coloured), corresponding to the Aigüestortes and Alt Pallars SCIs. The points of the extensive study are represented, where HCIs have been georeferenced, in different colours according to altitude category; and the complex systems where intensive study has been conducted: 1, Bassa Nera-Era Planhòla; 2, Ruda-Locampo-Saboredo; 3, Plan de Tor; 4, Clots de Rialba; 5, Ribera de Boldís; 6, Plans de Sotllo; 7, Estanyeres; 8, Cabana de Parros; 9, Portilhon de Marimanha; 10, Ras de Bonabé; 11, Pleta de Molières; 12, Vall de Conangles; 13, Trescuro.

MATERIAL AND METHODS

Study Area

The study has been carried out at the Aigüestortes (ES0000022) and Alt Pallars (ES5130003) SCIs, both located in the northwest of Catalonia (Figure 2). The Aigüestortes SCI occupies an area of 56,033 ha, where the high mountain is very well represented; the altitude varies between 1200 and 3023 m. As for geological materials, there are mostly granite (which forms the core of the SCI and its most emblematic landscape) and slate, with limestone materials appearing in the eastern sector. The Alt Pallars SCI occupies a larger area, with a total of 77,112 ha, and extends from approximately 600 m to 3143 m in altitude. Shale and schist predominate in the high mountains, but other types of substrates (granite, limestone) are also present. Both natural areas, at high levels, have landscapes moulded by ancient glaciers, where tarn lakes and morainal deposits are common. These forms promote the appearance of wet areas, either directly by flooding or by the presence of springs.

General cartography of mires

In order to locate the main sampling areas, throughout 2014 and 2015 we developed an extensive mapping of the high mountain wetlands of the two SCIs, which includes the mire systems of over approximately 1,500 m2. This mapping was done manually by interpretation of detailed orthoimages (scales 1:2,500 and 1:5,000), both in colour and infrared (ICGC, 2019; http://www.icc.cat/vissir3/index.html? 4v6vMyiv5). According to the slope and shape of the wetland, we assigned them to the category “Valley” when located in a valley floor (and it was noted if it coincided with a lake system), or “Spring” when it appeared in a spring or following a rivulet. This mapping is approximate and has not been subsequently validated in the field.

Extensive sampling of Habitats of Community Interest

To carry out this sampling, we selected 15 main valleys (Figure 2) based on the following criteria: 1, Proximity to mires mapped at scale 1:50,000 (Cartography of the Habitats of Catalonia; GEOVEG, 2019) and with the mapping prepared by us; 2, Presence of calcareous substrates (scarcer than siliceous ones); 3, Areas under Atlantic influence.

We started field sampling in 2014 and finished in 2019. During the field campaigns, all in summer to ensure the correct development of the vegetation, the mires in the 15 valleys were visited, and the GPS coordinates were recorded (accurate to within 10 m) for all the mire habitats that could be attributed to an HCI. At each point or coordinate, the following was noted: a) CORINE Habitat, b) HCI Code, c) Disturbances, d) Threats, and e) Endangered or endemic species.

The HCIs considered are the following (Carreras et al., 2015):

- 3160 Natural dystrophic lakes and ponds
- 4020* Temperate Atlantic wet heaths with *Erica ciliaris* and *Erica tetralix*
- 7110* Active raised bogs
- 7140 Transition mires (quaking bogs)
- 7220* Petrifying springs with tuf formation (*Cratoneurion*)
- 7230 Alkaline minerotrophic fens
- 7240* Alpine pioneer formations of *Caricion maritimae*
- 91D0* Bog woodland

The disturbances assessed correspond to those caused by large herbivores, mostly due to domestic livestock. We divided them into disturbance by trampling and disturbance by herbivory, and we assessed their intensity using a four-unit scale.

In the case of herbivory, the meaning of the scale is: 0, no herbivory; 1, low herbivory, presence of mild signs of herbivory; 2. medium herbivory, i.e. between 10 and 50% of plants showing signs of herbivory; and 3, high herbivory, i.e. more than 50% of the plants showing signs of herbivory at the time of the visit.

In the case of trampling, the meaning of the scale is: 0, no trampling; 1, low trampling, which causes soil compaction; 2, medium trampling, with compaction and onset of soil disorganization, with visible livestock tracks; 3, high trampling, with visible bare soil as a result pugging damage.

The threats considered were: 1, Bovine, equine and ovine grazing; 2, Hiking trails (tourism); 3, Presence of forest tracks; 4, Water catchments; 5, Spontaneous afforestation; 6, Erosion. More specifically, the simple presence of herds and tourists in the mire was considered a sign of potential threat, although it did not necessarily cause conservation problems at the time of sampling. We considered that the presence of trees (very often young) near the GPS point (within approximately 10 m) was a potential threat.

We also recorded the presence of species of interest, both vascular plants and bryophytes. Specifically, we considered the presence of species included in the Catalan government Order of threatened flora (Catalonia, 2008, 2015) or the "Red Book" of Sáez *et al.* (2010), and others because of their biogeographical or ecological interest, according to the authors criterium.

In order to know in more detail the ecological distribution of the mires, we characterised them considering the variables: 1, altitude (highly correlated with the average annual temperature); 2, geological substrate, from a simplified version of the "Map of lithological classes of forest importance" (Conesa *et al.*, 2010); 3, slope; and 4, continentality index, which is the average of the highest of the warmest month (July) minus the average of the minimum of the coldest month (January).

Intensive sampling of complex mire systems

For this sampling we chose thirteen locations where mires form particularly complex systems, in order to analyse the ecological conditions of these ecosystems more completely and accurately (Figure 2). These are systems that stand out due to their expansion and for the fact that they include an interesting diversity of mire habitats, which respond to complex environmental gradients. For system one we prepared detailed mapping of habitats, based on areas and points. This mapping was done using orthophotography from 2017, where field annotations were recorded, both of habitat identity and georeferencing GPS. Finally, the map of each mire system was transferred to digital format, working on screen, with the help of other photography formats (infrared orthophotography, field photographs) and field annotations.

From each habitat we chose a minimum of three sampling points, where we made two relevés of vegetation (vascular plants and bryophytes) of an area of 2.5 × 2.5 m, with an assessment of its coverage according to the 10 categories of the Domin scale. As environmental data associated with each relevé, in addition to physiographic and physiognomic descriptors, we extracted a soil sample (with half-round steel probe) to measure the underlying peat thickness and the depth of the water table. We also measured the pH and conductivity of the squeezed water from the upper part of the soil with a multiparameter field probe. Both from the edaphic water and the ground we preserved two fresh samples, which were later frozen for subsequent analyses.

RESULTS

Typology and Habitats of Community Interest

Through photointerpretation, we identified 921 mire systems larger than 1,500 m^2, which together occupy some 780 ha. However, each mapped mire system includes a significant portion of wet habitats, in addition to those that are strictly mires, with which they are dynamically related (such as the hygrophilous meadows of *Nardus stricta*). According to our estimates, the mire habitats occupy about 342 ha if we apply a correction based on the intensively visited locations (see below, Spatial complexity of the mires). Considering the area studied, the density of mires is approximately 18 m^2 per hectare of territory, i.e. 0.18% of the territory is covered by mires.

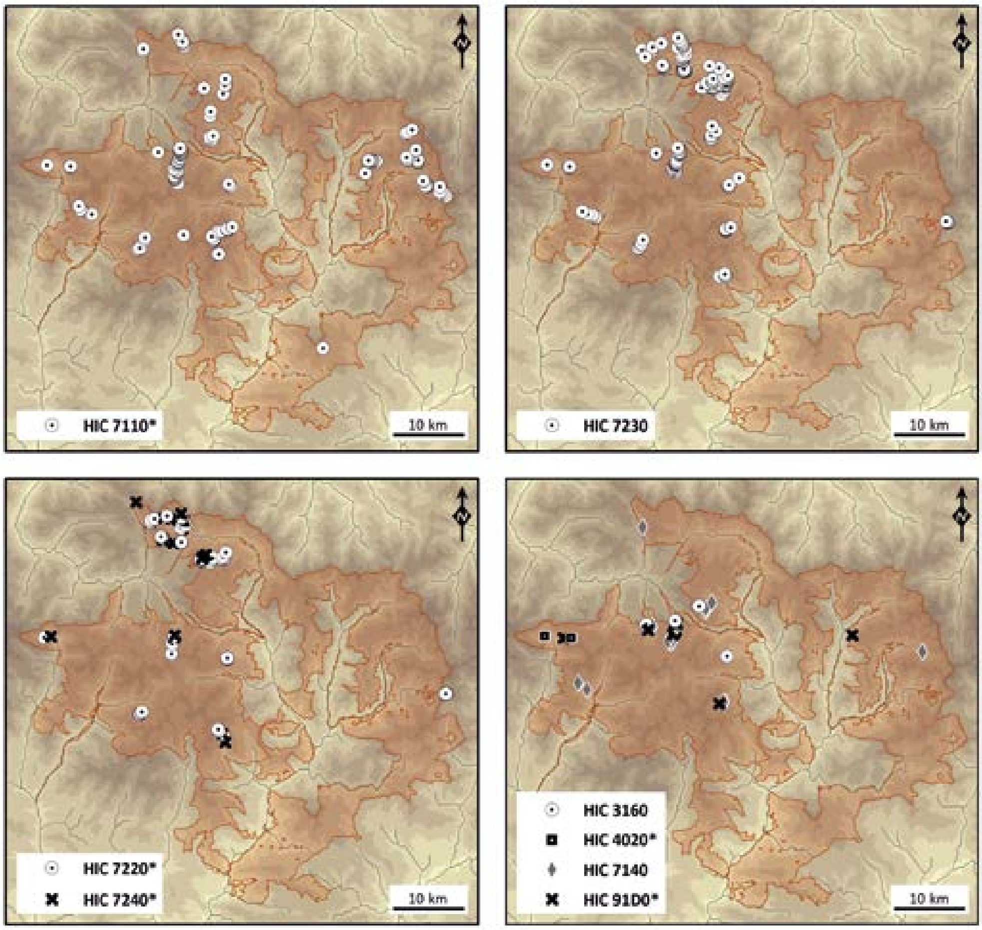
The cartography also shows a classification of mires related to springs and rivulets, or with valley floors, whether associated with lakes or not. The most frequent typology are mires associated with springs and rivulets, of which there are 557, while those associated with valley floors amount to 364 (among these, 97 are linked to lake shores). The mire systems associated with springs and rivulets occupy about 492 hectares, a value that allows us to estimate the total area of these mires as 197 hectares. The systems associated with valley floors occupy some 290 ha, of which 145 ha are strictly mires. Thus, the mapped mires occupy about 0.37 ha on average each (0.35 those of springs and rivulets and 0.40 those of valley floors).

In addition, as a result of extensive field sampling, we georeferenced a total of 604 points corresponding to mire habitats (Figure 2). Of these, 573 points could be attributed to some mire HCI; one HCI (4020* Temperate Atlantic wet heaths with *Erica ciliaris* and *Erica tetralix*) was not represented given its rarity (Table 1). Considering only the hydrological basins (main valleys), we obtained data from 18 basins with more than five HCI points recorded, in addition to eight more basins where the number of points was lower.

Considering the 573 points recorded, the most abundant HCIs were the 7230 Alkaline fens (with 249 points) and the 7110* -Active raised bogs (with 194 points; Figure 3). The majority of these HCIs correspond to the CORINE 51.1117 *Sphagnum capillifolium* hummocks (23.0%) and 54.24 Pyrenean rich fens (22.0%).

Regarding the distribution according to the geological substrates (Table 2), all HCIs can occur on acidic materials, but only 7230 Alkaline fens, 7220* Petrifying springs and 7240* Alpine pioneer formations are well represented on carbonate substrates. These three HCIs, although typically calcium, are also found on acidic substrates, especially in soligenous mires on granites.

The environmental descriptors in Table 3 express how all recognised habitats are typically located in high mountain levels and have an optimal distribution in the



▲ **Figure 3.** Distribution of the different mire HCIs in the studied area (Aigüestortes and Alt Pallars SCIs) from the 573 points recorded in the extensive study and the 13 locations of the intensive study. The HCI codes correspond to: 3160, Natural dystrohpic lakes and ponds; 4020*, Temperate Atlantic wet heaths with *Erica ciliaris* and *Erica tetralix*; 7110*, Active raised bogs; 7140, Transition mires (quaking bogs); 7220*, Petrifying springs with tuf formation (*Cratoneurion*); 7230, Alkaline minerotrophic fens; 7240*, Alpine pioner formations of *Caricion maritimae*; 91D0*, Bog woodland.

HCC	Number of points	Percentage of points
3016 Dystrophic lakes	4	0.70 %
7110* Active raised bogs	194	33.86 %
7140 Transition mires	31	5.41 %
7220* Petrifying springs	54	9.42 %
7230 Alkaline fens	249	43.46 %
7240* Alpine pioneer formations	30	5.24 %
91D0* Bog woodland	11	1.92 %

▲ **Table 1**. Number of points and percentage of the total where the different mire HCIs were recorded..

HCI	Acid schist and shale	Other acid siliceous materials	Carbonate materials	Calcium-rich schist
3016 Dystrophic lakes	0.00 %	75.00 %	0.00 %	25.00 %
7110* Active raised bogs	33.52 %	52.75 %	7.14 %	6.59 %
7140 Transition mires	13.04 %	86.96 %	0.00 %	0.00 %
7220* Petrifying springs	1.85 %	22.22 %	53.70 %	22.22 %
7230 Alkaline fens	3.29 %	32.51 %	53.50 %	10.70 %
7240* Alpine pioneer formations	3.33 %	16.67 %	80.00 %	0.00 %
91D0* Bog woodland	9.09 %	90.91 %	0.00 %	0.00 %
Total	**13.71 %**	**41.13 %**	**35.83 %**	**9.32 %**

▲ **Table 2**. Percentage of presence of each mire HCI in the different geological materials (according to the simplified map of Conesa *et al*., 2010), evaluated as a percentage of points over the total.

subalpine belt. However, neither the dystrophic lakes (3160) nor the bog woodland (91D0*) are found above 2300 m and, therefore, do not reach the alpine area. As for gradients, the vegetation linked to springs and rivulets (HCIs 7230 Alkaline fens, 7220* Petrifying springs and 7240* Alpine pioneer formations), has the highest values, followed by 91D0* Bog woodland, which often occupies relatively soft, but steep slopes, where water flows diffusely. Finally, since the continentality index was correlated with altitude, the HCIs that appear at higher altitude have the lowest values of this index.


