TEMPERATURE VARIABILITY, PREDATION PRESSURE AND ZOOPLANKTON COMPOSITION in Pyrenean high mountain lakes

Ibor Sabás Saludas
DOCTORAL THESIS
Temperature variability, predation pressure and zooplankton composition in Pyrenean high mountain lakes

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Doctoral Thesis
Portada, contraportada i portades interiors

*El laguito y el complejo estudio de sus bichejos*

Ra Paredes
Temperature variability, predation pressure and zooplankton composition in Pyrenean high mountain lakes

Variabilitat tèrmica, pressió de depredació i composició del zooplàncton en estanys d’alta muntanya dels Pirineus

Memòria presentada per Ibor Sabás Saludas per a optar al grau de doctor per la Universitat de Barcelona

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Mai, mira-me as mans; 
as trayo buedas, 
lasas d’amar...
Son dos alas
d’un viello pardal 
que no puede 
sisquiera bolar.

Mai, mira-me os güellos, 
n’o zielo perdíus 
n’n un fondo silencio...
Son dos purnas 
chitadas d’o fuego 
que no alumbran 
ni matan o chelo.

Mai, mira-me l’alma 
aflamada de sete, 
enxuta d’esperanza...
Ye un campo labráu 
an no i-crexen qu’allagas 
que punchan a vida 
dica qu’a matanm

Mai, mira-me à yo. 
Me reconoxes , mai? 
Fué o tuyo ninon... 
Uey só un home 
que no sé como só. 
Mai, me reconoixes? 
¡MAI!, ni sisquiera tu?

Ànchel Conte, *No deixez morir a mía 
voz* (1972)
Agraïments

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Contents

General abstract ........................................................................................................................................ 3

1. General introduction ............................................................................................................................. 5

   Objectives ............................................................................................................................................ 13

PART I Temperature and ice phenology of Pyrenean lakes .................................................. 17

   2. Modelling surface thermal conditions of Pyrenean high mountain lakes
   for spatial and climate change projections ......................................................................................... 17

   3. Modelling ice phenology in high mountain lakes of the Pyrenees and its
   influence on summer water temperatures ....................................................................................... 45

PART II: Relative effects of temperature and predation on zooplankton ............ 67

   4. The relative importance of predation and temperature on the
   zooplankton of Pyrenean high mountain lakes ................................................................................. 67

5. General Discussion .............................................................................................................................. 101

6. General Conclusions .......................................................................................................................... 113

Bibliography .......................................................................................................................................... 117

Appendix ................................................................................................................................................. 133
General abstract

The current context of anthropogenic global change, and particularly climate change and biological invasions, stress the importance of assessing the effects of biotic and abiotic factors on the communities. Therefore, it is fundamental to have a wide knowledge about variables related to these stressors, in order to better understand communities’ composition and future changes. One of the most certain effects of climate change is temperature warming, to which Pyrenean lakes are susceptible. Moreover, though lakes of the Pyrenees are remote, they have been introduced with fish in great proportions. In addition, zooplankton is sensitive to both temperature and predation.

The aim of this thesis is to unveil the factors which explain water temperature during the ice-free period and ice phenology in Pyrenean lakes to have further a better understanding of their effects on zooplankton, together with predation pressure, of fish and other natural predators, in relation to the environmental factors which explain zooplankton composition.

In order to understand the temperature variability in Pyrenean lakes, we conducted a monitoring of 59 lakes from 2001 to 2014, from which we could study temperature conditions during the ice-free season and the ice phenology. Mixed models were developed with these data in order to know which environmental variables can explain main thermal variables and ice phenology. With these models, it was possible to project temperatures and ice-cover periods, along the Pyrenees, and, in the future, by downscaling air temperatures projections, of Global Climate Models.

Following the models’ projections, accumulated degree-days are expected to increase more in lowlands, whereas maximum temperatures are expected to show greater increases at higher altitudes. Mean ice-free period surface water
temperatures are expected to increase an average of 2 °C from current mean temperatures of 11.3 °C by 2090 in Pyrenean lakes.

All the studied lakes froze in winter. Mean ice-cover duration was of eight months among the Pyrenees, ranging from 4 months to 11. Using the models, future decrease in ice-cover duration of between 23 and 32 days by 2090 was foreseen, mainly due to the advance in ice-off.

Regarding zooplankton communities in the Pyrenees, we found that the dominant crustacean species were *Daphnia longispina*, *Cyclops abyssorum*, *Eudiaptomus vulgaris* and *Chydorus sphaericus*, and the dominant rotifer genera were *Kellicottia*, *Keratella*, *Asplanchna*, *Polyarthra* and *Conochilus*. The zooplankton composition was principally explained by geomorphological and chemical/phytoplankton variables. Also, introduced fish species, temperature and ice phenology explained zooplankton. On Pyrenean scale, salmonids explained zooplankton variability, whereas, when analysing fish abundance effects on zooplankton, minnows were the ones to show a negative effect. Different predation pressures were observed among predators of zooplankton, having minnows a greater impact. We could also observe differences in zooplankton sizes due to selective fish predation. Moreover, fish had a negative effect on fecundity, whereas fecundity could be recovered by lakes’ restoration removing invasive fish.
1. General introduction
Global change: a global context

The Earth system has changed through time, and so have done particularly geological formations, atmosphere, climate and living organisms. On particular occasions since the beginning of life, the Earth’s history has faced dramatic changes reflected in great mass extinctions, five of which involved the loss of more than 75% of the known species at the time. They were caused by various synergistic events, but climate changes were somehow involved (Barnosky et al. 2011). Nowadays, we are living in an interglacial epoch named Holocene, which started approximately 11,000 yr BP, after the last glacial era. In this time, human societies have been inducing changes in the environment at a greater scale than any other organisms. Important changes started with the Neolithic revolution, since 8000 yr BP, with the development of agriculture and stockbreeding, which through the centuries modified vast territories. The massive arrival of Europeans in America since the XVth century caused an increased dispersal of the biota through colonization, trade and transportation, which also had a deep impact in American human populations. Industrialization, which began by the end of the XVIIIth century with the invention of the steam engine, caused a great increase in human population, especially since the 1950s, with the development of fertilizers and the improvement of public health. Global changes have gone so far that, even the beginning of a geological new epoch, named Anthropocene, is being discussed (Steffen et al. 2007; Lewis and Maslin 2015). Two of the features of current human-caused global change are climate change and biological invasions, which, in turn, can also induce biological loss of diversity (Vitousek et al. 1997). Recent reports describe that global temperature has increased by 1 °C since pre-industrial times (1850-1900) due to greenhouse gas (GHG) emissions, and it is likely to reach 1.5 °C by 2030-2052 following current trends (IPCC 2018a). The worst scenarios include an increase in global temperature up to 4.8 °C (IPCC 2013b). The loss of species is higher than the background rates and, if conservation measures are not taken, we would be facing a 6th mass extinction (Barnosky et al. 2011).
Climate change effects

As mentioned before, one of the most notorious components of global change is climate change. The climate has changed in the past due to variations in the orbit of the Earth known as the Milankovitch cycles, as the Earth’s orbit changes in eccentricity, obliquity and precession, with the consequent effects on insolation. Another factor of climate change is volcanic activity, which, through the high emission of particles, decreases temperature, reducing the radiation arriving at the surface of the planet (Angell and Korshover 1985). If we focus on current climate change, there is no doubt about the anthropogenic origin of it, as the emissions of CO₂ and other greenhouse gases have increased as a consequence of the increasing combustion of fossil fuels by a human growing population. These gases absorb and emit infrared radiation, keeping the atmosphere warm; in fact, they are responsible for the mild temperatures which allowed the development of life. However, as their concentration increases, air temperature rises. One clear example of these emissions are the rise in CO₂ concentrations recorded in Mauna Loa in Hawai (Keeling et al. 1995), and the measured air temperature increase (IPCC 2013b). Air temperature is only one of the effects of Climate Change, the most certain, but other effects are derived, such as acidification due to the increased CO₂, which has a great impact in the oceans as coral reefs are very vulnerable to acidification (Hoegh-Guldberg et al. 2007), but lakes can also be affected. Air temperature increase is reducing glaciers and permafrost extension, reducing solid precipitation in favour of rain and thus reducing ice-cover in lakes and rivers. The increased water volume is causing, as a consequence, a sea level rise. Moreover, there are evidences of atmosphere circulation patterns moving towards the poles. Precipitation is more difficult to measure, so the confidence in future projections is lower. Humidity and precipitation have increased globally and are projected to increase, whereas, in the Mediterranean basin, precipitations are decreasing and are expected to do so by the end of the present century. In addition, the increase in extreme heat, drought and storm events’ magnitude and frequency are expected (IPCC 2013b). We are exceeding the time needed to prevent further climate change and its derived effects, being thus in a state of climate emergency (Lenton et al. 2019).
Biological invasions

Biological invasions are not a new phenomenon. Since Neolithic times, humans started to manipulate the habitats with the development of agriculture and cattle stocking and the consequent movement of species. Through increasing populations, trade and transportation, species introductions have increased (Hulme 2009). One tipping point in the history of introductions was the discovery of America by Europeans, which lead to increased species introductions around the globe (Lewis and Maslin 2015). The routes created by man allowed the movement of species through them; a perfect example of this is that in the Mediterranean Sea most of the alien species entered through the Suez Canal, so they are called Lessepsian introductions. Ship transportation is one of the most important sources of alien species in the sea, together with those coming from aquaculture (Streftaris et al. 2005). In the case of freshwater systems, mostly in Europe, they have been mainly introduced with allochthonous species through aquaculture, pet or aquarium trade, stocking and shipping. Most of the alien species were of European origin (Nunes et al. 2015). In Europe freshwater habitats, fishes have been the most introduced group.

For an alien or introduced species to become invasive, not only does it have to be transported to a location, but it also has to show reproduction and spread in the new habitat and produce negative effects on it. Invasive species not only cause impacts, but they can also have negative effects on the economy of human societies (Sakai et al. 2001; Pimentel et al. 2005). The invasibility of an ecosystem depends both on the introduced species and the ecosystem. Alien species can be invasive by having similar traits as native species, or the other way round, by occupying inexistente niches.
Global change: the Pyrenees

Pyrenean lakes date back to the end of the Pleistocene and the beginning of the Holocene, at the end of the last glacial period, when the glaciers retreated and left glacial valleys with over 3,000 lakes and ponds in existence nowadays. Lakes have changed since their formation, for instance, in the productivity variations of Redon lake; at the beginning, during 2 thousand years, productivity was high due to the phosphorous entrance from the catchment. Later, during 6 thousand years, productivity decreased due to the development of vegetation in the catchments and consequent phosphorous retention. Since 1500 yr BP productivity augmented in relation to the increased sheep stocking (Buchaca 2009). Other past reconstructions from lake sediments indicate changes in communities of the lakes, such as diatoms or chironomids, which responded to climatic variations in temperature (Pla and Catalan 2005; Tarrats et al. 2018). They showed a warming trend during the early Holocene (10000-9000 yr BP), with warm temperatures in the mid Holocene (9000-6000 yr BP), then temperatures decreased showing low temperatures at the beginning of the last Holocene (4000-2000 yr BP) and an increase in temperature in the last 2 millennia. Reconstruction of air temperatures in altitude from instrumental records showed air temperature increases during the 20th century (Agusti-Panareda and Thompson 2002). Sediments also had pollen and charcoal records from which land cover could be reconstructed. They showed humid conditions with higher water levels in Basa de la Mora lake during the mid-Holocene, and increasing aridity until present. Also, anthropogenic influence in the land covers could be seen in the last 700 years (Perez-Sanz et al. 2013).

Although remote, the Pyrenees have been for long occupied by humans. First remnants of human presence date back to late Pleistocene (18600 BC) in Central Pyrenees, and they were hunter-gatherer groups. First evidences of farming and livestock in the peripheries of the Pyrenees began around 5500 BC. Since then, these practices were extended to higher altitudes (Gassiot Ballbe et al. 2017). Human populations changed the vegetation through fires to favour their use of the land. Later, in the bronze and iron ages, mining in this
region lead to an increase in heavy metal concentration in lakes around 3000-2500 BC. Anthropogenic pressure also increased since the middle ages until the XIXth century (Gassiot Ballbe et al. 2014). Linked to human populations in this area, the first historical record of fish introductions in Pyrenean lakes dates back to 1371 A. It consists in a written document, which regulated fishing rights in lake Evol (Miró and Ventura 2011). More documents like this were found dating from the XIVth and XVth centuries, which are informative of trout introductions and their commercial use. By 1900, 26.5% of high mountain lakes had already been introduced with brown trout (Salmo trutta), which was normally introduced from nearby rivers. An important fish introduction wave took place between 1960 and 2000, which resulted in a 52% of lakes being introduced with fish. These introductions were related to fish management by governmental agencies, hydroelectric companies and local angling societies related to recreational fishing of trout. Not only was brown trout introduced, but also other salmonids such as the rainbow trout (Oncorhynchus mykiss) and brook trout (Salvelinus fontinalis), which were introduced from North America (Miro and Ventura 2013). Another fish species which was introduced was the minnow (Phoxinus sp.), which was used as a live bait for trout angling. Minnow introductions were done mostly in lakes which already had salmonids and affected a 27% of the lakes (Miró and Ventura 2015). Fishing bans in protected areas, such as the Aigüestortes i Estany de Sant Maurici National Park founded in 1988, prevented further introduction of fishes at the expense of surrounding areas, where introduced lakes still increased (Miro and Ventura 2013). Another impact, which started in the XXth century was the impoundment of some lakes in order to obtain hydroelectric power, which may result in alteration of littoral and benthic biota due to water level changes, for instance, in macrophytes (Gacia and Ballesteros 1996). Recent sources of pollution include heavy metals, especially increased lead (Pb) concentrations from leaded gasoline combustion, nitrogen and sulfate oxides, which can acidify the lakes, polycyclic aromatic hydrocarbons (PAHs) increased by fossil fuel combustion, and newly synthesized organochlorines (OCs). This pollutants can travel long distances through the atmosphere from their source and, although they may not appear in high concentrations, they can bioaccumulate in organisms and through the trophic net (Catalan et al. 2006).
Objectives

Main objective

The main objective of this Thesis is to evaluate the importance of abiotic and biotic factors, and more specifically, temperature, ice phenology and predation by invasive fish on the zooplankton communities of the Pyrenees.

Specific objectives

Part I Temperature and ice phenology of Pyrenean lakes

Modelling surface thermal conditions of Pyrenean high mountain lakes for spatial and climate change projections

Whereas the thermal characteristics of the lakes have been described for many regions, and empirical models have been developed to have a better understanding of the thermal functioning of the lakes, the Pyrenees are still underrepresented, and, if we want to analyse the effect of thermal variables on zooplankton in present day and in the future along the Pyrenees, it is of paramount importance to have a better knowledge on them. Therefore, in chapter 2 the objective is to describe the main thermal variables of the monitored lakes water surface. These are accumulated degree-days, mean temperature, maximum temperature, diel temperature range and temperature oscillation in three-day lapse. Then, mixed models were developed to select which variables explained the temperature variability to know the relative importance of spatial and temporal variables. Finally, the aim was to use these models to make projections of present and future thermal
variables along Pyrenean lakes and ponds using observed or projected air temperatures of Global climate models (GCMs).

**Modelling ice phenology in high mountain lakes of the Pyrenees and its influence on summer water temperatures**

Organisms living in high mountain lakes are adapted to live between 4-6 months under the ice, thus developing most of their cycle during the ice-covered period. Under the current climate change, the ice phenology might change substantially, and therefore, affect local species. Despite the fact that there are some preliminary models of Pyrenean ice phenology (e.g. Thompson et al. 2009), there is a lack of robust models and knowledge on the factors affecting the ice-cover variability in the Pyrenees. In chapter 3 the objective is to describe the ice-cover phenology of Pyrenean lakes and ponds. Moreover, the aim is to develop mixed models to explain these variables and to know which variables and to what extent they can explain ice-cover variability. The intention is to explore the relation between air temperatures, ice-cover and summer water surface temperatures. The final purpose is to make projections of present and future ice phenology along the Pyrenees using these models introducing present air temperature observations and future air temperatures projections of the Intergovernmental Panel on Climate Change (IPCC).
Part II Relative effects of temperature and predation on zooplankton

The relative importance of predation and temperature on the zooplankton of Pyrenean high mountain lakes

Whereas the negative effect of invasive fish is well known and there are many studies on the effects of temperature and ice-cover in lakes, further evidence is needed to understand their combined effects with other abiotic variables. Consequently, in chapter 4 the aim is to describe the zooplankton of the Pyrenees and know the effects of abiotic and biotic variables on zooplankton composition and, particularly, the effects of temperature and introduced fish. The intention is to see the relations among zooplankton groups and how they are affected by both natural and introduced predators. Additionally, the aim is also to analyse the effects of introduced fish on the sizes and fecundity of zooplankton.
PART I Temperature and ice phenology of Pyrenean lakes

2. Modelling surface thermal conditions of Pyrenean high mountain lakes for spatial and climate change projections
Abstract

In the current context of climate change, it is of paramount importance to know the variables which can be used for modelling and making projections of present and future scenarios in water bodies as crucial drivers of ecological and biological processes. In this study, we describe the regional and interannual variability in water temperature of Pyrenean lakes and ponds and we use mixed models to identify the key environmental variables for modelling mean and maximum summer temperature, accumulated degree-days, diel temperature ranges and three-days oscillation. We used 59 lake-temperature series measured from 2001 to 2014 in Pyrenean lakes and ponds. We found that altitude was the primary explicative variable for accumulated degree-days, mean and maximum temperature, whereas lake area had the most relevant effect on the diel temperature range and temperature oscillations, with a lower effect of altitude. Altitude, lake area, catchment morphology and solar radiation together, were more explanatory than interannual variability in air temperature for all thermic variables. We ran the models to predict present and future temperatures under different warming scenarios, the most conservative RCP2.6 and the one with the highest emissions RCP8.5 until 2090 for 2,267 water bodies of the Pyrenees. Accumulated degree-days are expected to increase more in smaller lakes and ponds and at lower altitudes, while maximum temperatures are expected to increase more at higher altitudes. For the warmer scenario, projected mean summer water temperature in Pyrenean water bodies can increase on average 2.0 °C from the present mean of 11.3 °C by 2090.
Introduction

The air temperature increase is one of the features of current climate change (IPCC 2013b). Temperatures have been increasing since the beginning of the industrial revolution, and they are expected to increase between 0.3 °C and 4.8 °C globally, depending on the scenarios of greenhouse gas emissions, for the 2081 to 2100 period (IPCC 2013). The air temperature rise translates into a lake water temperature increase (Livingstone 2003). This warming is expected to be greater in high mountain areas (Beniston 2006). In this sense, the Pyrenees constitute an excellent case study as there are over 3,000 lakes and ponds. Past air temperatures have been reconstructed in this region at some sites using statistical extrapolations for recent centuries (Catalan et al., 2002; Agusti-Panareda & Thompson, 2002) or using lake sediments records (Pla and Catalan 2005; Tarrats et al. 2018). However, lakes in this mountain region and their variation have not been addressed as a whole, even though some research has been done relating the Pyrenean lakes with those of other mountain regions in Europe (Thompson et al. 2009). Here we go further presenting a temperature data series from 2001 to 2014, with nine years of complete summer water temperature data of high-mountain lakes and developing models which help us to understand present climate, as well as future and past climate change.

A precise knowledge of water bodies’ temperature is of great importance to study the biology of aquatic organisms. Temperature is a key environmental variable in lakes, since it accelerates biochemical reactions, and therefore, increases the rates of many biological and ecological processes including photosynthesis and respiration (Allen et al. 2005; Peck et al. 2009; Yvon-Durocher et al. 2012; Gillooly et al. 2017), organic carbon mineralization (Gudasz et al. 2010) and methane emissions (Yvon-Durocher et al. 2014), organism growth (Balcells 1956; Balcells 1957; Angelier and Angelier 1968; Honsey et al. 2018), biomass production (Ernest et al. 2003), organism size (Charnov and Gillooly 2003), and ecological processes as it influences thermal niche and species distribution (Magnuson et al. 1979), biodiversity (Allen et al. 2002; Pinel-Alloul et al. 2013), and trophic cascades (Symons and Shurin 2016).
More specifically, accumulated degree-days (ADD), which is the temperature integrated in time over a determined threshold, explain organisms’ development (Dupuis and Hann 2009; Honsey et al. 2018), while maximum water temperatures limit growth rate (Butterwick et al. 2005) and warming tolerance (Peck et al. 2009; Duarte et al. 2012), essential in explaining lifehistory traits, as some specialist organisms, occupying a narrow temperature range, present a higher performance (Richter-Boix et al. 2015). Temperature oscillations, which can operate daily or among different days, are important as they can increase poikilotherms growth, or can affect them negatively when temperatures are extremely high (Balcells 1956).

Many studies have dealt with thermic variables monitoring and modelling of lake surface water temperature (LSWT) at different temporal scales: daily (Livingstone and Lotter 1998; Livingstone et al. 1999; Kettle et al. 2004; Sporka et al. 2006; Livingstone and Padisak 2007), monthly (Livingstone et al. 2005) or seasonal (Blenckner et al. 2007; Strong and Maberly 2011), maximum annual temperature (Sharma et al. 2008), daily minima and maxima (Wilhelm et al. 2006), the start and end of the significative diel temperature range (Woolway et al. 2015b) and diel temperature range (DTR) (Woolway et al. 2015a; Woolway et al. 2016). Also, ADD, and temperature oscillations have been used for physiological studies of freshwater organisms (Balcells 1956; Balcells 1957; Angelier and Angelier 1968; Honsey et al. 2018), and ADD has also been modelled (Winslow et al. 2017; Honsey et al. 2018). However, no studies have attempted to develop models of a handful of thermic variables using the same methodology.

It is crucial for performing empirical models to identify the key variables which can be used as predictors, how they behave in a mountain region like the Pyrenees, and what importance they have in predicting water temperatures. LSWT depends on heat exchange processes within the water column and at the air-water interface, being the latter where most of the processes occur, including short and long wave emissions, conduction, condensation and evaporation (Edinger et al. 1968). Among these processes, absorbed radiation by water is independent of water temperature, but may be modified by
cloudiness, while longwave emissions, conductive and latent heat exchange depend on water and air temperature, and also on humidity and wind. Water temperature tends to an equilibrium at which heat exchange between air and water is zero (Edinger et al. 1968). This equilibrium temperature changes depending on those heat exchange processes, which are strongly dependent on the air-water temperature gradient and the wind speed.

Air temperature near a given water body is thus the variable most closely related to LSWT, and both respond coherently in time at broad scales, showing a similar response over time due to synoptic processes which can affect the whole region (Livingstone and Lotter 1998; Livingstone et al. 1999; Benson et al. 2000; George et al. 2000; Livingstone and Dokulil 2001). Temperature temporal variation is large, and also warming trends have been described in many regions (Livingstone 2008). In mountain ranges air temperatures decrease with altitude, due to the decrease in pressure, defined as the adiabatic lapse rate, LSWT decreases consequently in altitude (Livingstone et al. 1999).

Incoming solar radiation can also influence LSWT (Kettle et al. 2004). Insolation in water bodies depends on latitude, time of the year, altitude, cloudiness and topography. Topography can be an important factor in mountain ranges, affecting at a local scale (Livingstone et al. 1999; Fu and Rich 2002). Also, solar radiation is not as coherent as temperature is at the regional scale (Benson et al. 2000). Insolation reaching the lake surface directly increases the water temperature producing maximum temperature peaks above equilibrium temperature, causing a diel temperature oscillation. For instance, in the case of soils, Fu & Rich (2002) found that temperature oscillation was correlated with insolation. Similar effects could be expected for water bodies.

Other meteorological parameters, such as humidity, precipitation and wind, can modify LSWT, but they are rarely considered in LSWT empirical models (Kettle et al. 2004). One exception is the study of Sharma et al. (2008), where
they found that precipitation and cloudiness were not good predictors of maximum temperatures. In the case of wind, it can produce errors in the estimations of the models during strong wind events on a daily scale (Kettle et al. 2004). Morphological factors such as lake area and depth in the Pyrenees are correlated (Catalan et al. 2009), and they are also correlated with wind fetch and mixing depth, which influence the thermal inertia of the water bodies, having a slower response to air temperature changes as the volume of mixed water is bigger (Kettle et al. 2004). Other factors may be locally important as water turbidity and color (Snucins and Gunn 2000; Edmundson and Mazumder 2002), river and groundwater inflows and heat exchanges with sediment.