Drosera longifolia.

HCI	Minimum altitude (m)	Average altitude (m)	Maximum altitude (m)	Average slope	Continentality index
3016 Dystrophic lakes	1783	1828.25	1958	5.16	25.15
7110* Active raised bogs	1751	2144.77	2498	6.54	23.7
7140 Transition mires	1810	2032.35	2257	1.69	24.41
7220* Petrifying springs	1802	2156.54	2431	16.65	23.31
7230 Alkaline fens	1555	1986.96	2564	12.65	24.12
7240*Alpine pioneer formations	1798	2161.17	2434	22.7	22.72
91D0* Bog woodland	1754	1859.55	1990	11.99	24.76
Total	**1555**	**2064.39**	**2564**	**10.83**	**23.87**

▲ **Table 3**. Average values of the environmental variables considered for each HCI, and for the whole.

Spatial complexity of mires

Pyrenean mires that occupy larger areas, either because they are found in lake areas, or because they depend on wide seepage systems, form systems with strong variations in environmental properties and in biological content, and thus they take the form of complex landscape mosaics. This is evident in the systems intensively studied, where the relationship between environmental parameters and habitats was analysed. In this section we will comment on general aspects observed in these complex systems, while for the more specific results we refer to other works (cf. Pérez-Haase & Ninot, 2017; Colomer *et al*., 2019).

These mire systems are mostly found in the lower half of the subalpine belt, linked to bottoms of glacial valleys or circuses (Table 4). Sometimes they clearly correspond to sediment-filled lakes or wide margins of lake infilling (Figure 4), others to seepage systems on gentle slopes (such as Cabana de Parros) and others to the combination of these situations, as occurs in the most extensive system

▲ **Figure 4.** . In some appropriate places, such as Plans de Sotllo, there are systems formed by complex mosaics, where different types of mires and other non-hydrophilic habitats are combined.

Location	HCIs	Typology	Altitude (m)	Area (ha)	Threats	Status	Species of interest
1. Bassa Nera-Era Planhòla	3160 7110* 7140 7230 91D0*	Lake and valley floor mire	1840-1900	16.19	Afforestation (M) Grazing (B-M) Trampling (B)	Very good	*Carex lasiocarpa* *C. limosa* *C. diandra* *Comarum palustre* *Menyanthes trifoliata* *Utricularia minor* *Sphagnum magellanicum*
2. Ruda-Locampo-Saboredo	3160 7110* 7140 7220* 7230 91D0*	Lake and valley floor mire	1720-2190	4.39	Grazing (B) Hydroelectric activity (B)	Very good	*Drosera anglica* *Menyanthes trifoliata* *Utricularia minor*
3. Plan de Tor	7110* 7140 7230	Valley floor mire	1960-2005	5.95	Grazing (B-M) Tourism (B-M)	Good	*Sphagnum fuscum*
4. Clots de Rialba	3160 7110* 7140 7230	Lake mire	2070-2090	2.81	Grazing (B) Winter sports (M)	Very good	*Carex limosa* *Comarum palustre* *Drosera anglica*
5. Ribera de Boldís	7110*	Glacier floor mire	2190-2200	1.40	Grazing (M)	Good	*Eriophorm vaginatum* *Sphagnum russowii*
6. Plans de Sotllo	7110*	Glacier floor mire	2183-2190	1.67	Grazing (M-A)	Good	*Sphagnum papillosum*
7. Estanyeres	7110* 7220* 7230	Carbonate source mires	1930-1950	2.62	Grazing (A) Trampling (A)	Average	*Cochlearia pyrenaica* *Utricularia australis*
8. Cabana de Parros	7220* 7230	Carbonate source mires	1750-1850	1.98	Grazing (M)	Average	*Eriophorum latifolium*
9. Portilhon de Marimanha	7220* 7230 7240*	Carbonate source mires	2220-2350	2.31	Grazing (M)	Average	*Carex frigida* *Sphagnum warnstorfii*
10. Ras de Bonabé	7220* 7230	Carbonate source mires	2000-2100	4.94	Grazing (M)	Average	*Juncus balticus* *Equisetum variegatum* *Sphagnum warnstorfii*
11. Pleta de Molières	4020* 7110* 7220* 7230 7240*	Valley floor mire	1600-1650	6.23	Tourism (M)	Average	*Erica tetralix*
12. Vall de Conangles	7110* 7230 91D0*	Valley floor mire	1820-1840	1.13	Grazing (B) Avalanches (B) Afforestation (B)	Good	*Erica tetralix* *Saxifraga aquatica*
13. Trescuro	7110* 7140 7230 91D0*	Lake and valley floor mire	2045	1.42	Grazing (B) Hydroelectric activity (B)	Very good	*Sphagnum magellanicum*

▲ **Table 4**. Characterisation of the 13 intensive study locations (located in figure 2) based on the mire HCIs present, the geomorphological and hydrological status, altitude, the area occupied by the mire habitats (including HCIs and other mire habitats, but not free bodies of water), an assessment of potential threats (B, low; M, medium; A, high) as well as conservation status and the species of interest observed (vascular plants and bryophytes).

of all at Bassa Nera-Era Planhòla.

Some of these systems are formed by a few different mire habitats, notably those found in acid schistose substrates and also those derived from seepage systems in some carbonate-rich slopes (Figure 5). However, those that include diverse hydrological situations (lake shores, rivulets, gentle slopes) and also different substrates are much more diverse, as is the case of Clots de Rialba, Pleta de Molières and Trescuro.

Impacts caused by large herbivores

Throughout the extensive sampling we found that the majority of mire HCIs had been affected by domestic livestock (Tables 5 and 6). Only 11.6% of the points showed no signs of grazing. Although in most cases the degree of herbivory was low (43.9%), the medium trampling levels was the most frequent (37.8%). Considering each HCI separately, we detected a medium or high level of grazing in more than half of the points of HCIs 7240* Alpine pioneer formations (61.9%), 7230 Alkaline fens (59.7%) and 7220* Petrifying springs (58.8%). These were also the three habitats with the highest affectation percentages in terms of medium and high trampling levels (47.1%, 67.2% and 61.8% respectively).

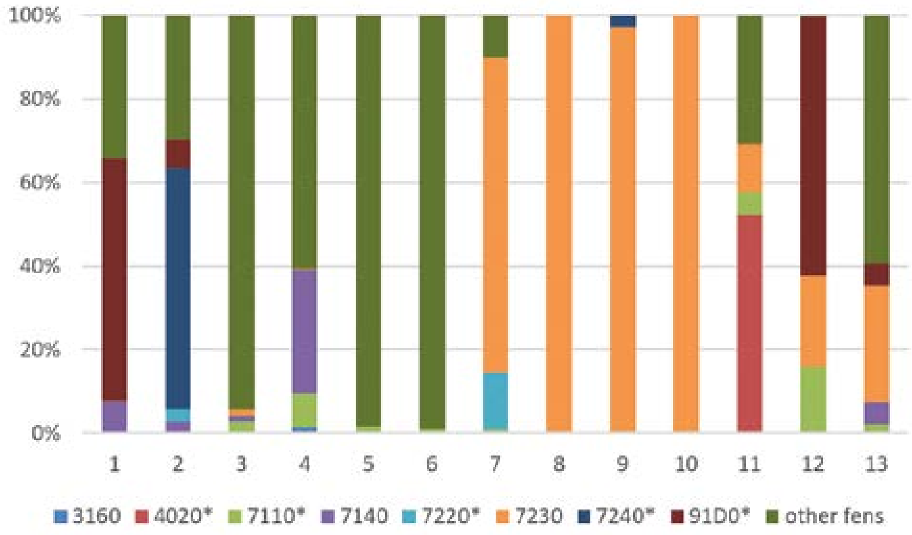


Figure 5.
Percentage of surface occupied by the different mire HCIs and other types of hydrophilic ecosystems (excluding free waters) in the different complex systems (see the numerical correspondence and its total expansion in Table 4).

HCI	No herbivory	Low herbivory	Medium herbivory	High herbivory
7110* Active raised bogs	11.65 %	51.46 %	34.95 %	1.94 %
7140 Transition mires	18.18 %	72.73 %	9.09 %	0.00 %
7220* Petrifying springs	11.76 %	29.41 %	52.94 %	5.88 %
7230 Alkaline fens	5.65 %	34.68 %	46.77 %	12.90 %
7240* Alpine pioneer formations	14.29 %	23.81 %	57.14 %	4.76 %
91D0* Bog woodland	0.00 %	100.00 %	0.00 %	0.00 %
Total	**9.40 %**	**41.61 %**	**41.95 %**	**7.05 %**

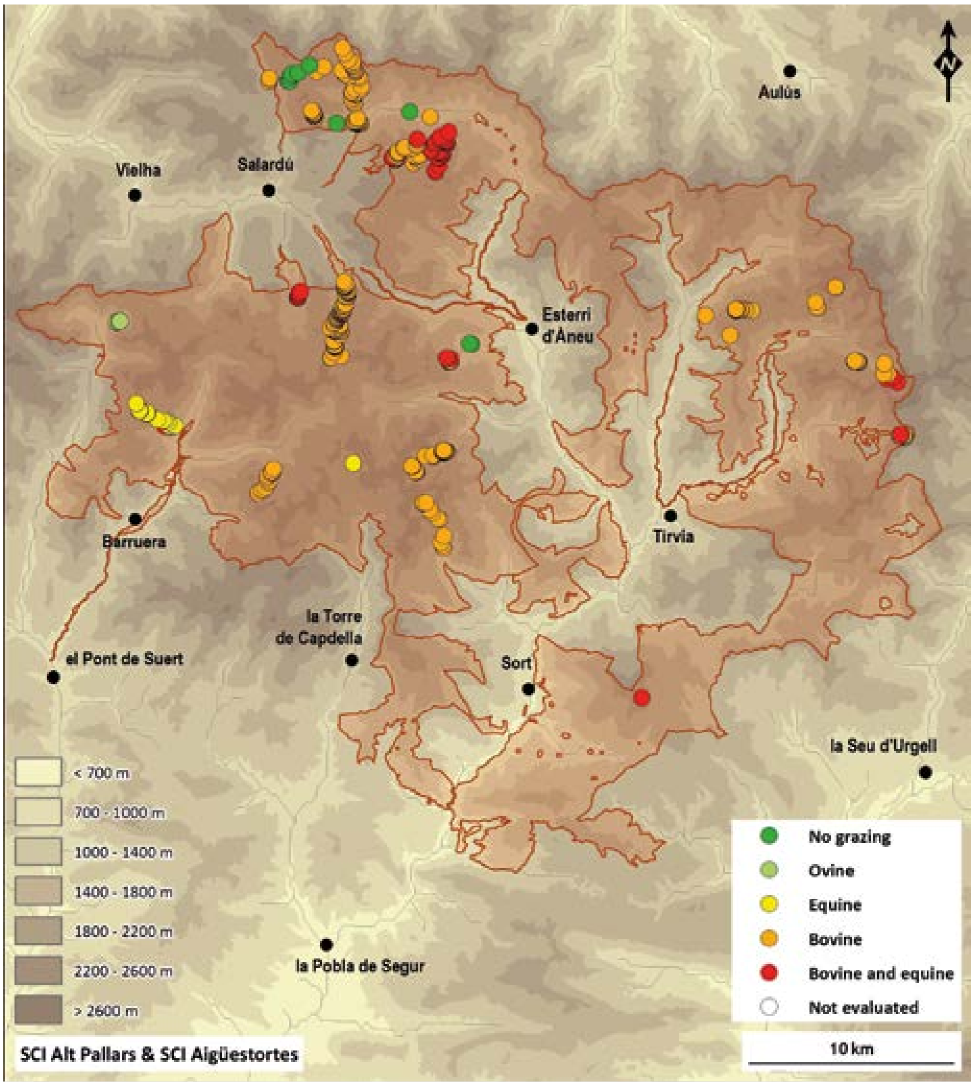
Table 5. Level of affectation of mire HCIs due to herbivory by large herbivores, as a percentage of points of each level of herbivory compared to the total points of each HCI and of the whole.

HCI	No trampling	Low trampling	Medium trampling	High trampling
7110⁎ Active raised bogs	16.67 %	46.97 %	30.30 %	6.06 %
7140 Transition mires	18.18 %	63.64 %	18.18 %	0.00 %
7220⁎ Petrifying springs	11.76 %	26.47 %	50.00 %	11.76 %
7230 Alkaline fens	4.69 %	28.13 %	46.88 %	20.31 %
7240⁎ Alpine pioneer formations	11.76 %	41.18 %	29.41 %	17.65 %
91D0⁎ Bog woodland	0.00 %	100.00 %	0.00 %	0.00 %
Total	**9.58 %**	**36.40 %**	**39.85 %**	**14.18 %**

▲ **Table 6**. Level of affectation of mire HCIs due to trampling by large herbivores, as a percentage of points of each level of trampling compared to the total points of each HCI and of the whole.

Threats

In almost all the points assessed (97%) we considered that there were potential threats to conservation. In the vast majority of cases (93.6%) we detected the presence of herds of cows, horses or sheep (Table 7, Figure 6), that considered separately represented a threat in 87.5%, 25.8%, and 1.6% of the cases, respectively.



▲ **Figure 6.** Potential threats to the conservation of mire HCIs, derived from the presence of cattle, horses and sheep, at the 573 points recorded in the extensive study.

HCI	Bovine	Equine	Ovine
3160 Dystrophic lakes	100.00 %	0.00 %	0.00 %
7110* Active raised bogs	90.00 %	13.92 %	1.25 %
7140 Transition mires	85.71 %	22.58 %	0.00 %
7220* Petrifying springs	78.00 %	29.63 %	0.00 %
7230 Alkaline fens	89.13 %	26.51 %	1.74 %
7240* Alpine pioneer formations	89.29 %	0.00 %	0.00 %
91D0* Bog woodland	90.91 %	0.00 %	9.09 %
Total	**88.27 %**	**20.24 %**	**1.39 %**

▲ **Table 7**. Level of threat to mire HCIs due to livestock (bovine, equine, ovine), as a percentage of threatened points compared to the total points of each HCI and of the whole.

HCI	Spontaneous afforestation	Tourist routes	Erosion
3160 Dystrophic lakes	0.00 %	0.00 %	0.00 %
7110* Active raised bogs	1.25 %	5.15 %	0.00 %
7140 Transition mires	0.00 %	0.00 %	0.00 %
7220* Petrifying springs	0.00 %	7.41 %	2.00 %
7230 Alkaline fens	3.04 %	16.47 %	1.30 %
7240* Alpine pioneer formations	0.00 %	13.33 %	10.71 %
91D0* Bog woodland	0.00 %	0.00 %	0.00 %
Total	**1.79 %**	**10.30 %**	**1.39 %**

▲ **Table 8**. Level of threat to mire HCIs due to spontaneous afforestation, tourist traffic and erosion, as a percentage of threatened points compared to the total points of each HCI and of the whole.

Disturbance	Bovine grazing	Equine grazing	Ovine grazing	Spontaneous afforestation	Erosion
Medium herbivory	89.68 %	26.98 %	0.00 %	0.79 %	3.17 %
High herbivory	100.00 %	47.62 %	0.00 %	0.00 %	9.52 %
Medium trampling	86.67 %	23.81 %	0.00 %	0.95 %	0.00 %
High trampling	91.89 %	45.95 %	0.00 %	0.00 %	0.00 %

▲ **Table 9**. Incidence of the five types of threat in mire HCIs in cases where they are disturbed by grazing, as a percentage of points threatened with respect to each type of disturbance.

Apart from grazing, the other potential threats considered all at once were recorded in 13.5% of all points with HCIs (Table 8). Among these, the presence of hiking trails is the most significant threat. The presence of forest tracks and wildlife are considered threats in only one HCI point each. Water catchments did not affect any HCI, but did affect one acid mire.

In cases of high disturbance due to grazing (either by medium or high herbivory, or by medium or high trampling) the main threat corresponds to cattle (Table 9). Spontaneous afforestation was not reduced in the case of high levels of herbivory, while erosion is higher in cases of high herbivory, but not in those of high trampling.

Species of interest

Throughout the field sampling we have documented the presence of 19 species of interest (Table 10), of which fourteen are vascular plants (very divided between groups, although there are two *Carex*) and five are bryophytes (four of the *Sphagnum* genus). Of the total, twelve are in some category of threat in the aforementioned references, and seven have been included in the evaluation because of their biogeographic or ecological interest.

Species of interest	**3160**	**7140**	**7230**	**7110***	**7220***	**7240***	**91D0***	**Total**
Cardamine pratensis subsp. *crassifolia* (ce)			3					3
Carex curta (ce)		7						7
Carex lasiocarpa (df, rb)		8						8
Carex limosa (df, rb)		1						1
Cochlearia pyrenaica (df)					5			5
Drosera longifolia (df, rb)		3	1	2				6
Equisetum variegatum (ce)			14	4		1		19
Erica tetralix (df)			6	5				11
Eriophorum vaginatum (ce)				3				3
Juncus balticus subsp. *pyrenaeus* (df)			6					6
Menyanthes trifoliata (df)		2						2
Salix hastata (df, rb)			1					1
Saxifraga aquatica (ce)			1		7	3		11
Scorpidium scorpioides (df)		1	1					2
Sphagnum fuscum (df)				1				1
Sphagnum magellanicum (df)				1				1
Sphagnum squarrosum (ce)							1	1
Sphagnum warnstorfii (ce)			1	1				2
Utricularia minor (df)	4	2						6
Total presence	**4**	**24**	**34**	**17**	**12**	**4**	**1**	**96**
Total species	**1**	**7**	**9**	**7**	**2**	**2**	**1**	**19**

▲ **Table 10**. Presence of species of interest in the different HCIs (number of times they appear in each HCI, over the total points of this HCI, see table 1). The abbreviations that follow the name of each species in parentheses indicate that it is contained in threat categories of the "Decree on flora conservation" (Catalonia, 2018, 2015) (df), of the "Red Book" (Sáez *et al*., 2010) (rb), or they are of high biogeographic or ecological interest, according to expert criteria (ce).

DISCUSSION

The archipelago of mires

The high alpine mountain hosts numerous representations of wet systems, but these are always small and scattered elements, a fact that is particularly pronounced in the Pyrenees (Ninot *et al.*, 2017). Thus, the distribution and dimensions of the mires in the cartography obtained, allows them to be understood as an archipelago of small scattered islands, which conditions the processes related to biodiversity maintenance (Hájek *et al.*, 2011). The fact that both the size of each mire system and the global area occupied by the mires are so small, determines that they are habitats particularly fragile to disturbances. The same also points to a low adaptive capacity of the vegetation in case of climatic changes, given the difficulty of finding favourable habitats by dispersing seeds. As a consequence, other groups of organisms associated with mires may also have conservation problems in relation to their colonising capacity. For example, flying animals are expected to reach more favourable new habitats more easily than those with short-distance dispersal, such as molluscs (Steinitz *et al.*, 2006).

From the mapping of mires we can point out that a significant part of the species could not maintain stable populations over time in each mire separately. Rather, they must function under active metapopulation dynamics (Heegaard, 2000) using the island system to maintain viable populations. It also follows that those species present in only a few mires are particularly fragile, and in quite a few cases, we can consider them relics from colder times (Jiménez-Alfaro *et al.*, 2016).

In the absence of specific studies, it is advisable to keep the entire archipelago of mires in good condition, and not to focus efforts on a few specific mires. In order to properly manage these habitats, detailed specific mapping of them is needed.

Grazing

The presence of livestock does not necessarily represent a conservation problem in grassland vegetation. In fact, many types of mires are dominated by graminoid plants that recover well after being grazed (Diaz *et al.*, 2007). In addition, the typical community structure (diversity and abundance of vascular plant and bryophyte species)

▲ **Figure 7**. Large livestock (cattle and horses) tend to concentrate in wetlands, as in Marimanha, where they can profoundly alter these fragile ecosystems.

often depends on herbivore activity (Ausden *et al*., 2005). However, when grazing pressure is high or very high (Figure 7), excessive herbivory and trampling cause damage to ecosystems, which in some cases can be difficult to reverse (Morris & Reich, 2013). In Pyrenean mires we detected an excess of herbivory or trampling at approximately half of the study points. Thus, the intensive consumption of some plant species may lead them to disappear locally (Olff & Ritchie, 1998), although this fact is documented enough with our data. In addition, in many cases, frequent trampling by livestock caused the presence of compacted and disorganized soils. These alterations cause changes in the processes linked to runoff and water retention capacity, so important in wetlands (Couwenberg & Joosten, 1999).

The affectation by livestock was not the same at all the habitats. Overall, the effects of trampling are greater when the water table is close to the surface or when mosses dominate, as detected in other regions (Morris & Reich, 2013). For example, we can see how the expansion of sphagnum (7110* Active raised bogs) are especially vulnerable to the trampling of equine livestock, although them prefer to graze in other habitats. Therefore, the most affected habitats must be carefully studied in order to plan efficient corrective management measures. Alkaline fens (7230), Alpine pioneer formations (7240*) and Petrifying springs (7220*), which present excessive grazing levels, should be a conservation priority in relation to livestock.

Potential threats

Among all threat categories considered, herds of cows and horses are the most significant by far. In fact, we considered bovine herds a threat to conservation in more than 75% of cases in all habitats except one. From the perspective of habitat conservation, the negative effects of livestock should be considered when making management decisions. In any case, our study cannot be conclusive when determining which livestock burden is harmful. In addition, it should also be considered that an adequate livestock load can contribute to the proper conservation of mires (Olff & Ritchie, 1998; Stammel *et al*., 2006). In fact, in some cases we find that visiting cattle may be related to the attenuation of the threat of spontaneous afforestation, which in certain habitats may be a major cause of loss of heritage value of mires (Colomer *et al*., 2019).

Also, tourism is a cause of concern for managers of natural areas. In particular, the Aigüestortes i Estany de Sant Maurici National Park and the Alt Pallars Natural Park are visited by a very large number of tourists. In this study we have found that hiking trails are a potential threat in a significant part of the mires. However, we found that they caused significant degradation only on a few occasions (as in the Molières and Sotllo systems, subject to corrective measures in the LIFE+ LimnoPirineus project). In most cases we interpret the trails as narrow linear elements that cause irrelevant degradation in wet systems. We must also consider the threat due to the installation of sports infrastructure, such as ski resorts, which can affect mires located in nearby areas. If we leave aside the alterations due to hydroelectric dams, not considered in this work, there are no more relevant potential threats in the context of the Central Pyrenees, although some areas intensely visited by livestock suffer erosion.

In addition, spontaneous afforestation proved to be an insignificant conservation factor. However, when we have approached detailed studies based on GIS techniques (Galobart *et al*., 2019) We have detected that in the subalpine area there may be a threat in some mires. The evaluation of spontaneous afforestation is more precise when the whole mire is considered and not only the area immediately surrounding a georeferenced point (as we have done in this work). It seems, therefore, that in order to detect the dynamics of spontaneous afforestation, this process must be studied on a relatively large spatial and temporal scale, including each mire system over at least 30-50 years.

CONCLUDING REMARKS

The set of wetlands studied includes a good variety of mire habitats, including eight HCIs, hosting 19 plant species that are found in different threat categories or that are of high biogeographic and ecological interest. Both rare HCIs and specialist species are often very dispersed over the complex mosaic of Pyrenean ecosystems within the study area, which is a challenge to conservative management.

The mires of these two protected natural areas are, as a whole, in an acceptable and, in many cases, good state of conservation. However, approximately half of them suffer excessively from visiting livestock. Among all the disturbances analysed, we believe that the level of high trampling, especially as a result of visiting cattle, is the one that most affects the correct conservation state of the mires. Consequently, it is recommended that the managers of the natural spaces aim to reduce the impacts of overgrazing in those points most affected. Potential threats that could damage the conservation status of mires also have to do with grazing livestock. Apart from livestock, the rest of the threats evaluated have a low or irrelevant effect, which should lead to focusing conservation efforts on livestock management. The management of spontaneous afforestation must be considered separately, once its effects have been measured through specific studies, based on a particular methodology different from that used here.

Therefore, managers must look further into the causes of the degradation of mires and promote applied work that seeks to mitigate the damage caused by livestock. Some experiences with livestock exclusion fences may help to base the management of mires with minimal damage to livestock activity, which, as we have stated, also has beneficial effects on biodiversity.

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~ Petrifying springs of Montseny de Pallars ~

EVALUATION OF THE PERCEPTION OF HIGH MOUNTAIN AQUATIC ECOSYSTEMS BY VISITORS AND NEARBY RESIDENTS OF THE NATIONAL PARK

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ABSTRACT

The study analysed the perception that visitors and nearby residents of the Aigüestortes i Estany de Sant Maurici National Park and the Alt Pirineu Natural Park have with respect to high mountain aquatic ecosystems. The study has allowed the identification of the different degrees of social knowledge and evaluation (from the ecological, landscape, and leisure points of view) of the four types of aquatic ecosystems analysed, with the lakes the best known, followed by rivers and streams, while carbonated springs and peat bogs are relatively unknown.

INTRODUCTION

This study is part of the LIFE+ LimnoPirineus project - "Restoration of lentic habitats and aquatic species of community interest" (2014 to 2019). Within the framework of this project, the study considered analysing the relationship between the habitats subject to intervention by the LIFE+ LimnoPirineus project (the high mountain aquatic ecosystems of the Catalan Pyrenees: lakes, rivers and streams, peat bogs and carbonated springs) and the society that lives nearby (the residents of the areas surrounding the project's study area) or who visit the places where we find these ecosystems (the visitors).

Thus, the main objective of this study when developing it, was the empirical knowledge of the perception of high mountain aquatic ecosystems in the case of the Catalan Pyrenees, both by the population residing within the environment of these ecosystems as well as the visitors who frequent these ecosystems (tourists and hikers). More specifically, the study aimed to know the different perceptions that may exist between the two social groups (visitors and residents), but also between the two main territorial areas under study: firstly, the Aigüestortes i Estany de Sant Maurici National Park, and secondly, the Alt Pirineu Natural Park.

The research questions raised by the study are the following:

- What type of high mountain aquatic ecosystem is the best known?
- What type of high mountain aquatic ecosystem is the most valued?
- What differences in perception can be identified in relation to the sociodemographic profile of those questioned?
- What is the level of social evaluation of the actions proposed within the framework of the LIFE+ LimnoPirineus project for the restoration of high mountain aquatic habitats?
- What is the level of knowledge regarding the problem of invasive species, especially in high mountain lakes?

CONTEXTUALIZATION

The relationships between aquatic ecosystems and humans are complex. It is widely known that they provide a vast range of services that contribute to the human wealth (for example, water supply and treatment, climate regulation and reduction of flooding risks, source of hydroelectric energy, maintenance of biodiversity, leisure opportunities and nature tourism, scientific knowledge, amongst many other ecosystem services) (Millennium Ecosystem Assessment, 2005; Borja et al., 2012). Regarding those services related with leisure and tourism – within those services named cultural services - recently there has been an increasing emphasis on the importance of the visits in natural areas and therefore, to the aquatic ecosystems, for the human health and welfare (Bowler et al., 2010; Hartig et al., 2014; Kaplan, 1995; Romagosa et al., 2015; Dustin et al., 2018).

However, these ecosystems degrade more rapidly than other ecosystems types. Knight and Cole (1990) already warned three decades ago that aquatic ecosystems such as lakes and rivers were probably the most threatened since they are both attractive and scarce, and therefore have a higher recreational pressure and suffer harmful uses for the environment. Despite the elapsed time, the situation has not changed substantially. In the case of aquatic ecosystems in Spain, for example, in recent decades a change in the aforementioned ecosystem services has been identified, which has manifested itself in the proliferation of invasive species, an increase in water pollution and the effects of climate change (increased drought, overexploitation of aquifers, etc.) (Borja et al., 2012).