Figure 2.1: Map of the study area in the Pyrenees. The lakes and ponds with measured water temperatures are represented in white. All the lakes and ponds of the region are painted in blue and in yellow triangles are depicted the automatic weather stations.

The main objective of this study was to monitor and model thermic variables of biological interest, including ice-free period mean and maximum temperature, ADD, diel temperature range (DTR), and temperature oscillation (Tosc; here defined as three-days lapse difference in maximum temperatures), for the case of the Pyrenees. We have developed empirical models of these thermal variables, measured in a set of 59 water bodies for 9 years, applying mixed models, using explicative variables which were expected to affect water
temperatures, such as altitude, air temperature, morphological variables and radiation. We also wanted to evaluate which variables can explain thermic variables and the importance of each one on the response. Moreover, we aimed at having a more comprehensive knowledge of the lakes and ponds temperatures in the Pyrenees, both on spatial and temporal scales. In addition, we intended to see the interannual variation in thermic variables not explained by air temperatures variation. Finally, our goal was to make spatial - across the whole set of lakes in the region- and future thermic variables projections, particularly, focusing on how the thermic habitat of aquatic organisms may change.

Methods

Temperature and environmental parameters

A set of 59 lakes and ponds were selected to deploy minilog-thermistors with attached dataloggers (Vemco Minilog-T) spread along the Central - Eastern Pyrenees (42 and 43° N and between 1° W and 3° E; Fig. 2.1), to consider a wide range of water temperature variability. The lakes and ponds were selected following key environmental variables known to affect high mountain lake water temperatures such as altitude, lake and catchment size, residence time, and radiation (See Appendix 1 and Appendix 5) for a description of their ranges and comparison with the Pyrenean water bodies). Thermistors provided us with complete summer water temperatures for 2001, 2002, 2004 just in Lake Redon, and from 2009 to 2014 for the whole set of water bodies, constituting 9 years of recorded summer water temperatures. Thermistors were deployed at 1.5 m depth and temperature measurements were taken at 90-minute intervals. For the ice-free periods, defined as the periods over 4.0 °C, mean temperatures (Tmean), maximum temperatures, the average of DTR and Tosc (calculated as a moving average of the three days lapse differences in maximum temperature) were calculated.
ADD were calculated over two different temperature thresholds \((T_T)\), 4.0 °C, and 7.6 °C, the first (ADD4) representative of the ice-free period, and the latter (ADD7.6) as an animal development threshold (Balcells 1975). First, the daily heat accumulation (degree-days; DD) were calculated from daily maximum and minimum temperatures and assuming the single sine curve as an approximation of the diurnal temperature curve (Zalom et al. 1984). Degree-days were only calculated when maximum temperature was above \(T_T\). Two different formulae were used for the calculation (Baskerville and Emin 1969). When the minimum temperature \((T_{min})\) was greater than \(T_T\), the DD were calculated from the difference between mean daily temperature and \(T_{max}\):  

\[
T_{min} > T_T \rightarrow DD = \frac{T_{max} + T_{min}}{2} - T_T \quad (1)
\]

When minimum temperature was below \(T_T\) the formula used was:

\[
T_{min} < T_T < T_{max} \rightarrow DD = \frac{1}{\pi} \left( \frac{T_{max} + T_{min}}{2} - T_T \right) \left( \frac{\pi}{2} - \theta_1 \right) + A \cos(\theta_1) \quad (2)
\]

Where:

\[
\theta_1 = \arcsin \left( \frac{T_T - \frac{T_{max} + T_{min}}{2}}{A} \right)
\]

\[
A = \frac{T_{max} - T_{min}}{2}
\]

Once the daily DD were calculated for each water body, we calculated the ADD during the ice-free period by adding DD values. Finally, we transformed the ADD measured at 1.5 m to those at 0.1 m, which is a more representative depth for many aquatic organisms’ habitat. The transformation was done using linear relationships of ADD at both depths, which were adjusted with data from five lakes for which we had measures; (ADD7.6_0.1 = 1.10 ADD7.6_1.5 + 15.83; \(R^2 = 0.99, p < 0.001\)) and (ADD4_0.1 = 1.02 ADD4_1.5 + 41.56; \(R^2 = 0.99, p < 0.001\)).

Mean spring (March – May) and summer (June – August) air temperatures were calculated for years 2000 to 2015 from available daily data of the Catalan
Meteorological Service in 14 altitude automatic weather station (AWS) located in the Catalan Pyrenees (Fig. 2.1; Appendix 2). Air temperatures used in the models were those of Lake Redon meteorological station, which is located in a central situation in the Pyrenees and has a long temperature series, given that all temperature series were highly correlated (Appendix 6), and it was representative of each year seasonal air temperatures in the region.

Lake surface area (Larea), direct catchment area (Dcatchment), which is the area where water flows directly to the water body (e.g. not passing through another lake), and total catchment (Tcatchment) were digitalized and calculated following existing topographical cartography (ICGC 2012c) and aerial photography (Casals-Carrasco et al. 2009; ICGC 2012a). From these variables we calculated two ratios describing the geomorphology of the catchments; Dcatchment/Tcatchment, which is inversely related to water retention in the upper lakes of the catchment, and Tcatchment/Larea, which is a surrogate of water renewal time. Theoretical radiation data for each lake and its catchment were calculated from a digital elevation model (15 m of pixel resolution) of the Catalan Pyrenees (ICGC 2012a). We calculated monthly radiation using the Area Solar Radiation algorithm (Fu and Rich 2002). This analysis tool calculates insolation across a landscape for specific locations based on methods from the hemispherical viewshed (Fu and Rich 2002). Global, direct and diffuse radiation and the sun hours were calculated for both the lake surface and the whole catchment. Radiation was calculated considering a solar constant of 1367 W m\(^{-2}\), considering solar track, atmosphere attenuation and topography. Radiation variables were highly correlated among them (Appendix 7). Therefore, we chose the sun hours in the total catchment as a representative of them all, as, in a preliminary analysis, the radiation variable was performing better.

**Statistical modelling**

Mixed models were constructed relating thermic variables (ADD7.6, ADD4, Tmean, Tmax, DTR, and Tosc) to altitude, lake area, geomorphology, X and Y coordinates, radiation (as the sun hours in the total catchment) and air
temperatures of spring (Tspring) and summer (Tsummer). The geomorphology variables included the ratio between direct and total catchment (Dcatchment/Tcatchment) and the ratio between total catchment and lake surface area (Tcatchment/Larea) as a surrogate of lake water renewal time. These two ratios and Larea were normalised by logarithmic transformation. We checked that there were not high correlations among explicative variables (r < 0.71) in order to avoid collinearity effects (Appendix 8). All variables were standardized to z-scores subtracting the mean and dividing by the standard deviation. Random variables considered in the models were year and water body type (classified as pond <0.5 ha and lake >0.5 ha).

Table 2.1: Summary of the coefficients of the mixed models. The coefficients of the models were calculated from z-scores obtained from the original values. The coefficients of the fixed part are Radiation: Sun hours in the total catchment (hours per day); Dcatchment: Logarithm of direct catchment area (ha); Tcatchment: Logarithm of total catchment area (ha); Dcatchment/Tcatchment: Logarithm of the direct catchment area / total catchment area ratio; Tcatchment/Larea: Logarithm of the total catchment area / lake area ratio; X: X UTM 31N coordinates (m); Y: Y UTM 31N coordinates (m); Larea: Logarithm of water body area (ha); Altitude (ha); Tspring: Spring air temperature (°C); Tsummer: Summer air temperature (°C); their interactions (Larea:Altitude and Altitude:Tspring) and the standard deviations of the models: sd fixed: Standard deviation of the fixed term; sd water body: Standard deviation due to the water body type; sd year: Standard deviation due to the year; sd residuals: Standard deviations of the residuals, R² conditional and R² marginal for mixed models (having both fixed and random part) and R² ordinary and R² adjusted for multiple regression models (only with the fixed part) are represented for seven thermal variables of the lake: ADD7.6: accumulated degree-days over 7.6 °C (°C day); ADD4: Accumulated degree-days over 4.0 °C (°C day); Tmean: Mean epilimnetic temperature of the ice free period (°C); Tmax: Maximum epilimnetic temperature (°C); DTR: Diel temperature range (°C); Tosc: Oscillation in maximum temperature in a three-day lapse (°C).
<table>
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<tr>
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<th>Tmax</th>
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\(R^2\) marginal/ordinary\(^a\) 0.75 0.75 0.52 0.60 0.45\(^a\) 0.50  
\(R^2\) conditional/adjusted\(^a\) 0.84 0.82 0.63 0.78 0.44\(^a\) 0.53  
AIC 322.45 299.83 493.11 399.08 536.14 507.80  
BIC 363.39 336.06 530.39 436.61 560.29 528.32  
RMSE 181.31 237.14 1.21 1.89 0.55 0.68
Figure 2.2: Frequency distribution of the morphological, radiation and thermic variables calculated in the 59 studied water bodies (See Table 2.1 for the description of the variables, here the variables are not transformed).
The model selection used followed the ten steps protocol described by Zuur et al. (2009). It consisted of a first selection of the variables in the fixed part, first adjusting multiple linear regression models with the “lm” function, then, selecting variables by stepwise selection, choosing the model with the minimum Akaike Information Criterion (AIC). We considered two different model selection methods for each variable, using forward or backward selection. We also considered interactions between the most relevant variables (Altitude, Larea, Tspring and Tsummer). Later, the random part of the models was compared using “gls” and “lme” functions of the package “nlme” (Pinheiro et al. 2013) adjusted by restricted maximum likelihood (REML). The null random structure was tested with the year, the water body type and the year nested in the water body type. The structure with the lowest AIC was selected. The following step was to test the optimal fixed structure using the likelihood ratio test with the mixed model estimated with maximum likelihood (ML) to determine the significance of the variables and drop the non-significant. Finally, the model was refitted with REML and tested for homogeneity of variance and independence. From the four models calculated for each variable, the one with the lowest AIC was kept (all models tested are in Appendix 3). Summary statistics of $R^2$ marginal ($R^2_m$) and $R^2$ conditional ($R^2_c$) were calculated with the package MuMIn (Nakagawa and Schielzeth 2013), where $R^2_m$ concerned the fixed part of the model, while $R^2_c$ referred to both the fixed and the random part. When the resulting model had no random part, ordinary $R^2$ ($R^2_o$) and adjusted $R^2$ ($R^2_{adj}$) were calculated, the latter penalized by the number of coefficients. RMSE calculation was performed using k-fold cross-validation of the model (k=5).

We used the models presented here to make present and future projections of the thermic variables along the Pyrenees. To do so, we multiplied the coefficients of the fixed part of the models with morphological and radiation available data from 2,267 lakes and ponds in the Pyrenees, observed current temperature and future projections of air temperatures. Then we added the random effects (the coefficients computed for each year and/or water body type). For current projections, air temperatures series of Lake Redon from 2000 to 2015 were used. Future air temperatures increase was estimated from Global Climate Models (GCMs) of the CMIPS (described in Appendix 4) for the
nearest point to Lake Redon AWS and considering two different greenhouse gases global emission scenarios until the year 2095, RCP2.6 considering lower emissions and RCP8.5 with a higher increase in temperature (IPCC 2013). As we have mentioned before, random coefficients were added in the calculations of the thermic variables predictions, when making projections for the years within the models. In contrast, random yearly variability was calculated making simulations for years out of the models, following a normal distribution with 0 mean and sd of year (Table 2.1), and then added to the estimates of the fixed part of the models.

Results

Thermic characteristics of Pyrenean lakes and ponds

Thermic variables showed a wide range in the monitored Pyrenean water bodies during the ice-free period (Fig. 2.2, Appendix 1). Tmean ranged between 5.9 and 15.3 °C, being 11.0 °C the average temperature for all water bodies. Tmax reached a value of 27.3 °C, while the lowest Tmax registered was only 8.4 °C, having a mean Tmax of 17.9 °C. DTR had a mean of 1.5 °C and ranged between 0.6 and 4.6 °C. Tos (maximum: 2.8 °C) was smaller than the maximum DTR, but was similar for the mean and minimum (1.1 and 0.5 °C respectively). Some water bodies had low ADD, having a minimum of 18.1 °C day ADD7.6 and 41.6 °C day ADD4, while mean ADD (697.2 ADD7.6 and 1161.8 °C day for ADD4) and maximum ADD (2126.5 °C day for ADD7.6 and 2931.5 °C day for ADD4) were much greater.