In protected environments, such as those that are part of our study, this degradation is obviously more limited, thanks to conservation and management actions. However, human activity also alters these habitats and causes a number of impacts that their managers have to deal with, many of which result from increased tourist and leisure visits (Newsome et al. , 2002; Romagosa, 2008) that ends up affecting the services of cultural ecosystems (Taff et al., 2019).

The aim of this study of social perception is to analyse mountain aquatic ecosystems. Perception is an essential part of how people experience and use ecosystems and the environment in general (Relph, 1976). Perceptual studies on the natural environment are abundant internationally (e.g., Barber et al., 2003; Mayer-Smith and Burgess, 2011; Khew et al., 2014; Weiler and Moyle, 2017; Colley and Craig , 2019). In the case of Spain, for example, studies have been carried out on the perception of the landscape, especially from the perspective of the use of the natural environment as a place of leisure. For example, Schmitz et al. (2007) addressed this issue in the province of Madrid, while Atauri et al. (2000) did so for the specific case of the Guadarrama mountain range, also in Madrid. Múgica and De Lucio (1996) had previously analysed the landscape preferences of visitors to protected areas as a reference for improving attitudes towards these areas.

In the case of the social perception of high mountain ecosystems, there is no evidence of studies that have specifically analysed this type of habitat in Spain, although some international studies have analysed certain aquatic ecosystems, such as coastal environments, both marine and fluvial, as is the case with a series of studies conducted by Springett-Walker et al. (2016) in different parts of Europe and Oceania. The study by Faggi et al. (2013), on the other hand, may be one of the few studies that have examined the assessments of «water landscapes» by residents and visitors to a certain place, in this case, the natural areas that have elements of water in the metropolitan area of Buenos Aires (Argentina). More specifically for mountain areas, a recent study by Wlejaczka et al. (2018) analysed residents' perceptions of a proposed dam and reservoir in the Indian part of the Himalayas.

In terms of its geographical area (Aigüestortes i Estany de Sant Maurici National Park and Alt Pirineu Natural Park), this study is unprecedented. There have been previous studies on the profile of visitors from both protected areas focussing on one of the social groups studied in this project, visitors (Farias et al., 2005; Farias, 2011), but neither has compared visitors and residents, and even less has analysed aquatic ecosystems.

METHODOLOGY

The first step in developing the study was to design the survey to be conducted on visitors and residents of the two fields of study. The study's technical team prepared an initial survey proposal based on previous experience in other studies of social perception and the environment. This proposal was outlined and finalised from the contributions of those responsible for management and the technical staff of the two natural areas subject to the study and the LIFE+ LimnoPirineus project technical team.

The survey finally conducted on visitors of the parks and residents of the areas surrounding the parks has as its central axis the perception of these two groups on the high mountain aquatic ecosystems and, more specifically, on the problem of fish proliferation in the lakes. In this sense, the first part of the survey asks visitors and residents of the area for their assessment of the level of knowledge and evaluation of these ecosystems, as well as the conservation actions proposed by the LIFE+ LimnoPirineus project. In order to facilitate the answer to the questions referring to these aspects, a Likert scale from 1 to 5, from less to greater importance, was used.

Those responsible for management and the technical staff of both protected areas worked together to establish and agree the protocol for conducting the field survey, with the aim of ensuring a correct selection of the sample as well as the maximum representation of the sample group, which is the set of visitors for the two protected areas, as well as the resident population in these environments. Regarding the location of the sampling points for conducting the survey, several points were chosen for each park where it would be relatively easy to access visitors. As for the residents, the surveys were conducted mostly in the towns themselves (Espot and Boí, in the case of the National Park, Àreu, Alins, Tavascan, Lladorre, Ribera de Cardós and Llavorsí, in the case of the Natural Park).

The method for conducting the field survey was the random approach to potential respondents by the interviewer, who briefly explained the objectives of the study and asked them to answer the questionnaire, ensuring confidentiality and anonymity in the answers. The answers were, for the most part, numerical in nature, so they could be subsequently processed with the SPSS statistical analysis program (version 21). In this sense, the statistical analyses carried out consisted, essentially, in the calculation of: 1) the percentages of the sociodemographic profile of the respondents and of the different numerical answers to the questions included in the survey; 2) the mean values of the subjective evaluations of different aspects included in the survey; and 3) the correlations

(Spearman's rho) between the sociodemographic profile of the respondents and the subjective assessments of different aspects included in the survey.

The survey was conducted in two batches (summer 2016 and Easter 2017), reaching a total of 519 people surveyed, distributed as follows:

- Aigüestortes i Estany de Sant Maurici National Park: 318 questionnaires (258 visitors and 61 residents).
- Alt Pirineu Nature Park: 201 questionnaires (150 visitors and 50 residents).

RESULTS

The results of the first part of the survey showed the high subjective evaluation that visitors and residents have of high mountain aquatic ecosystems. Specifically, the water spaces, together with the high mountain landscape, were the best valued element or characteristic of the park, above forests and meadows, wildlife, cultural heritage, or other elements. This evaluation was slightly higher in the case of the National Park than that of the Natural Park (Table 1).

The survey asked visitors and residents about the level of subjective knowledge they had about the different types of high mountain aquatic ecosystems: a) lakes; b) rivers and streams; c) peat bogs, and d) natural sources/springs. The results show different levels of knowledge of the four types of aquatic ecosystems analysed, with the lakes the best known, rivers and streams in second place, significantly above the carbonated springs and peat bogs (the latter turned out to be the least known by all respondent groups). Residents showed a higher level of knowledge compared to visitors for all four ecosystems, both in the National Park and the Natural Park (Table 2).

In addition to the level of knowledge of the high mountain aquatic ecosystems, the survey also asked visitors and residents about their overall evaluation of each of the four types of ecosystem analysed. It was specified that the value given from the ecological, landscape and leisure points of view was understood as a general evaluation. Visitors to both parks rated the four types of aquatic ecosystem with almost the highest score (average rating of 4.9 out of 5), while residents also gave them almost the highest score, although slightly lower than that of the visitors (average rating of 4.7) (Table 3).

The survey included an issue that explicitly referred to the LIFE+ LimnoPirineus project, with the aim of knowing the evaluation of visitors and residents for the conservation activities of the high mountain aquatic ecosystems proposed within the framework of this European project. The average assessment of the LIFE+ LimnoPirineus project actions by the total respondents was 4.7 on a scale of 1 to 5. In general terms, visitors tended to value project actions more positively (4.8) than residents (4.4). A slightly better evaluation of the actions was also observed by the respondents at the Aigüestortes National Park compared to the respondents in the Alt Pirineu Natural Park, although the differences were not significant (Table 4). The only significant difference, from the point of view of the sociodemographic profile of respondents, was that older residents tended to value the conservation actions proposed by the project less positively (Table 5).

The second part of the survey allowed more depth, with further detail in aspects more directly related to the specific problem of the proliferation of fish in high mountain lakes. Specifically, the survey asked about the knowledge of the different types of animal species that respondents believed could be found in high mountain lakes, giving frogs, insects, crustaceans, fish, newts and a final category entitled "others" as possible options. The purpose of including this question in the survey was, in addition to making it easier for respondents to recognise the existence of high biodiversity in high mountain lakes, to identify the importance of the perception of the association of fish in the lakes. In this sense, the results showed that this association exists, since the fish were precisely the most mentioned group among the possible answers provided. Specifically, nine out of ten respondents (visitors and residents almost alike) took for granted that fish are types of species that can naturally be expected to find high mountain lakes. The other answers given, after fish, were insects and frogs, and at a much lower rate, the newt, others and crustaceans. In all cases, although the differences between visitors and residents are not significant, the visitors mentioned a greater number of species compared to residents (Table 6).

The following question was, in fact, in three parts, given that by viewing the lake at Closell before (2013) and after (2015) the actions carried out within the framework of the LIFE+ LimnoPirineus project, which consisted of previously eliminating fish introduced by human action (mainly minnow) that had led to high eutrophication and turbidity of the water, respondents were asked (without any details about the images) in which of the two situations they believed that: a) the lake was in a more optimal condition; b) greater species diversity could be expected; and c) one might expect to find fish.

The answers showed that the main perception was that the lake with the clearest and most transparent water is mostly associated with the one with the most optimal condition (85.5%), which has the greatest diversity of species (73.9%), and where one could expect to find fish (85.5%). It must be said that of the three perceptions mentioned, the last one is the one furthest from reality, since this image is precisely of the lake without fish.

Finally, the survey asked visitors and residents of the territory about the knowledge of the problem of invasive species in high mountain lakes, and specifically about the case of minnow. A vast difference of perception between residents and visitors became evident here. While more than half of the residents stated that they knew or had heard of the problem (34% and 28.3%, respectively), the visitors mostly did not know it (82.3%) (Figure 1).

CONCLUSIONS

In conclusion, the study showed how there is a fairly wide perception among visitors and residents of high mountain lakes as biodiverse ecosystems, where the presence of fish is a specific attribute of these, without knowledge of, to a large extent, the problem of invasive species, particularly fish.

Likewise, the study has highlighted the importance of the need to document and disseminate the characteristics, values, status and problems of high mountain aquatic ecosystems.

The study has contributed to provide knowledge from a more social perspective of an essentially ecological matter, such as that addressed by the LIFE+ LimnoPirineus project, in line with the call from the scientific community advocating the growing need to incorporate social sciences in the analysis and management of ecosystems in general or of aquatic ecosystems in particular and the challenges they currently face, especially from the perspective of ecosystem services.

Finally, the study has also allowed social dissemination of the project objectives, through the two batches of surveys, which have reached more than 500 people, between visitors and residents of the field of study, enhancing the connection between society and ecosystem conservation and management.

	Aigüestortes NP		Alt Pirineu NP	
Elements or characteristics of the park	Visitors	Residents	Visitors	Residents
Forests	4.9	4.8	4.9	4.6
Meadows	4.7	4.7	4.6	4.1
Water spaces	5.0	5.0	4.9	4.7
Wildlife	4.8	4.6	4.8	4.3
Livestock	4.6	4.4	4.6	4.4
High mountain landscape	5.0	5.0	4.9	4.8
Cultural heritage	4.8	4.7	4.7	4.2
Clean air and environment	5.0	4.8	4.9	4.8
Peace and tranquility	4.9	4.7	4.8	4.6
Park equipment and services	4.5	3.6	4.0	3.3
Activities organised by the park	3.8	3.8	3.5	3.2

▲ **Table 1.** Subjective importance of the elements or characteristics of the park (average score, scale 1-5)

	Aigüestortes NP		Alt Pirineu NP	
Type of aquatic ecosystem	Visitors	Residents	Visitors	Residents
Lakes	2.7	3.9	2.8	3.7
Rivers and streams	2.0	3.5	2.6	3.7
Peat bogs	1.5	2.9	1.9	2.6
FNatural sources/springs	1.8	3.1	2.1	3.1

▲ **Table 2.** Level of subjective knowledge of the different types of high mountain aquatic ecosystems (average score, scale 1-5)

Type of aquatic ecosystem	**Aigüestortes NP**		**Alt Pirineu NP**	
	Visitors	Residents	Visitors	Residents
Lakes	4.9	4.7	4.9	4.7
Rivers and streams	4.9	4.7	4.9	4.7
Peat bogs	4.9	4.7	4.8	4.6
Natural sources/springs	4.9	4.7	4.8	4.7

▲ **Table 3.** General evaluation (ecological, landscape and leisure value) of the different types of high mountain aquatic ecosystems (average score, scale 1-5)

Aigüestortes NP		**Alt Pirineu NP**	
Visitors	Residents	Visitors	Residents
4.8	4.5	4.7	4.3

▲ **Table 4.** Average evaluation of the LIFE+ LimnoPirineus project conservation actions (scale 1-5)

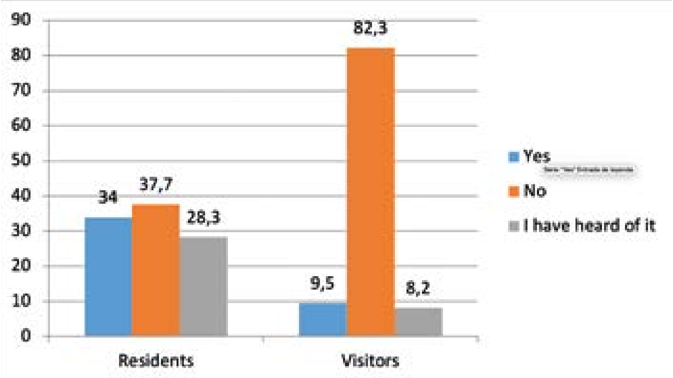
Age		**Level of education**		**Gender**	
Visitors	Residents	Visitors	Residents	Visitors	Residents
-0.10	-0.28*	0.10	0.02	0.05	-0.06

* The correlation is significant at level 0.01 (bilateral).

▲ **Table 5.** Correlations between the level of agreement regarding the LIFE+ LimnoPirineus project conservation actions and the sociodemographic profile of the respondents

	Visitors	**Residents**
Fish	89.1	88.7
Insects	84.4	79.2
Frogs	70.7	62.3
Newts	29.9	28.3
Others	29.9	26.4
Crustaceans	12.9	7.5

▲ **Table 6.** Level of knowledge regarding the species present in high mountain lakes (percentages)



◀ **Figure 1.** Level of knowledge regarding the problem of invasive species in high mountain lakes (percentages)

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~ Cap del Port de Peguera ~

~ Pyrenean sculpin (*Cottus hispaniolensis*) ~

CONSERVATION STATUS OF THE PYRENEAN SCULPIN *(COTTUS HISPANIOLENSIS)* IN THE ARAN VALLEY

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ABSTRACT

The Pyrenean sculpin (*Cottus hispaniolensis*) is one of the most threatened freshwater fish in Catalonia and Spain. Its status in the Aran Valley drastically deteriorated as a result of the floods of 2013, to the point of reaching its practical disappearance. Since then, their populations have been progressively recovering, with a slow increase in their average density and occupied localities. In part, this recovery has been produced thanks to actions framed within the LIFE+ LimnoPirineus project, which have been specifically designed for the recovery of this species within the Natura 2000 network space "Aigüestortes" (ES0000022).