Models of thermic variables

Mixed models of thermic variables resulted in good adjustments (Fig. 2.3b, Table 2.1; R², 0.53-0.84). Only the model for DTR fitted better without the random part in the model, and therefore, it was adjusted as a multiple regression model and resulted in a lower adjustment (R², 0.45). The rest of the models included the year as a random variable except Tmean, which only
included the water body type (lake or pond). The water body type was also included in the model for ADD7.6 and ADD4 together with the year.

The models for ADD7.6 and ADD4 had the best performance ($R^2_c$ 0.84-0.82) and were mostly explained by the fixed part of the model, since $R^2_m$ was the highest of all the models (0.75) (Fig. 2.3b, Table 2.1). The Tmean model had the lowest adjustment for the fixed part ($R^2_m$ 0.52), while Tmax had the most important effect of year and water body (Fig. 2.3b). Tosc had the lowest performance among the mixed models ($R^2_c$ 0.53).

The primary variable explaining the variation in thermic variables was altitude, whose mixed models coefficients were between -0.27 and -0.81 depending on the response variable (Fig. 2.3a; Table 2.1): Altitude had a negative effect on all thermic variables; it affected more ADD7.6, ADD4 and Tmax and it had a lower effect on DTR and Tosc (Fig. 2.4). In contrast, lake area was more important than altitude in explaining DTR and Tosc (-0.61 and -0.69 respectively) (Fig. 2.3a; Table 2.1). It also had a negative effect on all variables (Fig. 2.4). Larea was also important for explaining the maximum temperature (-0.42) (Fig. 2.3a; Table 2.1). Geomorphology had its most important role in mean temperature (-0.71), but it also affected ADD7.6, ADD4 and Tmax (-0.21 - -0.38) (Fig. 2.3a; Table 2.1). Greater Tcatchment/Larea and Dcatchment/Tcatchment produced a decline in water temperature, and therefore, a negative effect on ADD7.6, ADD4, Tmean and Tmax but not on DTR or Tosc (Fig. 2.4). Tcatchment/Larea (which can be considered as a surrogate for water renewal time, given comparable precipitations among sites and time, as it describes the potential flow of water into the water body) explained most of the effects of geomorphological variables. Radiation had a similar positive effect on all thermic variables (0.16 - 0.26). Coordinates had a limited effect (-0.09 - 0.15), with an increase of ADD7.6, Tmax and DTR to the west and a decrease of ADD4 to the north.
Interannual variability in air temperatures, represented by the seasonal air temperatures in Lake Redon, had a substantial positive effect on ADD7.6, ADD4 and Tmean. On Tmax, it had a positive effect interacting positively with altitude and it had no effect on DTR or Tosc. Interannual variability in air temperature accounted for a lower effect than the sum of the other variables which depended on the lake characteristics, such as altitude, lake area, morphology or radiation (Fig. 2.3b). Spring air temperatures had a stronger effect than summer air temperatures on the thermic variables (Table 2.1).

![Graph showing coefficients and variance explained](image_url)

Figure 2.3: a) Coefficients of the fixed variables of the models classified in six categories plus the interactions. Where Air temperature includes Tspring and Tsummer, Coordinates are X and Y and Geomorphology includes Dcatchment/Tcatchment and Tcatchment/Larea (See Table 2.1 for variables description). b) Conditional ($R^2_c$) and marginal ($R^2_m$) /ordinary ($R^2_o$) $R^2$ of the models. Conditional $R^2$ account for the random variability of the model, whereas marginal $R^2$ account for the fixed part, ordinary $R^2$ is depicted in the case of multiple regression.
Figure 2.4: Partial effects of the explicative factors on modelled thermic variables. Only the three variables with more effect on the models are drawn (See Table 2.1 for the complete model description).
Figure 2.5: a) Predicted against observed ADD7.6. Predicted ADD7.6 are calculated from averaged predictions of the cross-validation (k=5). b) Observed and predicted ADD7.6 (°C day) for lakes Estanyet de Sotllo, Llebreta and Estanho de Vilac (from colder to warmer). Observed values are depicted with continuous lines, while predicted values are drawn with dotted lines. Standard deviations to the predicted values are represented by grey ribbons.

Projections of thermic variables

Projections in the lakes and ponds for the measured years using k-fold cross-validation allowed us to validate the models giving a value of RMSE (Table 2.1). Predicted values were in good agreement with the observed ones (Fig. 2.5a); Predictions for ADD had lower errors than for Tmax and Tmean while DTR and Tosc showed higher prediction errors. Predicted time series followed the same temporal pattern as observed thermic variables and generally observed values were within the error of the predicted thermic variables or close to it. Biases of the predictions were related to extreme events and to the fact that DTR and Tosc were not explained by interannual variability in air temperatures (Fig. 2.5b).
Figure 2.6: Projected ADD7.6 (°C day) for Pyrenean water bodies (n=2,267) calculated from spring mean air temperatures in Lake Redon meteorological station for 2006-2015 decade in red, in green, calculated from the GCMs air temperature increase predictions described in Appendix 4 for the lower emission scenarios RCP2.6, and in blue, for the greater emission scenarios RCP8.5 a) Frequency distribution of the projections calculated with the mean air temperatures in the observations period and the expected air temperature for 2086-2095 decade. b) Projections temporal variation from 2000 to 2095.

Air temperatures projections of the GCMs at Lake Redon gave an increase in spring and summer temperature (Fig. 2.6). Projections for all the lakes and ponds along the Pyrenees (n=2,267) gave us a Tmean average for all water bodies of 11.27 °C, ADD7.6 of 826 °C days, ADD4 of 1301 °C days, Tmax of 19.63 °C, DTR of 2.08 °C and Tosc of 1.55 °C for the period between 2006 and 2015 (Fig. 2.6). Future projections under different warming scenarios gave an increase of Tmean of 0.48 °C for the most conservative situation (RCP2.6) and of 2.02 °C for the warmer scenario (RCP8.5) by 2090. For ADD7.6, the expected increase was 179 and 219 °C days (RCP2.6 and RCP8.5 respectively) and for ADD4, an increase of 58 and 356 °C days (RCP2.6 and RCP8.5 respectively) was expected. Tmax was expected to increase slightly; 0.04 and 0.23 °C (RCP2.6 and RCP8.5 respectively). DTR and Tosc were not expected to increase. In addition, future projections of ADD7.6 and Tmax resulted in different expected increases with altitude and lake areas. Whereas ADD7.6 was expected to increase more in lower water bodies, Tmax would increase more at higher
altitude water bodies (Fig. 2.7a). Also, ADD7.6 increase in lower altitudes would be more acute for ponds than for lakes (Fig. 2.7a).

Figure 2.7: a) ADD7.6 and Tmax changes in altitude from 2015 (blue crosses) to 2090 (red dots) for the higher temperatures scenario RCP8.5. b) Differences in the relation between ADD7.6 and altitude in ponds (<0.5 ha), and lakes (>0.5 ha) from 2015 (blue crosses) to 2090 (red dots) for the higher temperatures scenario RCP8.5.
Discussion

Among all the predictor variables, altitude was the variable which explained more variability in LSWT in the Pyrenean mountain range, since LSWT decreases linearly with altitude as the air temperature adiabatic lapse rate, but at a higher rate, as found in the Alps (Livingstone et al. 1999). This was the case for ADD, Tmean and Tmax. This relationship has been previously described in lakes of other regions situated in mountain ranges at daily and monthly scales (Livingstone and Lotter 1998; Livingstone et al. 1999; Sporka et al. 2006; Arp et al. 2010). In the case of maximum temperature, a weak relation with altitude was found in Canadian lakes (Sharma et al. 2008), probably because they considered a reduced altitude range compared to ours (1053 - 2978 m; Appendix 1). In contrast, we found that altitude was the main explicative variable for maximum temperatures in high mountain lakes. Previous studies have successfully modelled ADD in lowland areas (Midwest) of North America (Winslow et al. 2017). In this study we have shown the importance of altitude on ADD in mountain areas.

In the case of DTR and Tosc, lake area was the most explicative variable. This was also found by Woolway (2016), and it is a result of lake area being proportional to lake depth and mixing depth (Gorham and Boyce 1989; Catalan et al. 2009), which increase thermic inertia, and therefore, reduce DTR (Woolway et al. 2015a). In contrast to the latter study, we have found that altitude was also a significant variable explaining differences in DTR together with other variables of less importance, like longitude and insolation (Fig. 2.3). Altitude was also found to be the most explicative variable for the date when DTR starts to increase significantly after ice-off (Woolway et al. 2015b). Lake area was also relevant to model ADD, mean and maximum temperatures. Similarly to our results, but on a daily scales, lake area smoothed water temperature comparing to that of air (Kettle et al. 2004), as the lake morphometry is related to the heat exchange between water and air and heat storage.
In addition to lake area, morphological variables related to lake catchment features were found to be important for modelling water temperature. The total catchment to lake area ratio can be used as a proxy of the inverse of water renewal time, and therefore, higher ratios corresponded to lower ADD, mean and maximum temperature of the water bodies, as a faster flushing would prevent a warming up of the epilimnion of the lakes and ponds. The ratio between direct and total catchment may be inversely related to water retention in upstream lakes and ponds and increased heating in these water bodies, and thus higher ratios corresponded to lower water mean and maximum temperatures. Taking into account these variables may help us to understand the potential effects of precipitation on lakes’ water temperatures, as catchment morphology variables have not been considered in the development of empirical models of water temperature. In the case of precipitation, it is commonly used in mechanistic models of water temperature (Vinna et al. 2017; Winslow et al. 2017), but it is rarely used in empirical ones, and even no effects of precipitation were found (Sharma et al. 2008). In the Mediterranean region precipitation is expected to decrease (IPCC 2013b), which could lead to lower water renewal times resulting in an even higher warming in lakes. Testing the effects of precipitation in conjunction with the catchment morphometries could help us understand the thermic characteristics of lakes, which would require a good understanding of precipitation spatial and temporal distribution.

Solar radiation, as the sun hours reaching the water bodies’ catchment, had a notable positive effect on lake temperatures. In our study, we centered on the local differences in solar radiation instead of seeking temporal variations. Spatial modelling allowed us to find the effects of latitude, altitude and the shading by topography on solar radiation. It is already known that the effect of shading by topography was of great importance in high mountain ranges, where shaded lakes were cooler than expected by their altitude (Livingstone et al. 1999). Different approximations have been considered when introducing solar radiation into water temperatures models. For instance, theoretical clear-sky solar radiation has been used to model daily water temperatures in Greenland (Kettle et al. 2004), where altitude and latitude were used, but not the shading by topography. Another option is the use of radiation downscaled
from GCMs (Winslow et al. 2017). In such an approximation, spatial differences can be assessed although topographical shading may not be considered. Using solar radiation in empirical LSWT models showed contrasting results. Whereas solar radiation was not a good predictor for maximum temperature in Sharma, Walker & Jackson (2008), spatial and temporal changes in solar radiation were related to lake warming trends worldwide using satellite data (O'Reilly et al. 2015). The spatial variability in incoming solar radiation can vary from one study to another, as latitude, altitude and shading depend on the scale and the region studied. In the case of the Pyrenees, latitude might not be very relevant as the mountain range is oriented W-E, but altitude and topographical shading have an important effect on spatial variability on incoming solar radiation. Whereas radiation derived from GCMs or from satellite data are a good option to account for temporal and local variability in solar radiation, our work shows the importance of taking into account topography in mountain regions, when modelling water temperatures, as it has a great spatial precision, and it is commonly neglected. Integrating methods to account for more precise temporal and spatial calculations of radiation arises as an excellent option to further improve models like the ones presented here.