INTRODUCTION

The Pyrenean sculpin (*Cottus hispaniolensis* Băcescu and Băcescu-Meşter) belongs to the *Cottidae* family (O. Scorpaeniformes) which is made up of freshwater benthic fish species that mainly inhabit cold and oxygenated rivers and lakes (Tomlinson & Perrow, 2003). It is endemic to the basin of the Garonne River (southwest France and Aran valley, northern Catalonia) and is restricted to its headwaters (Freyhof et al., 2005). The Garonne fish community in the Aran Valley is formed almost exclusively by Pyrenean sculpin and brown trout (*Salmo trutta* L. 1758), although minnow (*Phoxinus sp.*) and Alpine char (*Salvelinus umbla* L 1758) have also been introduced in some reservoirs and high mountain lakes (Aparicio, 2015). The Pyrenean sculpin was historically distributed in continuous populations along the main course of the Garonne River and in the lower parts of some tributaries (Sostoa et al., 1990; Doadrio et al., 2011). These populations have experienced strong regression in recent decades (Doadrio et al., 2011; Sousa-Santos et al., 2014). In addition, a strong flash flood in June 2013 in the Garonne and its tributaries further debilitated the precarious status of the Pyrenean sculpin (Rocaspana & Aparicio, 2017). For this reason, the species is currently listed as endangered on the Catalonia and Spanish red lists of Endangered Species (Doadrio et al., 2011; Aparicio et al., 2016). Just like *Cottus gobio* (former synonym), it is also included in Annex II of the European Directive on habitats 92/43. EEC.

In 2004, a first global diagnosis was made of the conservation status of the Pyrenean sculpin population in the Aran Valley (Rocaspana et al., 2004). Subsequently, periodic inventories were carried out that provided a guide to its evolution. However, the conditions caused by the strong floods of June 2013 led to the start of various diagnostic projects and conservation actions for the Pyrenean sculpin (Aran, 2014a, 2014b) that consisted of population censuses, the analysis of the degree of affectation of the flood and the evolution of populations throughout the year and a half following the flood.

Alongside these latest studies, the LimnoPirineus project (LIFE+ Natura LIFE13 NAT/ES/001210) was initiated to improve the conservation status of species and aquatic habitats of European interest in the Pyrenean high mountains. This project had the Pyrenean sculpin as one of its target species, planning various actions focused on the improvement and conservation of this species within the Natura 2000 "Aigüestortes" network space


▲ **Figure 1.** Image of a Pyrenean sculpin (*Cottus hispanoliensis*) individual from the river Garonne in the Aran valley.

(ES0000022). Among these actions, the most prominent was the consolidation of two population nuclei in the Ruda and Aiguamog rivers by releasing specimens from populations in good condition located in other sectors of the Aran Valley. These translocations also had to contribute to reducing the effects of genetic isolation suffered by these population nuclei located within the "Aigüestortes" area.

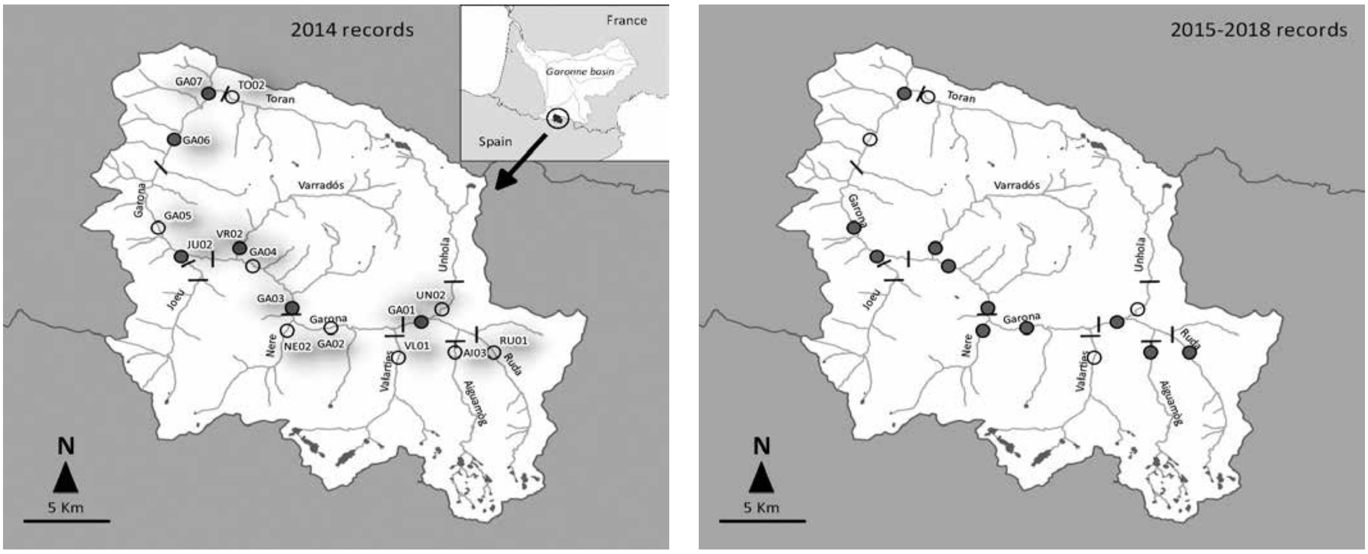
At the same time, regular monitoring of all the valley populations was planned in order to determine their status and trends. In addition, this monitoring has allowed increased knowledge of its biological and ecological traits.

METHODS

The monitoring of the Pyrenean sculpin populations consisted of sampling at 16 stations from 2015 to 2018. The sampling stations (80 to 200 m long) were selected according to the information of the historical distribution of the Pyrenean sculpin, *i.e.* the main course of the Garonne river and the lower parts of its main tributaries (Figure 1). Fish were captured by two pass electrofishing (pulsating direct current). All fish captured were anaesthetised with clove oil, identified at the species level, counted, measured (fork length FL, mm), weighed (g) and released at the same catch site. Abundance (N) of fish was estimated as $N = C_1^2 (C_1 - C_2)^{-1}$, where C_1 is the number of fish caught in the first pass and C_2 is the number of individuals caught in the second. The probability of capture was calculated as $p = (C_1 - C_2) (C_1)^{-1}$ (Seber and Le Cren, 1967). Fish density (individuals · ha^{-1}) was calculated by dividing the estimated number of fish by the sampled area. After sampling the fish, the composition of the riverbed substrate was visually estimated according to a modified Wentworth scale: boulder (particle size> 256 mm), cobble (> 64-256 mm), pebble (> 16-64 mm), gravel (> 2-16 mm), sand (0.1-2 mm) and silt (<0.1 mm). The percentage of the different mesohabitats present (pools, runs and riffles) were also recorded. A multiple linear regression (Stepwise multiple linear regression) was used to study the effect of habitat (substrate composition, mesohabitat) on Pyrenean sculpin density. For statistical analyses, fish densities were transformed into $\log_{10} (x + 1)$ and the percentage data was standardised by a square root transformation.

The translocations of Pyrenean sculpin specimens for the reinforcement, improvement and consolidation of the populations located within the Natura 2000 "Aigüestortes" network space, they had to be delayed in relation to the initial plans, due to the precarious state of all the populations of the Aran valley. But they were finally carried out between 2017 and 2018 (see Table 2). These releases were carried out using partly wild specimens directly from the natural environment and partly specimens from a captive breeding program that has been developed alongside the LIFE+ LimnoPirineus made by ADEFFA.

We were able to genetically characterize 203 of the captured individuals. By microsatellite analysis we were able to determine the distribution of the different genetic lineages throughout the Garonne course in the Aran valley. Genomic DNA was extracted using the Hotshot method (Montero-Pau et al. 2008), in a total volume of 100 ml. Each fish was genotyped for 24 microsatellite loci selected from the literature: Cott150, Cott113, Cott144, Cott164, Cot158, Cott153, Cott138, Cott105, Cott152, Cott173, Cott112, Cott130, Cot132, Cott154, Cott119, Cott118, Cott170, Cott163, Cott100, Cott127, Cott146, Cott128, Cott149, Cott175 (Nolte et al. 2005). Forward primers were labelled using four different fluorescent dyes and divided into three multiplexes. PCR amplification was performed in a reaction volume of 11 ml containing 1 ml of extracted DNA and 5.5 ml of Qiagen Multiplex PCR mastermix and primers. PCR amplification was performed with the Applied Biosystems 2720 thermal cycler. The PCR cycle protocol for all three multiplexes



▲ **Figure 2.** Map of the Garonne basin in the Aran valley and location of the sampling stations (see codes in table 1) showing the area of distribution of Pyrenean sculpin (*Cottus hispaniolensis*): (◦) indicates sampling locations and (•) indicates places where *C. hispaniolensis* has been positively recorded. The physical barriers (weirs and dams) are marked as perpendicular lines to the river.

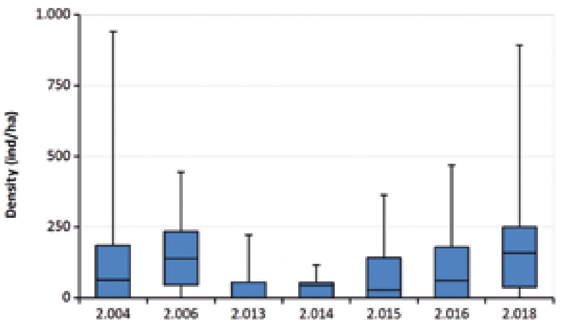
Station	Location	2004	2013	2014	2015	2016	2018
GA01	Garonne at Tredós	31		42	140	0	157
GA02	Garonne at Casarilh	0	0	0	19	0	27
GA02a	Garonne at EDAR Naut Aran				0	0	38
GA03	Garonne at Vielha	45	0	69	81	430	189
GA04	Garonne at Aubert	53		0	93	133	38
GA05	Garonne at Bossòst	0	111	0	31	173	285
GA06	Garonne at Les	0		44	0	0	0
GA07	Garonne at Pont de Rei	0	0	56	14	0	159
RU01	Ruda at Pont de Vaqueira	0	0	0	0	0	251
AI03	Aiguamòg at reservoir	218	0	0	0	59	269
UN02	Unha				0	0	0
VL01	Valarties at pont d'Aiguanera	0		0	0	0	0
NE02	Nere at Vielha				150	468	892
VR02	Varradós at the mouth	156	222	67	363	202	167
JU02	Joeu at the mouth	940	0	122	0	64	52
TO02	Toran at pont de Canejan	0		0	0	0	0

▲ **Table 1.** Sampling stations during 2015-2018 and Pyrenean sculpin densities (ind ha^{-1}) in the censuses carried out within the framework of the LIFE+ LimnoPirineus project (2015-2018) and in diagnoses of previous years (shaded in grey: not sampled).

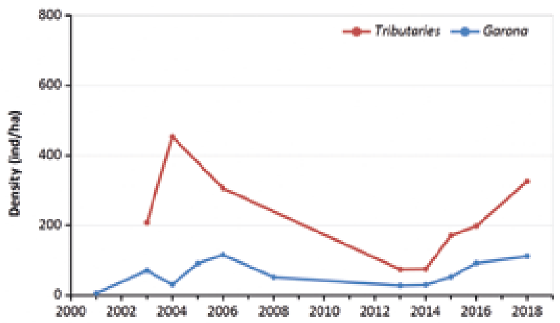
consisted of an initial denaturation of 15 minutes at 95 °C, followed by 38 cycles with 30 seconds at 94 °C, 90 seconds at 60 °C, 60 seconds at 72 °C, and a final extension of 30 minutes at 72 °C. PCR products were diluted 1:10 for all multiplexes and run on the analyser with 48 Capillars 3730xl DNA (Applied Biosystems). Alleles were flagged using the Geneious 11.1.4 program, and each genotype was checked with naked eye to detect badly named peaks. The genetic structure and genetically homogenous groups of all analysed individuals were obtained using the DACP program (Jombart et al. 2010).

RESULTS

The Pyrenean sculpin populations of the Aran Valley, evaluated by monitoring sampling, were not abundant (Table 1), confined to highly localised areas and mostly isolated due to the presence of artificial barriers (Figure 2). Brown trout coexists with the Pyrenean sculpin and is dominant in density at all points where the latter is present. Pyrenean sculpin density in the period evaluated (2015-2018) shows a slow recovery trend, after the demographic shortage of 2013 that almost led to its extinction in the Aran Valley (Figure 3). Sculpin density in the tributaries remained higher than that of the main course of the Garonne and the recovery trend is also higher (Figure 4).



▲ **Figure 3.** Box plot (median, 25th and 75th percentiles, maximum and minimum) of Pyrenean sculpin densities (ind ha^{-1}) between 2004 and 2018.



▲ **Figure 4.** Evolution of the average Pyrenean sculpin density (ind ha^{-1}) between 2001 and 2018 in the Garonne river and its tributaries.

Regarding habitat preferences, the linear regression showed significant relationships between Pyrenean sculpin density and the composition of the substrate, explaining 64% of density differences between sampling sites. The percentage of boulders was positively related to density (Coefficient: 0.52, *P*=0.011), while the percentage of pebbles had a negative relationship (Coefficient: -0.47, *P*=0.020). The rest of the substrate variables, as well as the mesohabitat variables (pools, runs and riffles) did not show statistically significant relationships with sculpin density.

The translocation and release of specimens in the Aiguamòg and Ruda rivers, within the Natura 2000 "Aigüestortes" network space, have produced positive results, with the restoration of a population in the Ruda river and improvement in the Aiguamòg river population (Table 2).