Interannual variability in air temperatures was represented by the seasonal temperature data series of Lake Redon, which represented air variation on a regional scale. This temporal change in air temperatures explained ADD and Tmean, and also Tmax in higher altitudes, whereas it did not explain DTR or Tosc. This may be related to a higher unpredictability of the last three variables (Fig. 2.3b; Table 2.1), and because of a probable increase in minimum water temperatures during summer for DTR and Tosc. Spring air temperatures explained more variability than summer ones. Summer air temperature was only significant to explain mean water temperature. This may be due to the effect of thawing of spring temperature on ice-off and the onset of LSWT warming, as in the Tatra mountains, where the LSWT began to show an altitudinal gradient in late spring (Sporka et al. 2006). Mean annual temperature, in addition to summer temperature, was significant to explain water maximum temperatures in Canada (Sharma et al. 2008), indicating that air temperature is influencing LSWT also beyond summer, especially during spring.
The results of applying future temperature scenarios to the whole set of lakes of the Pyrenees highlighted that lakes and ponds located at lower altitudes would experience higher ADD7.6 increases compared to lakes at higher altitudes (Fig. 2.7a). We found a negative interaction between altitude and spring air temperature on ADD7.6 (Table 2.1), which meant a different effect of interannual air temperature differences on higher and lower lakes and ponds. This was probably a result of a greater advance in ice-off date at low altitude than at higher altitudes, causing high altitude lakes to be disconnected during more time from spring air temperature, as the ice-cover has an insulating effect on lake water (Sporka et al. 2006). In contrast, maximum water temperatures, which take place at the middle of summer, are expected to increase more at lakes from higher altitudes, as they depended on the positive interaction between interannual air temperature and altitude (Table 2.1). This may be due to a greater increase in air temperatures in altitude, as described in a review by Pepin (2015). In addition, ADD7.6 in ponds (surface areas < 0.5 ha) had a steeper decreasing slope with altitude than in lakes (> 0.5 ha) (Fig. 2.7b), as lake area interacted positively with altitude on ADD7.6, an interaction which was also found for Tmean, Tmax and DTR (Table 2.1). Low altitude ponds may accumulate more heat because of earlier ice-off and a quicker response to air temperature rise in comparison with low lakes. High altitude ponds, in turn, may be more affected by cooling in autumn, whereas lakes may remain warmer at high altitudes due to higher thermic inertia. Besides, differing warming trends between small and big lakes have been described (Winslow et al. 2015). This can be indicative of different behaviours of ponds, small lakes and large lakes, showing different responses to global warming.

We have shown that, for a nine-year period, the effects of variables which depend on the spatial distribution of the lakes (altitude, lake area, catchment morphology and radiation) were more important than the effects of interannual air temperature differences for all thermic variables. These results may vary in a wider window of time, as the measured period was of slight temperature increase in comparison to the last half century (Appendix 6), when temperature had a steeper increase (Dokulil 2014), and may continue to do so with the current scenario of climate change thus rising upper
temperatures (IPCC 2013b; IPCC 2018a). However, mean summer air temperatures from this study ranged between 8.2 and 11.3 °C, while it was found to be between 6.1 and 11.6 °C from 1781 to 1997 in the Pyrenees, derived from instrumental records (Agusti-Panareda and Thompson 2002), comparable to the range found in our study. On the other hand, air temperatures were not significant in explaining DTR and Tosc, which indicates an increase also in minimum water temperatures during summer. Therefore, they would not be expected to change in the future.

The water temperature increase in Pyrenean lakes is expected to be lower than air temperatures. Projected future mean summer temperatures (Tmean) indicate an increase per decade between 0.06 and 0.25 °C, while summer LSWT increase in European lakes has been between 0.4 and 0.66 °C per decade during the last century (Dokulil 2014), or a mean global increase of 0.34 °C per decade (O'Reilly et al. 2015). In comparison, future warming seems lower than those that occurred in the past decades. This may be because the current decade, taken to make the models, has been of a lower temperature increase compared with the past ones (IPCC 2013b), this trend could change in the future as well as the relation between air and water temperatures. Also, this warming can be more pronounced in high altitudes (Pepin et al. 2015; Loomis et al. 2017). Tmax is expected to increase slightly and at high altitudes, probably because of a higher interannual random variability (Table 2.1). As for DTR and Tosc, they are not expected to increase, as we found no relation with regional air temperature, this was also related to a worse performance of the models (Fig. 2.3b; Table 2.1), since a lower relation with interannual air temperatures makes these variables more unpredictable in time.

Temperatures increase can drive changes in water bodies’ communities, such as functional traits, composition, biomass or abundance. For instance, in Canadian lakes, it was found that water temperatures were negatively related to zooplankton body size, a similar effect to the one produced by fish predation, and these combined effects would be non-additive (Redmond et al. 2018). Therefore, lakes warming would favour smaller zooplankton species. Combined fish predation with warming may increase small zooplankton by
predation on big zooplankton (MacLennan et al. 2015), causing an increase on producers’ abundance due to less efficient consumption of small zooplankton (Brooks and Dodson 1965). Moreover, higher lakes would be more sensitive to warming since they have less functional diversity than lower ones (Redmond et al. 2018). This functional diversity would move upwards, as species may move their distribution ranges to higher elevations (Walther et al. 2002). At high elevations, cold stenothermal species are more vulnerable as they have a restricted distribution range (Marziali and Rossaro 2013), and they could be lost as a consequence of climate change (Jacobsen et al. 1997). A temperature increase can also advance zooplankton phenology (Velthuis et al. 2017). ADD can be modelled to predict recruitment and abundance of fishes (Hansen et al. 2017), which may be benefited by increasing ADD. Whereas maximum temperatures may induce thermal stress to certain species (Duarte et al. 2012), influencing then species composition (Jacobsen et al. 1997). These effects would have a more significant impact on higher water bodies, where we have foreseen a greater increase in maximum temperatures. The knowledge about thermic variables and the models developed here enables making projections of water thermic variables which are fundamental for disentangling ecological and conservation issues in a global change context.
3. Modelling ice phenology in high mountain lakes of the Pyrenees and its influence on summer water temperatures
Abstract

Ice phenology is of great importance for the thermal structure of lakes and ponds and the biology of the lake species, especially for their phenology and change in their habitat. Under the current climate change, water bodies’ ice-cover duration has been reduced by an advance in ice-off and a delay in ice-on and future projections foresee that it is continuing to do so. Here, we have described the ice phenology of Pyrenean lakes and ponds, including ice-cover duration, ice-on and ice-off date. We have used mixed models to identify the variables which can explain ice phenology in the region, to make current and future projections along the mountain range. Ice phenology was obtained from temperature series of 59 lakes and ponds for seven years, including 2002 and from 2009 to 2014. We found a greater predictability of ice-off and ice-cover duration in comparison to ice-on. Altitude was the most important variable explaining the ice phenology of lakes and ponds. Lake area was significant for ice-on and ice-cover duration, catchment size was significant for ice-on, whereas interannual variability of air temperature and radiation were remarkable for ice-off and ice-cover duration but not for ice-on. In addition, ice-off influenced accumulated degree-days (ADD) in summer, which in turn affected the following ice-cover. All the measured lakes and ponds froze in winter during the studied period, as it was also found for the whole set of lakes following the present projections. In relation to future projections, we foresee an advance in ice-off between 23 and 32 days by 2090, whereas no changes in ice-on are expected. In spite of the projected reduction in the ice-cover, Pyrenean lakes and ponds will still freeze in winter.
Introduction

Ice phenology has a great importance in temperate climate lakes and ponds. It affects the chemistry of water beneath (Catalan 1989), it modifies thermal and light environment within the lake by impeding water heat exchange and penetration of light (Catalan 1989; Felip et al. 1999). It also has biological implications as it hosts a rich and active microbial community (Felip et al. 1999), it can modify the phenology of the organisms which live in lakes and ponds by modifying their peak timing and abundance. For instance, mild winters in Müggelsee (Berlin, Germany) with short ice-cover duration had higher temperatures in spring months, earlier algal blooms of greater magnitude and more variation in both timing and magnitude of the blooms (Adrian et al. 1999). An advancement in ice-off can increase zooplankton production and density (Carter and Schindler 2012). As a consequence, short ice-cover durations can cause an advance in the clear water phase (CWP) due to predation pressure on phytoplankton (Adrian et al. 1999). Some studies point out that the advancement in algal blooms and corresponding cladocerans growth might be uncoupled, causing a mismatch between predator and prey (Winder and Schindler 2004; Domis et al. 2007). Zooplankton predation by fish may increase due to longer periods of activity and higher metabolic rates of predatory fish. Also competition among fish species during the ice period may change with reduced ice-cover duration (Helland et al. 2011).

Under current climate change, the duration of the ice-cover has been reduced in water bodies of different regions (Magnuson et al. 2000; Ghanbari et al. 2009; Weyhenmeyer et al. 2011; Benson et al. 2012; Gebre et al. 2014; Surdu et al. 2014), by a delay in freezing (Sharma et al. 2016) and an advance in melting (Livingstone 1997; Weyhenmeyer et al. 2011; Sharma et al. 2013; Beyene and Jain 2015). Moreover, the trend towards a late ice-on, an early ice-off, and therefore, a shorter ice-cover period is steepening, with an increase in extreme warm events, since the beginning of the industrial revolution (Sharma et al. 2016) and in the last decades (Benson et al. 2012), and future simulations predict further shortening of the ice-cover period (Gebre et al. 2014). The
variability in this magnitudes has also increased in the past decades, and extreme warm events are increasing, but not as fast as cold events are reduced (Benson et al. 2012). The reduction of ice-cover is not equal, as the advancement in ice-off is greater than the delay in ice-on (Benson et al. 2012; Carter and Schindler 2012), because ice-off is more sensitive to air temperatures (Vavrus et al. 1996). In regions where air temperature falls close to below zero, the interannual variation in ice-off, ice-on and ice-cover duration is greater (Weyhenmeyer et al. 2011), and a greater variability is also related to a higher frequency of warm events (Benson et al. 2012). Also, the intermittency of ice cover would increase and even lead to ice free winters (Livingstone and Adrian 2009). In the Pyrenees, a region which is located in the southernmost location of these studies (Thompson et al. 2009), this could drive to years without ice in the lowest altitudes.

Ice-cover duration, especially ice-off date is also important due to its influence in water temperatures during the ice-free period, since earlier ice-off leads to higher surface water temperatures (Gerten and Adrian 2000), as during the ice-covered period the lakes are isolated from external forcing (Livingstone and Dokulil 2001; Czernecki and Ptak 2018), and advancing the start of stratification leads to a higher water temperature increase (Austin and Colman 2007). However, there are few studies focusing on the future influence of summer water temperature on the following ice-on date. But Sharma et al. (2013) found no evidence of memory in the ice-cover of the lakes as they did not find significant autocorrelations between ice break-off dates at different time lags.

Several studies report long term trends in the reduction of ice-cover duration, advancement in ice-off and delay in ice-on (e.g. Livingstone 1997; Magnuson et al. 2000; Sporka et al. 2006; Benson et al. 2012; Sharma et al. 2013), monitoring has also been done using remote sensing (Arp et al. 2010; Surdu et al. 2014). A relation within teleconnections with ice phenology variables has also been described (Ghanbari et al. 2009; Sharma et al. 2013; Beyene and Jain 2015). For predicting ice phenology, process-based models have been developed and used for simulating present or future scenarios of ice
phenology (Vavrus et al. 1996; Walsh et al. 1998; Lepparanta 2010; Gebre et al. 2014). Some empirical or semi-analytical models have been developed to relate air temperature with ice phenology in different ways; using a fixed-period regression (Palecki and Barry 1986; Assel and Robertson 1995; Livingstone 1997; Sharma et al. 2013) or accumulated melting degree-days (AMDD) for ice-off or accumulated freezing degree-days (AFDD) for ice-on (Livingstone 1997; Arp et al. 2010; Lepparanta 2010; Beyene and Jain 2015), both methods showed similar results (Livingstone 1997). Ice-cover duration can also be related to the duration of air temperatures under 0 °C (Livingstone and Adrian 2009).

Ice phenology variables can be calculated from air temperatures alone, indeed many models have been developed using only air temperature, and these variables are highly sensitive to air temperature changes (Palecki and Barry 1986; Vavrus et al. 1996), although other meteorological variables such as wind (Lepparanta 2010) and precipitation (Ghanbari et al. 2009; Sharma et al. 2013) have been used to develop individual lake models. The effects of precipitation on ice-cover are contrasting. A pattern of snowfall increase was related to an ice-cover period reduction and an ice-off advance (Ghanbari et al. 2009), whereas precipitation (both rain and snowfall) delayed ice-off date (Sharma et al. 2013). Lake morphometry variables (Livingstone 1997; Walsh et al. 1998), latitude and coastal influence can also be relevant (Palecki and Barry 1986; Walsh et al. 1998). In high mountain lakes, ice-off is known to last longer at higher altitude, while elevation has no significant effect on ice-on, and therefore, the effect on ice-cover duration is totally due to ice-off (Sporka et al. 2006). Moreover, other anthropogenic changes, such as population, flood control or hot springs may influence ice phenology, although air temperature change is the predominant factor (Sharma et al. 2013; Sharma et al. 2016).

In this study we aim at monitoring the current ice phenology in the lakes of the Pyrenees, and then at modelling ice phenology variables (ice-on, ice-off and ice-cover duration) to seek which altitudinal, seasonal air temperatures and morphological variables can explain their variation, and which is the relative importance of each explanatory variable on ice phenology. In order to analyse
that, we have used ice phenology dates from temperature data series of 7 years in 59 Pyrenean water bodies, and we apply an empirical modelling approach using mixed models. We also intend to disentangle the relation among seasonal air temperatures, ice phenology and water summer temperatures. With these models we aim at predicting ice phenology variables for all the Pyrenees in current conditions and future climate change scenarios, as ice phenology has also relevant consequences for physical and chemical properties of the lakes and for the organisms which live within.

**Methods**

We monitored a set of 59 lakes and ponds in Central-Eastern Pyrenees, between 42 and 43° N and between 1° W and 3° E (Fig. 3.1). They were selected comprising a wide range of altitudes, from 1,561 to 2,880 m, and different lake areas, from 0.015 to 24.6 ha, and also had a wide range of catchment areas and incoming radiation (Appendix 1, Appendix 5). Minilog thermistors with attached dataloggers (Vemco minilog-T) were deployed in the littoral of the lakes at 1.5 m depth. They registered water temperatures each one and a half hours, during seven years, the year 2002 and from 2009 to 2014.

The date of freezing (ice-on), and the date of melting (ice-off) was determined from the temperature data series, as the date when temperature decreased and stabilized, showing no diel temperature variations, for ice-on, and when temperatures began to show temperature diel variations above 4°C for ice-off, since the ice-cover prevented from daily temperature variations. Dates were calculated as the ordinal date and ice-cover was calculated as the difference between ice-off and ice-on. Accumulated degree-days (ADD) for the ice-free period were calculated over 4 °C from the temperature data series as described in chapter 2. Mean seasonal air temperatures were obtained from Lake Redon automatic weather station (AWS), as it had a long temperature series and a centric location amongst 14 meteorological stations in the
Pyrenees (Appendix 2), and it was representative of regional air temperature as air temperatures were highly correlated amongst Pyrenean AWS (Appendix 8).

Figure 3.1: Map of the studied lakes in the Pyrenees. The lakes and ponds with ice phenology data are represented in white. All the lakes and ponds in the region are painted in blue and in red are the automatic weather stations (AWS).

Lake surface area (Larea), direct catchment area (Dcatchment; proportion of catchment draining directly to the lake, not passing through another lake) and total catchment (Tcatchment) were calculated from the cartography of 2,267 lakes and ponds with their catchment areas. This cartography of Pyrenean lakes and catchments over the Pyrenees was elaborated from topographic cartography and ortophotography from the Institut Cartogràfic i Geològic de Catalunya (ICGC), Institut Geogràfico Nacional (IGN) and Institute Geographic National (IGN) as in Casals-Carrasco et al. (2009). Theoretical radiation data for each lake and its catchment were calculated from regional digital elevation models from the abovementioned institutions. The monthly radiation was calculated using the Area Solar Radiation algorithm (Fu and Rich 2002). It calculates insolation across a landscape for specific locations based on methods from the hemispherical viewshed (Fu and Rich 2002). Direct radiation and the duration of incoming direct solar radiation were calculated for each lake and catchment. Direct radiation was calculated considering a solar
constant of 1,367 W m\(^{-2}\), considering solar track, atmosphere attenuation and terrain orientation. Radiation variables were highly correlated (Appendix 7), therefore, we only used the number of sun hours reaching the catchment surface (Radiation).

Mixed models were developed for three ice phenology variables: ice-on, ice-off and ice-cover duration. The independent variables included in the initial models were Altitude, Larea, Dcatchment, Tcatchment, the ratio between direct and total catchment (Dcatchment/Tcatchment), which describes the relation between the water which is driven directly to the lake in relation to the water retained in upstream lakes, the ratio between total catchment and lake surface area (Tcatchment/Larea), as a proxy of water renewal time, Radiation, and the interannual variability of air temperature (Air temperature) averaged for the four seasons. Spring and summer air temperatures were included for ice-off and summer and autumn for ice-on. In the case of ice-cover duration, all seasons were included in the initial models. The variables Larea, Dcatchment/Tcatchment and Tcatchment/Larea did not adjust to a normal distribution, and therefore, were log-transformed. All variables were standardized to z-scores subtracting the mean and dividing by the standard deviation. There were no high correlations between explicative variables. All of them were <0.71, so collinearity effects among predictors were avoided (Appendix 11). The random variables considered in the models were year and water body (lake >0.5 ha or pond < 0.5 ha).

Model selection was done following the ten steps protocol described in (Zuur et al. 2009). First, the fixed part of the model was built by adjusting ordinary regression models using R language (R Core Team 2019) with the function “lm” and performing a stepwise variable selection, choosing the model with the lowest Akaike Information Criteria (AIC). This selection was done by both forward and backward variable selection. Then we tested the random part of the models. We compared the null model (no random structure), year, water body and year nested into water body models. To do so, we adjusted the models with “gls” (no random structure) and “lme” (random models) functions respectively (Pinheiro et al. 2013), using the restricted maximum likelihood
(REML) approach, and we selected the model with the minimum AIC. Later, the variables of the fixed part of the model were tested again using the likelihood ratio test with the mixed model estimated with maximum likelihood (ML), dropping the non-significant variables. Finally, the selected model was refitted with REML and tested for homogeneity of variance and independence. For each variable, we tested four models (using backward or forward selection, with or without interactions). Then we chose the model with the lowest AIC (all models tested are in Appendix 9). Summary statistics of $R^2$ conditional and $R^2$ marginal were calculated following Nakagawa and Schielzeth (2013). Root mean standard error (RMSE) calculation was performed using a k-fold cross-validation of the model ($k=5$).

![Histograms of various variables](image)

Figure 3.2: Frequency distribution of the morphological, radiation and ice phenology variables of the 59 studied lakes and ponds (See Table 3.1 for variable description).
To explore the relationship between ice phenology and summer water temperatures and how air temperatures and lake morphology influenced both ice phenology and water temperature, we performed a structural equation model. From an initial model (Appendix 12) we tested the significance of the variables and removed those with the highest p-value, and repeated this step until all variables left were significant (Summary statistics of the model are in Appendix 10).

Mixed models of ice phenology were used to make current and future projections of ice phenology variables. To do so, we multiplied the coefficients of the models with a database of morphological, radiation and air temperature variables. Morphologic and radiation variables derived from the cartography of 2,267 Pyrenean lakes and ponds. For present projections, the air temperature series of Lake Redon AWS from 2000 to 2015 was used. For future projections air temperatures were estimated considering an increase of Lake Redon air temperatures following future air projections of global climate models (GCMs), taking into account two different greenhouse effect gases global emission scenarios until year 2095, the RCP2.6 with a temperature increase of 0.94 °C and RCP8.6 with a higher increase of 3.57 °C (Appendix 4; IPCC 2013a). When making projections for the years within the models, random coefficients were added to the thermal variables predictions of the fixed part of the models. While for other years, random coefficients were calculated, following a normal distribution with sd of the random part of the model, and they were added to the predicted response variable of the fixed part.

Results

All the 59 sampled water bodies always froze in winter during the study period. Water bodies froze between 13th October and 20th December, and on average they froze the 12th November. They melted between the 2nd March and the 30th August, and on average they melted the 5th June (Fig. 3.2; Appendix 1).
Therefore, the duration of the ice-cover ranged between four and eleven months, being the mean eight months (Fig. 3.2; Appendix 1).

Table 3.1: Summary of the coefficients of the mixed models. Coefficients were calculated from z-scores obtained from original values. The coefficients of the fixed part (Radiation: Mean direct solar radiation duration in the total catchment (hours per day), Tcatchment/Larea: Logarithm of the total catchment lake area ratio, X: X UTM 31N coordinates, Y: Y UTM 31N coordinates (m), Larea: Logarithm of water body area (ha), Altitude (m), Tspring: Spring air temperature (°C), Tsummer: Summer air temperature (°C), Tautumn: Autumn air temperature (°C), their interactions (marked with “:”) and the standard deviations of the random part of the model (sd fixed: standard deviation of the fixed term, sd year: standard deviation due to the year and water mass type, sd residuals: standard deviations of the residuals) are represented for three ice phenology variables of the lake (Ice-on: freezing date of the lake (d), Ice-off: melting date (d), Ice-cover: Ice cover duration (d))

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</tr>
<tr>
<td>sd fixed</td>
<td>0.55</td>
<td>0.81</td>
<td>0.85</td>
</tr>
<tr>
<td>sd year</td>
<td>0.41</td>
<td>0.43</td>
<td>0.35</td>
</tr>
<tr>
<td>sd residual</td>
<td>0.73</td>
<td>0.46</td>
<td>0.45</td>
</tr>
<tr>
<td>R² marginal</td>
<td>0.30</td>
<td>0.62</td>
<td>0.69</td>
</tr>
<tr>
<td>R² conditional</td>
<td>0.47</td>
<td>0.80</td>
<td>0.81</td>
</tr>
<tr>
<td>AIC</td>
<td>554</td>
<td>354</td>
<td>345</td>
</tr>
<tr>
<td>BIC</td>
<td>575</td>
<td>378</td>
<td>372</td>
</tr>
<tr>
<td>RMSE</td>
<td>9.71</td>
<td>17.82</td>
<td>18.90</td>
</tr>
</tbody>
</table>
The adjustments of the models for ice-off and ice-cover were higher ($R^2_c$ 0.80 and 0.81 respectively) than for ice-on ($R^2_c$ 0.47) (Table 3.1; Fig. 3.3). However, the RMSE was lower for ice-on than for ice-off and ice-cover duration (9.7, 17.8 and 19 days respectively). This can be explained by the lower variability in ice-on. The adjustment of the fixed part of the models ($R^2_m$) was 0.30 for ice-on, 0.62 for ice-off and 0.69 for ice-cover duration. Therefore, the difference, associated with the random part of the model, was considerable, and it meant the interannual variability of meteorological parameters other than air temperature. Results of the cross-validation values against observed values and the temporal variation of projections are drawn in Fig. 3.4.

![Figure 3.3](image)

Figure 3.3: (a) Coefficients of the fixed part of mixed models calculated from z-scores classified in seven categories (b) Marginal and conditional R-squared representing the proportion of variance explained by the fixed and random variables respectively.
Altitude was the most important variable explaining the ice phenology in the Pyrenees, since it had a negative effect on ice-on (-0.42), as described by the standardized coefficients of the mixed models. Altitude had a positive effect on ice-off and ice-cover duration (0.73 and 0.74) in the mixed models (Fig. 3.3; Fig. 3.5; Table 3.1). Mean spring interannual variability in air temperature was the
following variable in importance for ice-off and ice-cover duration (-0.35 and -0.29 respectively), but interannual variability in air temperature was not significant for ice-on, changing forward, therefore, the date of ice-off and reducing the ice-cover duration. The ratio between total catchment and lake area was also important for explaining ice-on (-0.14), as water bodies with higher renewal time froze earlier. Radiation also explained ice-off (-0.18) and ice-cover (-0.19), by advancing the date of melting.