Date	Source	Destination	Number
4/12/2015	Centre Camadoca (Adeffa)	Garonne a Salardú (GA02) i riu Ruda (RU01)	75
25/07/2017	Garonne a Vielha (GA03)	Riu Ruda (RU01)	5
25/07/2017	Garonne a Les (GA06)	Riu Ruda (RU01)	5
26/07/2017	Garonne a Bossost (GA05)	Riu Aiguamòg (AI03)	4
26/07/2017	Riu Joeu (JO02)	Riu Aiguamòg (AI03)	2
22/12/2017	Centre Camadoca (Adeffa)	Garonne a Salardú (GA02) i riu Ruda (RU01)	80
29/08/2018	Garonne a Bossost (GA05)	Riu Ruda (RU01)	25
29/08/2018	Garonne a Bossost (GA05)	Riu Aiguamòg (AI03)	25

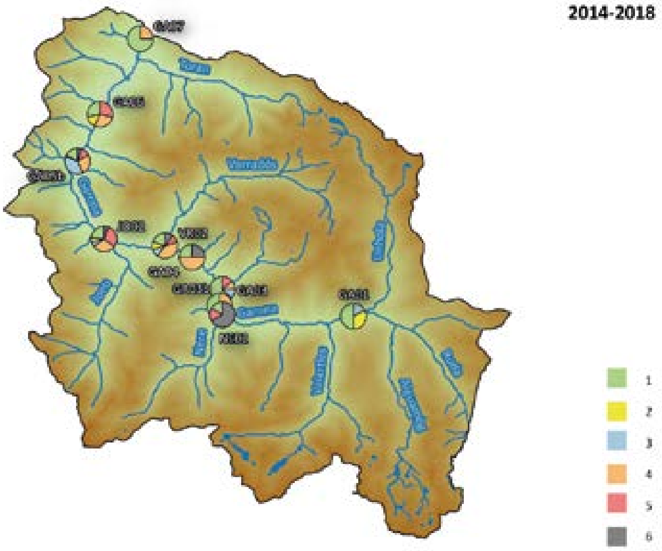
▲ **Table 2.** Releases of Pyrenean sculpin within the framework of the LIFE + LimnoPirineus project.

Genetic analyses indicate the existence of six differentiated genetic lineages, which are widely distributed along the river (Figure 5). Most lineages can be found at different points without a clear genetic structure. The sites located on both ends of the valley have the lowest proportion of lineages.

DISCUSSION

After the critical moment suffered by the Aran population of Pyrenean sculpin during 2013, due to the historical flood that occurred that year, the species continues increasing its distribution range, having been detected in the largest number of locations in 2018 since records began. At the same time, the population density has also continued to increase gradually, mainly due to the contribution of the tributaries (Figure 4). However, although part of this population improvement may be due to natural causes, it is important to keep in mind that in some of these tributaries, the Conselh Generau d'Aran [General Council of Aran] carried out translocations of specimens from the channel of the Bossòst hydropower station, a fact that undoubtedly influenced the densities observed. In the Garonne's main course, the average density seems to have stabilised at around 100 ind./ha (Figure 4). This could indicate that local abundance in each segment has reached a level that is already limited by habitat availability or by biotic interactions with other species (competition, depredation).

The presence of Pyrenean sculpin and its abundance is related to several habitat characteristics such as the substrate. The stations with the highest sculpin density had a higher proportion of boulders. Therefore, these types of substrate are positively selected by the Pyrenean sculpin and the stations with the highest proportion of rocks are more suitable for their development. These results are similar to those of previous studies (Legalle et al. 2005a, 2005b; Rocaspana & Aparicio, 2017),



▲ **Figure 5.** Canvis en la proporció de llinatges genètics (grup 1 al 6) de cavilat al llarg del riu Garona a la Val d'Aran.

and are probably related, firstly, to their reproductive requirements (Tomlinson & Perrow, 2003), and, secondly, with the microhabitat that forms under and behind the boulders, where the water velocity weakens and facilitates the movement of the Pyrenean sculpin ,that have a reduced natatory capacity (Legalle et al., 2005b).

As regards the improvement actions for Pyrenean sculpin populations located within the Natura 2000 “Aigüestortes” network space, the expected objectives have been achieved, with a general improvement of the two population nuclei now located within this natural area. Specifically, first, the reestablishment of the population nucleus in the Ruda river, which had long ago disappeared, has been achieved, and second, the improvement of the population of the Aiguamòg river, which was, in fact, about to disappear as a result of the great flood of 2013. However, it must be considered that both populations remain isolated from the other populations of the Aran Valley, due to artificial barriers or highly modified river stretches. Therefore, it will be necessary to monitor its progress, and to schedule new translocation actions in the medium term, if necessary.

Molecular results did not show a clear genetic structure of the different lineages along the Garonne as would be expected in a benthic species with a low mobility. Although a deeper analysis of the results is needed, it is possible that disturbances in the river, such as the 2013 flood with a drastic decrease in the population size of the species, have led to situations with strong bottlenecks that might explain the observed genetic pattern.

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~ Restauration of Font Grossa mires ~

RECOVERY OF MIRES AT THE RESERVOIR OF FONT GROSSA, ESPOT

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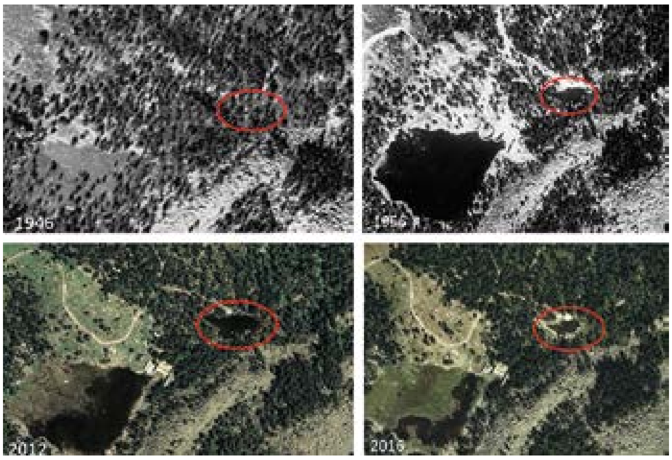
ABSTRACT

The Aigüestortes i Estany de Sant Maurici National Park has numerous hydroelectric infrastructures that modify the structure and function of the aquatic and semi-aquatic habitats. Within the framework of the LIFE+ LimnoPirineus project, we have taken the small reservoir of Font Grossa, disused for years, as an example of ecological restoration of mire habitats. Specifically, we have laid the foundations for the development of two types of Habitats of Community Interest, transition mires (HCI 7140) and sphagnum bogs (HCI 7110*). Given the little knowledge available regarding the restoration of Pyrenean mires, we have carried out a series of experiments focused on the structural plants of these types of mires, specifically *Carex rostrata, C. nigra, Juncus filiformis* and four species of *Sphagnum*. We have tested their growth capacity under different conditions of flood and competition, in order to select the most suitable for each situation. The experiments started under controlled conditions (growth chamber, greenhouse) and were finally developed at the Font Grossa site, under field conditions. Then, the restoration activities for the target HCIs were performed in the appropriate areas, which took place in the summers of 2017 and 2018. The monitoring and subsequent actions, carried out until summer 2019, corroborated good implantation of the *C. rostrata* population and foresee partially good implantation of the *Sphagnum* populations. However, the assembly of more or less stable communities is predicted to proceed slowly, constrained by extreme events such as strong increases in water flow (due to snowmelt in spring or heavy rains) or occasional extreme droughts.

INTRODUCTION

The use of high mountain lakes and rivers as hydropower producers has often led to alterations in the surrounding vegetation. The flooding of the lake shore, due to the regrowth of the bodies of water with the construction of dams completely eliminates the previous ecosystems, among which, different types of wetlands and humid meadows are often found. When you want to reverse this situation, and return to the natural water level, you have to face the difficulties of regenerating the vegetation and soils that were previously there (Moreno-Mateos *et al.*, 2015). To begin with, there is not usually enough information available about the previous ecosystems, and in addition the exposed wasteland that surrounds the lake is a space of very different characteristics to those that existed before the flood. Rebuilding a mire on the new shore of the lake will only be possible if the morphology is conducive and a more or less high water table can be maintained in the new soil. In addition, although there are sufficient experiences of restoring mires in boreal and Atlantic areas (Budelsky *et al.*, 1999; Caporn *et al.*, 2018; Quinty & Rochefort, 2003), there is very little information regarding Alpine systems, and none concerning the Pyrenees.

In the Peguera basin, in Espot, the elimination of the dam of the small reservoir called Font Grossa and its associated infrastructure in 2012, led to the opportunity to address mire restoration action (Figure 1). Thus, the LIFE+ LimnoPirineus project (2014) proposed that a demonstrative restoration of two mire Habitats of Community Interest (HCIs) be conducted at this location to serve as a real trial for other possible similar actions. Here it is necessary to consider the high number of hydrological alterations of different types found in the National Park and in the rest of the Pyrenees, which are likely to fall into disuse over the years, and therefore to be worthy of restoration.



▲ **Figure 1.** Ortho-images of the whole of Lladres and Font Grossa, in the Peguera basin, in Espot, on four key dates. In 1956 the two dams that generated the Lladres reservoir, which flooded a relatively large area of peaty meadow (1946 image) and that of Font Grossa, forested but probably also hiding another peaty area (1946 image) had already been built. A few years later, the Lladres reservoir progressed to its current status (lower images), which is usually almost empty. The reservoir at Font Grossa remained full until 2012, when it was reduced by the partial demolition of the dam (2016 image).

PURPOSE

The objective defined in the LIFE+ LimnoPirineus project is the restoration of transition mires and sphagnum bogs, two Habitats of Community Interest (HCI 7140 and HCI 7110* respectively). These are habitats of particular significance within the National Park for their relative abundance in this space and for their rarity in the Catalan Pyrenees (Pérez-Haase & Ninot, 2017). The two types of mire are well represented in the Trescuro lakes, located just above the Font Grossa and within the same basin, so it was decided to restore these two habitats and not others.

DIAGNOSIS

To carry out this restoration in a well-founded manner, we studied the characteristics of the Font Grossa environment, both of the strip exposed from under water in 2012 and the immediate environment. In the summer of 2014, we installed nine phreatometers to monitor the oscillations of groundwater and analyse it, we gathered vegetation relevés from the adjacent mire system, which is fed by lateral springs, and we drew up a detailed map of habitats (following the same methodology as Colomer *et al.*, 2019).

The strip exposed from under water following the elimination of the dam was quite irregular, containing numerous granite blocks. Among these, there was a set of relatively soft surfaces, formed by a sandy-silty substrate. Here, the water table remains relatively superficial, determined by the seasonal oscillation of the new water surface and subjected to sporadic highs and lows caused by the hydroelectric use of the water diversion to the Lladres dam. In fact, what would correspond to the Peguera ravine course runs through the bottom of the old reservoir. The water is retained by the base of the old dam and by rubble caused by its demolition, which leads to an almost natural pond. In addition, there is a significant upwelling between the granitic blocks of the SW, and some diffuse sources that already maintained a small mire prior to the reservoir, of which the part above the flood level was preserved.

In 2014, this sandy area was largely in the process of fairly active colonisation by more or less opportunistic mire plants, especially *Juncus articulatus*. Outside this exposed strip, a rather interesting wetland mosaic stretched up the eastern hillside, which included *Trichophorum cespitosum* fen (*Pediculari-Scirpetum cespitosi*), *Carex rostrata* populations (*Caricetum rostratae*), a few small stand of this carex with sphagnums (*Sphagno-Caricetum rostratae*) and remarkable sphagnum bogs with *Ericaceae* (*Vaccinio-Sphagnetum capillifolii*; Pérez-Haase *et al.*, 2012). Specifically, these last two communities correspond to HCIs 7140 and 7110*, targets of the restoration.

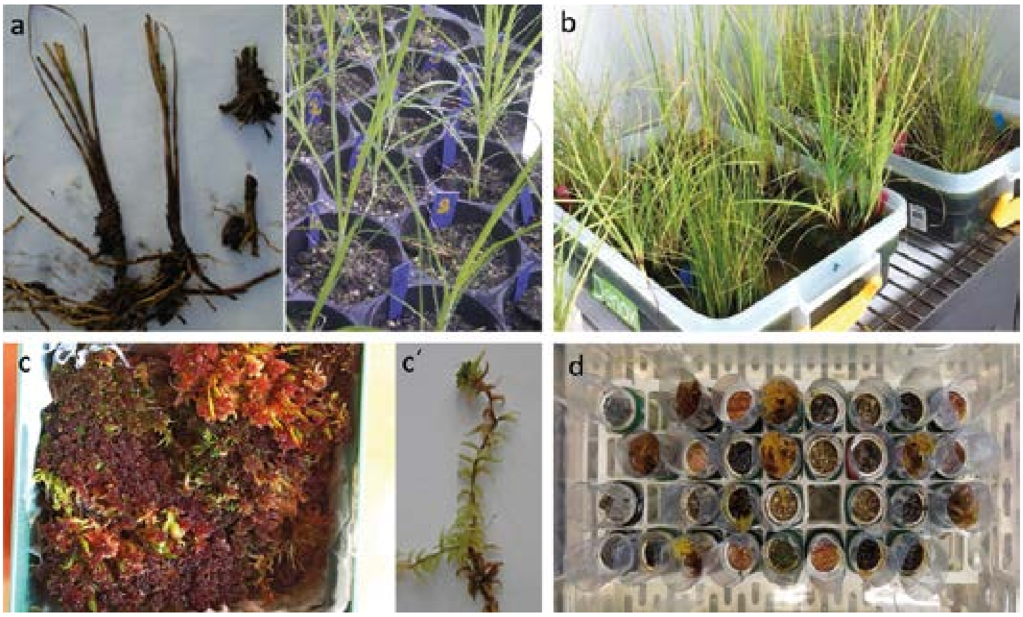
During the summer period of 2014, the water table remained between 35 and 47 cm below the surface above the sphagnum bogs, while in the rest of the communities it ranged mainly between 3 and 13 cm below the surface of the soil. It should be noted, however, that in the communities located at the lowest level (mainly those of *C. rostrata*) the water level would have been above ground until 2012, at least temporarily or partially, and so, that in 2014 and even now these plant communities respond in good part to past conditions.

As for the groundwater, these are poorly mineralised (between 17 and 67 µS/cm, most with only 5 to 15 ppm of calcium) and acidic (pH between 4.5 and 5.5).