Figure 3.5: Partial effects of the explicative variables on the ice phenology variables. (See Table 3.1 for complete information about the variables coefficients and variables description)
The structural equation model showed that water bodies freezing late melted earlier and had higher ADD, with a strong correlation between ice-off and ADD of -0.35. In turn, ADD was positively correlated with ice-on (0.31). Ice-off was negatively correlated with previous ice-on date (-0.31), advancing the years with later ice-on (Fig. 3.6). Altitude was positively correlated with ice-off (0.68) and negatively with ice-on, before the ice-off and after summer (-0.36 - -0.33) and ADD (-0.48). Interannual variability in air temperatures were positively related between winter and spring (0.57) and spring and summer (0.40). Autumn air temperatures were correlated positively with ice-on (0.29) while ice-off was negatively correlated with spring air temperatures (-0.62). ADD was positively correlated with spring air temperature (0.24).

The hindcasted mean ice-on date for the decade of 2006-2015 (calculated from measured air temperatures), was 4th November for the wide set of 2,267 Pyrenean lakes and ponds. Ice-on had no significant trend under any climate change scenario, as we found no relation with interannual air temperature variability. However, this variable had a great interannual variability, and also it had a great uncertainty, as the adjustments of the models were low (Table 3.1). Mean ice-off date was predicted to be on 8th July, and projections resulted in an advance of 23 days (15th June) under the RCP2.6 scenario and 32 days (7th of May) under the RCP8.5 scenario. As for the ice-cover duration, the mean value of current estimates for the whole set of Pyrenean water bodies was 211 days and future projections showed a reduction to 189 days under the RCP2.6 scenario and 179 days under the RCP8.5 scenario by 2090. Despite the expected advance in ice-off and reduction of ice-cover, projections indicate that lakes and ponds in the Pyrenees will still freeze in winter.
Discussion

Factors affecting ice-cover duration variability

Lakes and ponds in the Pyrenees are located in a wide mountain region with over 3,000 lakes and ponds located at the South of Europe at mid-latitudes (42 - 43 °N). Despite their southern situation, they always froze in winter during the studied period (Fig. 3.2; Appendix 1), as they were located at high altitudes, in comparison to the lower altitudes of some northern European lakes, such as the Müggelsee, which could show intermittent ice-cover or even winters without ice (Livingstone and Adrian 2009). The two events which delimit the ice-cover duration, ice-on and ice-off date, showed different temporal variability, being ice-off more variable in time (Fig. 3.2; Appendix 1). Both variables were explained by different variables or with different effects. Ice-off showed dependence on time, as it was affected by interannual variation in temperature, in contrast to ice-on, which only had random temporal variability. Furthermore, observed past trends of ice phenology showed a greater change in ice-off relatively to ice-on (Benson et al. 2012) and a higher sensitivity to air temperature changes (Vavrus et al. 1996). Ice-on was also found to be more unpredictable (Table 3.1), as the range of distribution was shorter, probably because local effects of lake and catchment morphology may play a major role, and generally ice-on has been found to be more unpredictable than ice-off (Sporka et al. 2006; Arp et al. 2010; Weyhenmeyer et al. 2011) this unpredictability may be enhanced by autumn air temperatures inversion episodes, which take place in mountain regions (Livingstone et al. 1999; Sporka et al. 2006). This would weaken ice-on dependence on altitude, as the effect of altitude on ice-on is smaller than on ice-off (Table 3.1).

Although the effect of altitude on ice-on was lower than on ice-off or ice-cover duration, altitude was still the most important variable explaining ice-on, as lake surface water temperature in summer and the beginning of autumn decreases with altitude, which is also related to air temperature lapse rate (Livingstone and Lotter 1998; Livingstone et al. 1999; Livingstone et al. 2005; Sporka et al. 2006). This contrasts with the results from Tatra mountains,
where no relation between ice-on and altitude was found (Sporka et al. 2006). Nevertheless, ice-on is more difficult to model. Lake area was the second variable with a higher effect on ice-on, as it is related to mixing depth, and therefore, the amount of heat accumulated in the epilimnion, which delays the ice-on of lakes and ponds. In addition, an important proportion of the variance was due to the random interannual variability, which can be associated to other meteorological variables out of air temperature which have not been included in the model, such as precipitation in form of rain or snow, humidity or cloudiness. We found that regional autumn air temperatures were not significant in explaining ice-on in the mixed models, similarly to what was found in the Tatras (Sporka et al. 2006). However, for longer time series of ice phenology, ice-on and ice-off were equally explained by air temperatures (Benson et al. 2012) also when lakes were analysed individually (Palecki and Barry 1986). In the structural equation model, we found an effect of autumn air temperatures on ice-on. However, summer ADD had a greater influence on ice-on. Therefore, the effects on ice-on are driven by summer water temperatures mainly, and secondly, by autumn air temperatures.

Ice-off, in contrast to ice-on, was mainly dependent on spring air temperatures at the site of the lake. In the Pyrenees, we found that altitude had the most important effect on ice-off, as air temperature decreases with altitude, being interannual variability in spring air temperature the second in importance (Table 3.1; Fig. 3.3). This shows the importance of air temperature in ice-off both in space and time, as shown in previous studies (Sporka et al. 2006; Beyene and Jain 2015). Similarly, ice-cover duration was mainly explained by altitude and interannual variability in air temperature, and a lesser effect of lake area, indicating a greater importance of the variables affecting ice-off. A greater effect of ice-off than ice-on on the ice-cover duration was also found for other mountain areas (Sporka et al. 2006). Ice-off was not only affected by spring temperature but also by winter air temperatures, as spring and winter air temperatures were positively related, but winter air temperature also had a contradictory effect on ice-off. In contrast, lakes on the east coast of North America showed a positive effect of milder winters on ice-off (Beyene and Jain 2015), or even in southern lakes in the Iberian Peninsula (Sanchez-Lopez et al. 2015), in our case the winter influence may be mostly driven by atmospheric
dynamics with a lesser direct effect of winter temperatures on ice-off, as ice-off takes place during spring in the Pyrenees.

ADD in summer were negatively correlated with ice-off date, as shown in the structural equation model (Fig. 3.6) and they were also related to spring air temperature, but not to summer air temperature, as could be thought (Fig. 3.6). Moreover, spring temperature was related to that of summer, so spring temperatures may influence ADD both by affecting ice-off dates, as lower lakes start to warm earlier (Sporka et al. 2006), but also influencing the following season and replacing the influence of summer on ADD, as was indicated in a previous study on lake summer temperatures (Chapter 2). An effect of spring air temperatures on maximum water temperatures was also found in Canada (Sharma et al. 2008), but there, summer was also significant, as it may be of more relevance in the case of maximum temperature.

ADD also influenced positively the ice-on date (Fig. 3.6), which indicates an effect of summer temperatures on winter ice-cover, as ice-on was also related to earlier ice-off. In addition, lake characteristics, such as altitude and lake area also affected ice-on. In the structural equation model we found that ice-on was also influenced by autumn air temperature (Fig. 3.6). This effect contrasted with the results of the mixed models where no effects of autumn air temperatures were found (Table 3.1; Fig. 3.3). Therefore, the ice-on depends more on ADD and morphological traits of the lakes (altitude and lake area) than on the interannual air temperatures, stressing the idea that ice-on depends more on lakes heat storage capacity than on temperature exchange with air, which had a secondary role. The results of the structural equation model point at a certain memory of the lakes as ice phenology and summer temperatures are related; this is, in contrast, the case of lakes Mendota and Monona, where no temporal autocorrelations were found (Sharma et al. 2013).
Figure 3.6: Relationship between the interannual variability of seasonal air temperatures, Altitude and Lake area (Larea) with ice phenology (Ice-on and Ice-off) and accumulated degree-days above 4 °C (ADD) for 59 Pyrenean lakes and ponds. Mean interannual air temperature variability of winter, spring, summer and autumn are abbreviated as Twinter, Tspring, Tsummer and Tautumn respectively.

*Ice-cover duration projections*

Ice-cover duration in Pyrenean lakes is expected to decrease in the following decades because of the advance in ice-off date, which is expected to advance between 22 and 32 days by 2090. Depending on the warming scenario, ice-cover is expected to decrease the same period of time, as ice-on is not expected to change significantly. Despite the reduction in the ice-cover duration, all lakes and ponds in the Pyrenees are expected to freeze. These results are comparable to other studies where the advance of ice-off was greater than the delay of ice-on. For instance, simulations of process-based
models of Fennoscandian lakes indicate an advance in 5-75 days on ice-off and a retard in 6-36 days in ice-on (Lepparanta 2010). Empirical models in the Great Lakes region in North-America project an advance in ice-off between 3 days later and 43 days earlier (Hewitt et al. 2018). We found no change in ice-on in future projections as all the temporal variation was considered to be random in the models and we found no significant effect of air temperatures on ice-on. Other studies project a delay in ice-on of 6-36 days (Lepparanta 2010) and 1-28 days (Hewitt et al. 2018). In a wider window of time, air temperature may have significance in our models, as the examined decade has been of great variation, but of a scarce warming trend compared to the global warming long-term trend (Dokulil 2014; IPCC 2018b). In addition, ice-on had a greater influence of the water body dynamics than ice-off, so it is expected to change less. Indeed, a greater change has been observed in air temperatures in winter and spring than in autumn in Fennoscandia (Gebre and Alfredsen 2014), demonstrating the greater sensitivity of ice-off in comparison to ice-on. Our projections show intermediate reductions in the ice-cover (22-32 days reduction) comparing to other regions; 21-85 days (Gebre et al. 2014), 5-75 days (Lepparanta 2010) in Fennoscandia and 14 to 34 days by 2070 in the Great Lakes region (Hewitt et al. 2018). However, the reduction of one month in ice-cover may have important implications for the ecosystem.

The predicted ice phenology changes in the Pyrenees may have important implications as their shifting is known to produce cascading effects driving to physical, chemical and biological changes in temperate lakes (Gerten and Adrian 2002). An advance of 22-30 days in the ice-off of the Pyrenean lakes may induce an earlier stratification, and therefore, an earlier phytoplankton bloom, as was found in Northern Germany (Adrian et al. 1999; Gerten and Adrian 2002). Also, zooplankton eggs hatching may advance and increase as experimental work has shown that earlier spring conditions of temperature and photoperiod enhance it (Caceres and Schwalbach 2001). In Central Europe the advancement in zooplankton bloom increases predation on phytoplankton leading to an increase in the transparency of the lakes, a phenomenon named clear water phases (CWP). These phases are expected to advance following earlier ice-off (Adrian et al. 1999) and air temperature increase in Central Europe (Straile 2002). In the Pyrenees, where the lakes are mostly oligotrophic
(Camarero et al. 2009), the occurrence of CWP is not so clear, and must be studied in detail. Moreover, some studies point at a mismatch between phytoplankton and zooplankton blooms driving to a decrease in zooplankton densities and an absence of a CWP (Winder and Schindler 2004). So, an advance in ice-off and the reduction of ice-cover may induce important changes in how species relate to each other driving to important and unknown changes in the communities.
PART II: Relative effects of temperature and predation on zooplankton

4. The relative importance of predation and temperature on the zooplankton of Pyrenean high mountain lakes
Abstract

Biotic and abiotic factors, including temperature related variables and predation are known for long to affect zooplankton assemblages. In high mountain lakes of the Pyrenees, fish introductions of trout and minnows have been extensive, affecting ca. half of the lakes in the mountain range. These fishes can also affect natural predators of zooplankton, so their interactions are also relevant in structuring the community. Moreover, future projections predict reduced ice-cover and increased temperatures in lakes. In this study, we aim at describing the zooplankton community, their species occurrence, abundances and body size. And further analyse how lake geomorphology, water chemical composition/phytoplankton, radiation, temperature and predation variables explain the zooplankton community composition, as well as how the interactions among zooplankton groups affect their community. We also wanted to assess the effects of fish predation on the size and fecundity of planktonic crustaceans. Our results showed a high abundance of the crustacean species Daphnia longispina, Cyclops abyssorum, Eudiaptomus vulgaris and Chydorus sphaericus, and of the rotifers genera Kellicottia, Keratella, Asplanchna, Polyarthra and Conochilus. The communities composition were explained, by order of importance, by morphology of the lake and the catchment (5.41 – 12.06 % of variance), water chemistry/phytoplankton, including nutrients, Chl-a and alkalinity (0 -12.61 %), predators (0.94 – 5.02 %), climate (2.41 – 4.20 %), radiation (0 – 2.82 %) and space (0 – 2.03 %). Among predators, we found a greater impact of minnows than of trout. Other predators such as newts reduced big size zooplankton abundances. The effect of fish predation is complex as it is transmitted through the food web. Fish predation caused a reduction in big and medium zooplankton, especially in the case of minnow, which also reduced D. longispina fecundity. The ice-cover duration was fundamental in zooplankton phenology, and there was a thermal niche differentiation between E. vulgaris, towards warmer ponds and cyclopoids towards colder lakes.
Introduction

Ecological systems are known to be regulated both by abiotic and biotic factors. Abiotic factors, principally nutrient loadings cause bottom up control of ecosystems while biotic factors, mainly changes in predators’ abundance, produce the bottom-up effects. In lakes, productivity depends on nutrient concentrations, especially total phosphorous (TP) (Wetzel 1983), but also on nitrogen (Buñay 2017). On the other hand, predators such as planktivorous fish produce changes in zooplankton composition, which are mostly consumers of primary producers, reducing then primary productivity in a top down control of the ecosystem (Brooks and Dodson 1965). Moreover, biomanipulation experiments, which consist of introducing or removing a superior trophic level, (e.j. piscivorous fish) could affect lower trophic levels affecting in the end primary productivity in what is called a trophic cascade (Carpenter et al. 1987). In this context, zooplankton plays a major role as it might control primary producers’ abundance and they serve as a food source to superior trophic levels, and, at the same time, their abundance and composition depend on both nutrients and predators. In addition, the way bottom-up and top-down control zooplankton assemblages is different, as bottom-up affects individual and population growth rates, whereas top-down effect cause changes in population density biomass and individual size (Gliwicz 2001). Predation can also alter reproduction due to selective predation of egg-bearing females, and produce indirect effects through life-history and behaviour changes in zooplankton (Gliwicz 1994).