PLANT MATERIAL FOR EXPERIMENTATION

Once the vegetation of mires around Font Grossa and Trescuro had been studied (Colomer *et al.* 2019; Colomer *et al.*, this volume), we selected some species as potential builders of the new habitats in the area to be restored. In all cases, they were species that have a structural role in one community or another, covering a certain range of the ecological gradient prevailing in mires, mainly that of flooding. In addition, they were clonal species, i.e. capable of forming large populations from the lateral expansion of one or few individuals (Keddy, 2010).

Most of the plant material was obtained from the surroundings of the Lladres reservoir, very close to Font Grossa (Figure 1). This reservoir serves to divert the water that flows down the Peguera river to the conduit channel in the Espot hydroelectric power station. According to the demand for energy, more or less water is released through the ravine towards Font Grossa, although most of the time the Lladres reservoir is almost empty. Therefore, its bed is colonised by populations of *C. rostrata* and other mire plants resistant to disturbance, mainly temporary immersion.



▲ **Figure 2.** Details regarding experimentation with plant material: *Carex rostrata* cuttings and *C. nigra* seedlings (**a**); chamber culture of *C. nigra* and *C. rostrata* under different flood conditions (**b**); small sward (**c**) and individualised plant (**c**) of *Sphagnum*; and chamber culture of *Sphagnum* fragments on different types of substrate (**d**).

In the case of vascular plants, we used *C. rostrata*, dominant in semi-flooded mires and on lake shores; *C. nigra*, abundant in mires of various types, generally less flooded; and *Juncus filiformis*, locally abundant in mires subjected to notable water table oscillation (Carrillo & Ninot, 1992). Of the three, in the autumn of 2014 we obtained hundreds of cuttings (basal regrowths with a rhizome fragment) from the populations that abound in the Lladres reservoir mire, as well as numerous infructescences with mature seeds from the same environment. During the following winter, in the greenhouses at the University of Barcelona, we produced numerous juvenile plants from the cuttings, and seedlings from the seeds (Figure 2a). As a general result, the low level of germination from the seeds obtained in all species and the long time it took to grow the seedlings led us to discard sowing in order to restore habitats with minimum efficiency. In contrast, most of the cuttings (between 49% and 89%, depending on the species) produced a juvenile plant, which in a few months had numerous leaves and had produced some regrowth.

Regarding mosses, we choose three sphagnum species: *Sphagnum teres*, typical of substantially flooded environments; *S. capillifolium*, typical of the upper part of the sphagnum bogs, in relatively dry conditions; and *S. magellanicum*, which is found in an intermediate spectrum of hydrological conditions, but which frequently form bogs. In the case of *S. magellanicum*, the fact that it is a species classified as Vulnerable in the Iberian Peninsula (Garilleti & Albertos, 2012) adds interest to the general restoration goal. From the three species we obtained small swards (few square centimetres) in the fall of 2014 and 2015, which we also grew and multiplied in the greenhouse (Figure 2c). Under these conditions, the sphagnum grew quite actively, both in height and forming new shoots.

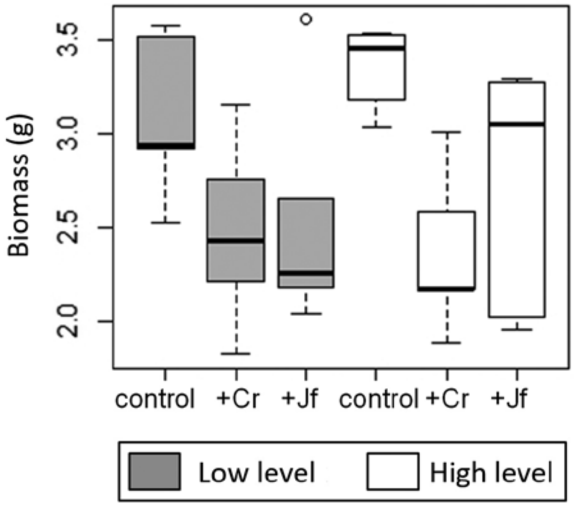
From the plant material produced, over the last four years we have evaluated the ability of the different species to germinate and grow, both in pure culture and in pairs, and under different ecological conditions, especially related to the level of flooding. A first block of experiments was conducted in culture chambers, with temperature and light conditions that mimicked those of early summer in the subalpine belt (16 hours of light at 22 °C, and 8 hours of darkness at 15 °C). Then other experiments were conducted directly in the field, in the natural environment and looking for the same conditions of the habitats we wanted to restore.

EXPERIMENTAL CULTURES

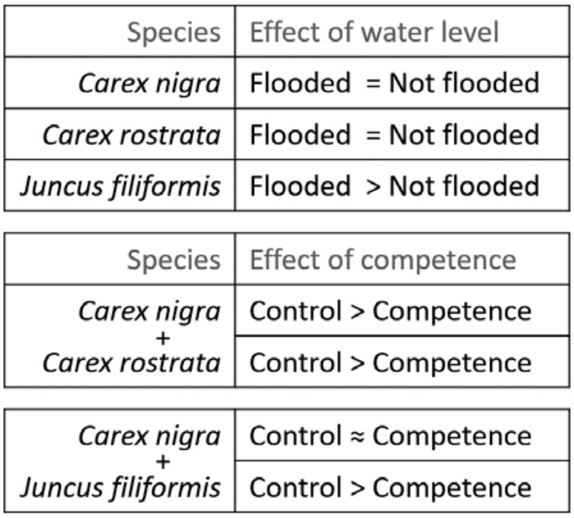
Sedges and reeds under controlled conditions

In spring 2015, 90 of the plants produced in the greenhouse of each of the three species (*Carex rostrata, C. nigra* and *Juncus filiformis*) and 90 seedlings of *C. nigra*, produced in the germination chamber, were used. They were placed in plastic containers with sterile and uniform substrate, in different combinations containing 3 + 3 plants of two different species; and batches of three plants of the same species were arranged in other containers as a control. All types were grown in a chamber for three months under two flood conditions, with water 2 cm above ground level and with water 5 cm below this level (Figure 2b).

At the end of the experiment, growth (leaves, rhizomes) was optimal in adult plants of the three species in pure culture. When two species coexisted, on the other hand, all species in general reacted to competition in the form of lower growth: the interaction between *C. nigra* and *C. rostrata* resulted in lower final values of aerial biomass (leaves) than those obtained in a control situation, while the interaction between *C. nigra* and *J. filiformis* caused lower growth in *J. filiformis*, but did not affect the sedges (Figures 3 and 4). The sedges were not affected by the water level, while *J. filiformis* grew less with a low water level. With respect to *C. nigra* seedlings, both flooding and interaction with adult plants affected them negatively, in the form of poor growth or mortality. We associate the inhibition of seedling growth to root anoxia in flooded conditions and the absence of light in the company of adult plants (Pladevall, 2015).



▲ **Figure 3.** Response of *Carex nigra*, evaluated as dry weight of the aerial part of the plants of each container, to the different cultivation conditions: pure population (control), with *C. rostrata* (+ Cr) or with *J. filiformis* (+ Jf); and with low or high water level.



Species	Effect of water level
Carex nigra	Flooded = Not flooded
Carex rostrata	Flooded = Not flooded
Juncus filiformis	Flooded > Not flooded

Species	Effect of competence
Carex nigra	Control > Competence
+ *Carex rostrata*	Control > Competence

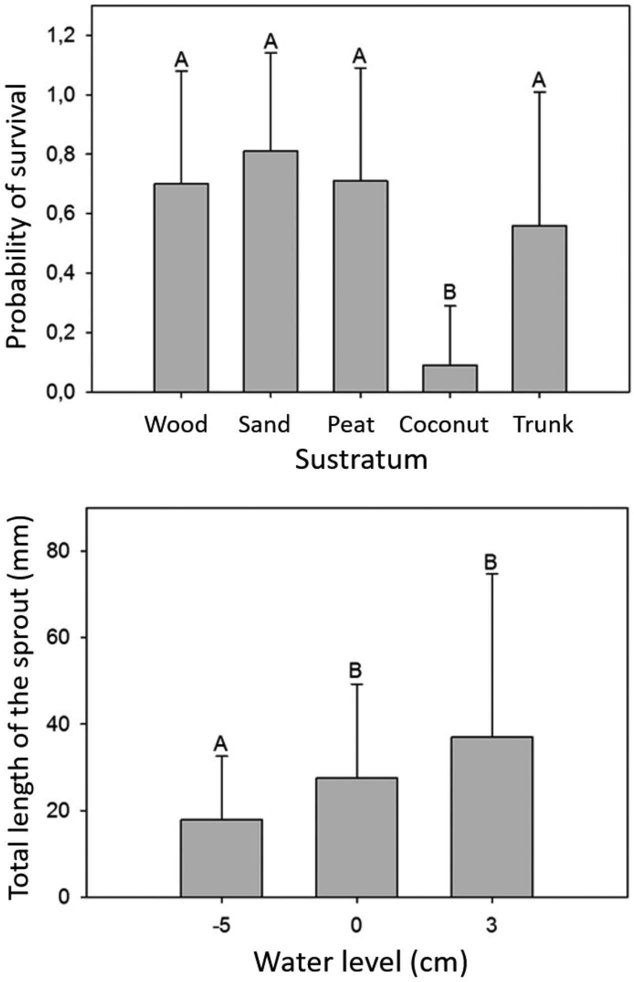
Carex nigra	Control ≈ Competence
+ *Juncus filiformis*	Control > Competence

▲ **Figure 4.** Summary of the response, relative to the dry weight of the aerial part, of the adult plants of the different vascular species tested, under distinct growing conditions.

Sphagnums under controlled conditions

In the spring of 2016, we assembled small culture containers, where fragments of sphagnum propagules were arranged, in the form of caulidia segments of 2 mm in length. Similarly, in the case of vascular plants, different combinations of pairs of species were arranged, based on 3 + 3 propagules for each container, and pure culture containers were also arranged for each species. With regard to the flood factor, three levels were established (5 cm below the substrate surface, at the substrate level, and 3 cm above). As the ability of the substrate to conduct water by capillarity is key in mosses, we experimented with different materials (new wood, partially decomposed wood, sand, peat and coconut fibre; Figure 2d).

After three months of cultivation, the survival of the three species was significantly affected by the type of substrate, with general preference for peat and sand. The type of substrate also gave differential growths in the two less hydrophilic species (*S. magellanicum* and *S. capillifolium*), which responded better to peat and wood, while *S. teres* responded to the water level, specifically growing more in more flooded conditions (Figure 5). In contrast, we did not detect effects of interaction between species, probably because they had not grown enough to exert competition.



▲ **Figure 5.** Response of *Sphagnum teres*, evaluated as plant survival and length, to different growing conditions; only the coconut fibre substrate and the lower water conditions gave significantly worse results than the other conditions.

To test the role of competition between the three species, a second experiment was conducted with a design similar to the previous one. In this one, larger fragments of

2.5 cm in length were used, which included the head (apical part of the sphagnum where the lateral branches are concentrated). The same three groundwater levels were established and only peat was used as a substrate. For all three species, the survival of the fragments was almost total. Growth was significantly affected by the level of flooding, and the three species grew more with water at the same level as the substrate. At this water level, the interaction between *S. magellanicum* and *S. capillifolium* affected them negatively, compared to when each of these two lived with *S. teres* (Espuny *et al*., 2018).

Based on the results of the two experiments, for restoration it is preferable to use large fragments, since they are more vigorous, and the type of substrate should be considered, since it has an important role in the survival of the fragments. A constant level of humidity favours growth of the three species, but the chemical composition of the water should not be ignored, because the *Sphagnum* species that form the bogs may be affected by the calcium in the lake water, although it is found in low concentrations. Competition between species may impair growth, and therefore should be considered when establishing location in restoration actions.

▲ **Figure 6.** Details related to field cultures: **a**, *Carex rostrata* plants at the end of summer 2015, the result of cuttings planted in June of the same year in different flood situations; **b**, planting of *C. rostrata* cuttings as the basis for the restoration of the transition mire; **c**, small peat containers with *Sphagnum* shoots ready to be transplanted; **d**, small population of *Sphagnum* growing from the containers through the protective mesh fixed with wooden stakes.

Cultures under field conditions

At the beginning of the summer of 2015, an experiment was set up with sedges and reeds considering two factors, flooding and competition, comparable to that carried out in the controlled conditions chamber, regarding adult plants of the same three species (Figure 6a). This experiment was monitored until the end of summer 2016.

Some of the blocks (where the soil was coarser-textured) showed very poor growth, including mortality of some plants, especially as a result of a dry season that occurred in mid-summer 2015. The rest grew well in both water level conditions. After two summer periods of growth, we could conclude that *C. rostrata* lives better in flood conditions, regardless of whether it has competition, while *C. nigra* grows less in the company of *C. rostrata*, in both situations. *Juncus filiformis* did not respond to different experimental situations. Therefore, *C. rostrata* showed good capacity for implantation and establishment from cuttings in the environment to be restored, both out of and in the water.

In mid-summer 2015, the rest of the plants produced in the greenhouse were also transplanted, mainly *C. rostrata* and *C. nigra*, but also *J. filiformis*, *Carex panicea* and other mire species. In late summer, 100 more cuttings of *C. rostrata* were used to transplant them to five blocks at different points that were conducive to them, in a status of mild flooding (few centimetres underwater).

In general, there was a good response from the three main species, for both survival and growth, until early summer 2017. However, some of the *C. rostrata* plants planted in the water towards the entrance of the reservoir disappeared, most likely dragged by the flow during the most flooded periods.

As for the sphagnum, small containers of pressed peat were prepared, each with four shoots of one species of *Sphagnum* or another (Figure 6c). In the summer of 2016, these containers were semi-buried in appropriate microenvironments on the shore, already planted the previous year with *C. rostrata*; and another set were placed in the same plots as the factorial experiment plants, once these were collected. The containers, in groups of four, were covered with a wide-mesh natural fibre hessian, and fixed to the substrate with untreated wood picks (Figure 6d).

Throughout the summer of 2016 there was a certain mortality in these sphagnums, affected by the surface drought of the substrate. In addition, between the autumn of 2016 and the spring of 2017, some were disturbed, or disappeared, due to episodes of greater intensity in the water current. This highlights that the restoration of populations of sphagnum encounters more obstacles than that of vascular plants, especially due to unfavourable episodes such as relatively dry periods in summer or overflows following thaws or strong storms.

Restoration actions

In summer 2017, most of the actions aimed at restoration were carried out, following a protocol based on the experiences described and documentation (Ferland & Rochefort, 1997; Quistberg & Stringham, 2010; Wittram *et al.*, 2015). This protocol defined the actions of conditioning and planting for different species, following a mapping scheme (Figure 7). Its main action was to establish lax populations of *C. rostrata*, which should be the construction base of the transition mire (HCI 7140), leaving the implantation of hydrophilic sphagnum for the second phase and, eventually, implantation of other mire plants. Also, we left for this second stage the creation of nodules for the bog development of *S. capillifolium* and *S. magellanicum* with *Ericaceae* (HCI 7110*).

▲ **Figure 7.** Plan of the restoration of Font Grossa, where the outermost blue line indicates the perimeter of the reservoir before demolishing the dam. Blue, free water; blue-green, planting area of *C. rostrata* semi-flooded; yellow, planting area of the same species out of the water, and creation of sphagnum bogs; grey, formerly flooded area, not suitable for target mire habitats (subject to spontaneous recolonisation by meadow and opportunistic plants).

In August 2017, the planting area was conditioned with stakes and boards of untreated wood, in order to protect the sediment from water currents during the rooting and growth period of *C. rostrata*, and a small lax palisade was also installed in the water, which will reduce the current during episodes of regrowth. As a preventive measure against trampling and herbivory by livestock, the entire restoring area was surrounded with electric fencing (Figure 8).

Meanwhile, about 5000 cuttings of *C. rostrata*, similar to those used in the experiments, were obtained from the neighbouring Lladres reservoir and transplanted in a lax plantation framework (about 15 cm between plants) on the surfaces to be restored (Figure 6b). These made a total of about 135 m^2, in the form of a strip of land, more or less wide according to the morphology of the shore, that has a flooded part and a part out of the water. In autumn 2017, small containers were transplanted with *S. teres* and *S. subsecundum* (a species ecologically similar to *S. teres*) protected by a hessian mesh. They were located in the middle of the *C. rostrata* plot, to ensure stability of implantation before the spring thaw.

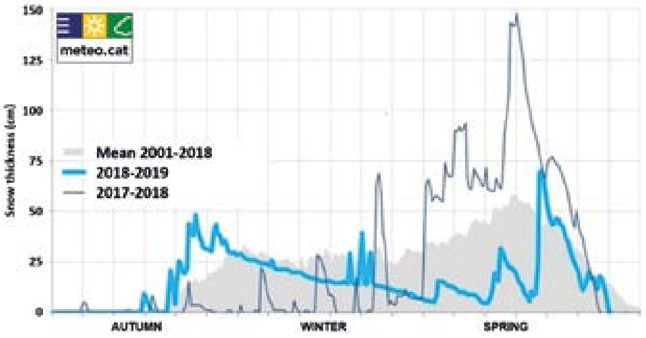
The creation of sphagnum bogs (HCI 7110*) began the same summer of 2017 with the accumulation of pieces of dead wood from the surroundings, interspersed between the replanted areas. We tried to place them on the outside of the *C. rostrata* formation and outside the direct influence of the reservoir water.

The definitive introduction of small swards of four different species of sphagnum, typical of the two HCIs to be restored, was done in late spring and early summer 2018, once the methodology used had been evaluated. In the transition mire (HCI 7140) we introduced small nodules (small groups of cuttings) in peat containers such as those already described, with *Sphagnum teres* and *S. subsecundum*. The containers were planted in groups of four and fixed to the substrate with hessian and wooden picks, as described above (Figure 6d). We arranged them following three levels of flooding, and in seven different sectors of the *C. rostrata* formation. In a part of the containers we added seeds and cuttings of species from

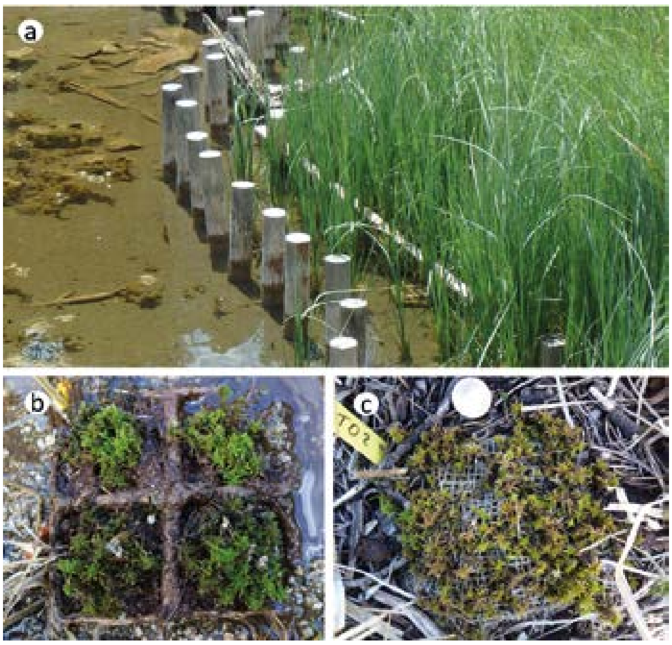
◀ **Figure 8.** Partial view of the restoration of Font Grossa at the end of summer 2017. Semi-submerged boards and stakes protect the transplanted population of *C. rostrata*, which occupies the soft edges from a few cm below the water to a few cm above. Electric tape protects the whole from herbivory and occasional trampling.

this habitat (*Carex canescens, C. rostrata, Viola palustris*).

For the sphagnum bogs, we use a methodology similar to that of the previous habitat, but using *S. capillifolium* and *S. magellanicum* as building species. In summer of 2018, we transplanted container blocks with different combinations of the two species, also fixed with hessian and picks; and we introduced some species from this habitat, both cuttings (*Calluna vulgaris*) and seeds (*Potentilla erecta, Drosera rotundifolia*). In this case, the containers were placed in the peripheral area of the pond, especially in the part where there are diffuse springs and on dead wood or sheltered by it, to act as a growth nodule of the ombrophilous sphagnum.



▲ **Figure 9.** Dynamics of snow thicknesses at the alpine station of Espot (2,519 m) for the two years of restoration work and, on average, the previous nine years (graph from the Servei Meteorològic de Catalunya [Catalonia Meteorological Department). The abundant snowfall of spring 2018 led to strong water currents and high levels at Font Grossa in early summer, fed by the rapid melting of snow. On the other hand, the low snow reserve of 2019, together with the little rain and high temperatures of the beginning of the following summer, caused a low water level from the beginning of that summer.



▲ **Figure 10.** Favourable development of structural species: **a**, *Carex rostrata* population densified and well-established in the summer of 2019, two years after implantation, and which in some places expands spontaneously (central part of the image) through water beyond the protection table; **b**, one of the sphagnum nodules already installed, just before covering it with hessian in June 2018; **c**, the same nodule, in May 2019, rising above the mesh.

Monitoring

The first results regarding the establishment of the *Carex rostrata* population (HCI 7140) were good. There was continuous monitoring, more frequent in 2017 and less so in 2018 and 2019, of different control plots and of the system in general, based on images.

There was rooting and new regrowth, and the plants survived two very strong floods in the summer of 2018: that from the thaw, which was very intense due to the large amount of snow accumulated in this year, and that caused by one extraordinary rainfall during the month of August. Both caused a sudden rise in level and, especially the second, a strong current of water that damaged a small part of the protections, moved large stones and dismantled part of the wood arranged to facilitate the creation of bogs. The sedges were all still in place, although in some sectors they were half buried by flood debries.

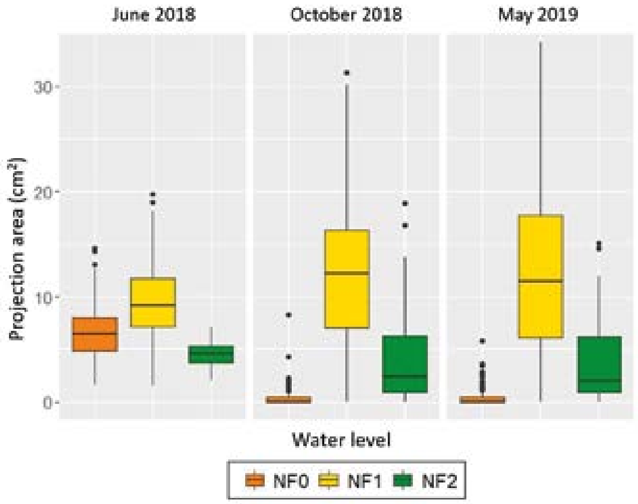
2019 had poor rainfall in winter and spring (Figure 9). The relatively low arrival of snow melting water, together with low rainfall and high temperatures during the first half of the summer made the water level in Font Grossa low throughout the summer, since the beginning. For this reason, the outermost strip of *Carex* had weak development, and the individuals in the most flooded sector continued to grow within the reservoir. Despite these extreme episodes, the dynamics of extension and densification, together with the fact that individuals introduced in 2017 have already flourished and fructified, allows us to consider the *C. rostrata* population consolidated and fully capable of structuring habitat 7140 (Figure 10a).

The sphagnum, despite being more recently introduced (June and July 2018) have remained in place, and grow by breaking through the hessian mesh that protects them. This protective mesh has been a key element to avoid the displacement of the small swards with the floods and to give some protection to the young plants against an excess of radiation and high temperatures.

One part (one in seven batches) of the Sphagnum introduced in the transition mire was buried by sand and gravel arriving with the flood of August 2018, and another small part was covered by a thin layer of sediment. The rest, and those of the bogs, reached autumn 2018 in good condition. At the end of May 2019, the different batches were monitored with overhead images and height measurement. In the case of the sphagnum located within the Carex rostrata plot, except for the samples affected by the flood last August, most were alive and growing well, although they showed differences between the three flood levels. Since the water level was generally high in the summer 2018, those further in the pond showed poor growth and some mortality. On the contrary, the high temperatures and the low water level of the summer 2019, meant that those farthest from the water had problems for growth and survival. Thus, the samples in the middle zone are the ones that have been best established; in some cases, the sphagnum, in addition to exceeding the hessian have begun to extend laterally out of the containers (Figures 10c and 11).

As for the sphagnum bog (HCI 7110*) regeneration nodules, a small part already presented problems at the beginning of summer 2019, mainly those that were established on dead wood, as opposed to those established on peat, which survived well throughout the first year. Most likely, the heat episode that has affected much of the Pyrenees and the European continent in July has caused difficulties in the growth and survival of the sphagnum in general. It should be considered that, although Sphagnum capillifolium, and even S. magellanicum, are sphagnum tolerant to temporary desiccation (they are usually found in places with low water level), they may not survive exceptional drought conditions, since they depend specifically on rain water. We have detected poor vitality and injuries in well-established natural bogs in areas near Font Grossa and in different places in the central Pyrenees.

Of the vascular species implanted in summer 2018 among the sphagnum nuclei, at the beginning of summer 2019 the Viola palustris and Calluna vulgaris cuttings had progressed reasonably well. On the other hand, very little generalised germination was observed, perhaps due to erosion of the seeds by water currents.

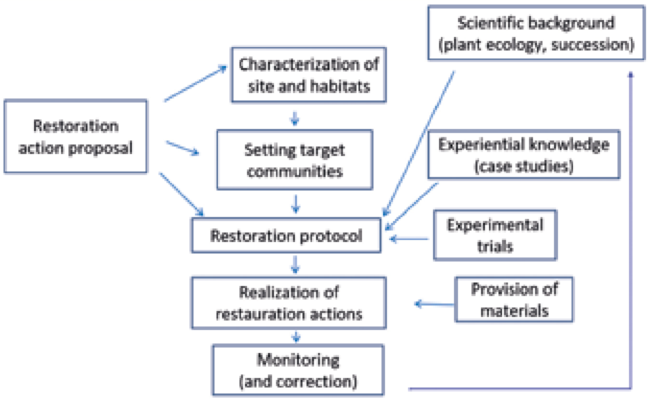


▲ **Figure 11.** Progression of the *Sphagnum* propagules used in the restoration experiments (measured as the projection area of the group planted in each container) throughout the first year, in the shoreline sand of Font Grossa, according to the three water levels considered: NF0, planted a few cm underwater; NF1, approximately at water level; NF2, few cm above the water.

Perspectives and general context

We expect both the *Carex rostrata* mire with sphagnum (HIC 7140) and the sphagnum bogs (HIC 7110*) to develop relatively slowly, depending on the different components of these plant communities. Thus, the *C. rostrata* populations were satisfactorily established in a short time, offering a relatively stable structure to the other components, while for the sphagnum populations we expect a rather slower and more random process, depending on environmental conditions. Simultaneously, several vascular wetland plants (*Carex flacca, C. lepidocarpa, Pinguicula grandiflora, Potentilla erecta,* etc.) were spontaneously introduced. In addition, even with a short monitoring period, the weight of extreme weather events in the course of succession has been verified. The temporality and intensity of these periods may substantially change the forecasts.

Here, we want to emphasize the importance of monitoring the peat system of Font Grossa, especially as we do not have similar experiences in the Pyrenees. This monitoring is key, not only to be able to assess the progress made, but to describe the plant succession in reasonable detail. In a more general context, it should be considered that restoration actions and ecological knowledge of natural systems must coexist and strengthen each other (Figure 12). Just as it is unreasonable to begin restoration actions without a minimum knowledge of the structure and function of ecosystems, this ecological knowledge also feeds from management and restoration actions. And it does so consistently, only if these actions are based on a reasonably precise protocol, and if proper monitoring of the system is conducted.



▲ **Figure 12.** Conceptual summary of the actions undertaken following the restoration of mires on the shores of the Font Grossa reservoir. From the characterisation of habitats to the monitoring, as well as the studies and experiments, most respond well to the proposed restoration target, while the monitoring and results of these studies are important as a test, and as a scientific basis in ecology of communities.

ACKNOWLEDGEMENTS

Volem fer arribar el nostre agraïment al personal del Parc We want to extend our thanks to the staff at Aigüestortes i Estany de Sant Maurici National Park, who have endorsed this action to recover mires. We must also thank several colleagues and trainees, who have helped in one task or another of the experiments or monitoring for their collaboration.

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