Zooplankton not only has to deal with fish predation, but also with other organisms such as newts (Ranta and Nuutinen 1985; Schabetsberger et al. 2006) or insects, as Chaoboridae or Corixidae, which can predate on zooplankton as well (Nyman et al. 1985). Predation pressure can differ depending on predator species, and interactions among them can induce also different predation effects than each predator alone. For instance, trout introductions in lakes with minnows reduced minnow abundance in the Alps, which lead to an increase in crustacean abundance and size. Moreover, newt predation had a minor effect on zooplankton (Schabetsberger et al. 2006).
Also, predation affected more conspicuous zooplankton, by their size (Gliwicz 2001; Tiberti et al. 2014a), coloration (Hairston 1979) or density (Gliwicz 2001), as fish select prey among their sighting distance (Gliwicz 2001). In the case of planktivorous fish, gill-rakers are related to the size of their preys (Brooks and Dodson 1965). Zooplankton also adapts to predation adopting particular behaviour and life-history traits to avoid predation. For example; fast swimming ability is given as the reason of calanoids advantage towards cladocerans and cyclopoids facing insect predation (Nyman et al. 1985).

Relationships among zooplankton species are complex and include both competition and predation, which can affect the assemblage of zooplankton species and their abundances. Both calanoids and cyclopoids are known to be in a higher trophic levels than cladocerans (Ventura and Catalan 2008). Cyclopoid species can predate on cladocerans, on rotifers. Early copepodite stages can predate on bigger *Daphnia* eggs entering their brood pouches, thus reducing their fecundity (Gliwicz and Stibor 1993), also late copepodite stages (IV-V) and adults can show cannibalistic behaviours, by inter or intraspecific nauplii predation (Vandenbosch and Santer 1993). Size-effective hypothesis states that bigger zooplankton has an advantage over the smaller organisms, so they become dominating in absence of predators, whereas smaller zooplankton may become dominant with high predation pressure (Brooks and Dodson 1965). Also, the competitive exclusion principle states that close related species cannot coexist (Hardin 1960), it was observed so in Pyrenean lakes, as species within the genera *Daphnia* and *Diaptomus* rarely coexisted in those lakes, as they would be too similar (Buñay 2017).

All these theories about top-down control in lakes also lead to research in the field of conservation biology, as even high mountain lakes have been introduced with fish in many regions, such as the Alps (Pechlaner 1984), Sierra Nevada, California (Rahel 1997) and Central Range (Toro et al. 2006), Cantabrian Range and the Pyrenees (Miro and Ventura 2013; Miró and Ventura 2015) in the Iberian Peninsula. A number of studies report local extinction of species and change in zooplankton composition due to fish introductions (Knapp et al. 2001b; Schabetsberger et al. 2009; Tiberti et al. 2014b), as well as
conspicuous insects (Knapp et al. 2001b; de Mendoza et al. 2012) and amphibians (Orizaola and Brana 2006; Miro et al. 2018), they can also alter nutrients recycling increasing consequently phosphorous loads (Schindler et al. 2001), and even affect subaquatic macrophytes through changes in water transparency (Gacia et al. 2018). Restoration actions have also been done in some regions to recover autochthonous populations and study resistance and resilience of these ecosystems (Knapp and Matthews 1998; Parker et al. 2001; Tiberti et al. 2019).

Climate change, in addition to biological invasions, is another important implication of current global change. One of the most certain features of climate change is air temperature increase due to greenhouse gases emissions. Air temperature has been increasing steeply since the beginning of the industrial revolution, and future air temperature rise is expected to increase between 0.3 and 4.8 °C globally in the period from 2081 to 2100 (IPCC 2013b). More recent reports state that we have already reached a global temperature warming of 1 °C since pre-industrial times (1850-1900), and it is likely to rise to 1.5 °C by 2030-2052 following current trends (IPCC 2018a). The report stressed the importance to reach zero net CO₂ emissions to halt anthropogenic warming, as greater temperature increases would imply a greater risk. This air temperature rise may cause an increase in mean water surface temperatures in the Pyrenees, as well as maximum temperatures in high altitudes (chapter 2) and reduce ice-cover (chapter 3). A temperature increase in the lakes may cause an increase in organisms’ metabolic rates (Gillooly et al. 2017), which could affect individual growth (Balcells 1956) and utterly affecting relations among species (Symons and Shurin 2016), also changes in ice-cover duration may induce changes in organisms phenology (Adrian et al. 1999).
In this study, we aim at describing the factors explaining Pyrenean lake zooplankton species distribution, with special emphasis on the importance of the environmental filter. Our objective was to understand the relative importance of climatic factors and predation patterns compared to other environmental factors to explain zooplankton composition and structure. To accomplish our objective, we worked at three scales: 1) understanding the variability of the factors in the whole biogeographic region (Pyrenees) and covering a full gradient of lake surface areas, from small ponds to large lakes; 2) focussing on the importance of different predation pressures, we selected a subset of lakes within a smaller geographical area (Central Pyrenees), being the presence of different predators the main expected difference amongst all these lakes; and (3) we explored the process of zooplankton community recovery by removing the top predators (different fish species) in eight lakes.
Methods

Study area

The study area comprised lakes and ponds located in the Pyrenees mountain range, which is located between 1° W – 3° E and 43° N – 42° N. The region includes up to 3,000 lakes and ponds situated at altitudes between 1,053 and 2,978 m. Most of the lakes and ponds in the region originated during the last glaciation, which also shaped their catchments (Catalan et al. 2009). The largest lake in the Pyrenees measures 160 ha, whereas the widest catchment measures 5,392 ha. Further characteristics of the lakes are described in Table 4.1. The sampled lakes contained lakes without fish, also referred to as natural, lakes with introduced minnows or trout, both fishes or lakes where fish have been removed within the conservation project (LIFE+ LIMNOPIRINEUS), referred to as restored. Lakes where water level is regulated by impoundments for hydroelectrical production were not included in this dataset.

Table 4.1: Description of the ranges of the main characteristics of the studied lakes and ponds. Mean values are given for altitude and lake area in parenthesis. Variables are described separately for each of the subsets used in this work. PYR for the biogeographic differences in zooplankton in the region, FISH for the analysis of fish predation and RES to study the effects of restoration.

<table>
<thead>
<tr>
<th></th>
<th>Pyrenean subset (PYR)</th>
<th>Fish effects (FISH)</th>
<th>Restoration changes (RES)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Longitude (*)</td>
<td>0.70 W – 2.21 E</td>
<td>0.81 – 1.68 E</td>
<td>0.81 – 1.33 E</td>
</tr>
<tr>
<td>Latitude (*)</td>
<td>42.42 – 42.97 N</td>
<td>42.52 – 42.77 N</td>
<td>42.53 - 42.51 N</td>
</tr>
<tr>
<td>Altitude (m)</td>
<td>1618 – 2880 (2283)</td>
<td>1618 – 2574 (2271)</td>
<td>1638 – 2521 (2182)</td>
</tr>
<tr>
<td>Lake area (ha)</td>
<td>0.0014 – 53.19 (4.03)</td>
<td>0.027 – 8.00 (1.91)</td>
<td>0.16 – 7.35 (1.76)</td>
</tr>
<tr>
<td>Samples nº</td>
<td>205</td>
<td>58</td>
<td>107</td>
</tr>
</tbody>
</table>
To develop this study we have used a total of 337 zooplankton samples of a total of 207 lakes and ponds of the Pyrenees, which were sampled in three main sampling occasions with different geographical extensions and lake area coverage (1996-2000 for the EMERGE project, Pyrenean scale, including only lakes; 2010-2013 for the INVASIVEFISH project, Pyrenean scale, including lakes and ponds; 2014-2018 for the LIFE LIMNOPIRINEUS project at Central Pyrenean scale, including lakes and ponds) (Fig. 4.1; Table 4.1). To answer the three main questions of this study, we combined the different sampling occasions in the following manner: 1) Pyrenean scale study: We combined the three studies at Pyrenean scale; 2) Fish effect study: We selected the group of lakes sampled within the LIFE LIMNOPIRINEUS project with different predator abundance (fish and newts) in order to answer the effect of introduced fish on zooplankton composition and structure. 3) Restoration effect study: Within this project, we removed the fish in a subset of eight lakes, referred to as restoration lakes (RES), which were sampled twice per ice-free period (July and September) during 5 years (2014-2018). In addition, we also sampled a subset of three lakes, which were used as natural controls (NAT), with similar characteristics as the RES lakes and Vilac Pond, a small lake where, in addition to removing trout, we also restored the hydrological regime.

For each lake, planktonic crustaceans and rotifers were sampled separately with two different techniques. Of the global set of samples 223 included both groups, while for the remaining only planktonic crustaceans were sampled. The first samples date back to 1998, whereas the last ones are from 2018. Samples were taken in the deepest point of the lake from an inflatable boat. The planktonic crustacean samples were taken with a Hansen type net with a mouth diameter of 0.022 m$^2$ and a mesh width of 200 µm, each sample consisted of three different vertical catches. For small ponds, samples were taken from the littoral either with the net or with a 2 m methacrylate tube. The rotifers and small crustacean (e.g. stages or species smaller than 200 µm) samples were taken with a UWITEC bottle taking almost 12 L from different depths integrating the whole water column and were subsequently filtered with a 50 µm width mesh. Samples were fixed with formol or lugol for posterior analysis.
Planktonic crustaceans were identified with binocular magnifying glass (Wild M3) and a microscope (Nikon Eclipse E200) with the manuals: Keifer (1978); Einsle (1993); Dussart and Defaye (1995) for the copepods and (Alonso 1996) for the cladocerans. Individuals from both groups where identified to species level. Rotifers were identified to species for most of the samples although for this work we kept the genera level. We used a microscope and followed Voigt (1956) for the taxonomic identification. Species abundances were counted by aliquoting the samples and at least 250 individuals were counted for each subsample of both crustaceans and rotifers with an inverted microscope (Olympus IMT-2). We measured approximately 15 individuals of each taxon and stadium in each sample. When there were females with eggs, we counted and measured them and calculated the clutch size and fecundity of *Daphnia longispina*, which was the only species with sufficient individuals to perform statistical analyses.

**Biological and environmental factors**

Potential predators were also sampled in the field within the last project, from 2014 to 2018; this included macroinvertebrates, amphibians and fishes, from which we could estimate their abundances. The macroinvertebrates taken into account were three genera of the family Corixidae: *Sigara*, *Arctocorisa* and *Glaenocorisa*. They were sampled in the littoral at a depth of approximately 0.8 m by kick sampling (Frost et al. 1971; de Mendoza et al. 2015). They were preserved in ethanol and identified and counted in a stereomicroscope (Olympus SZ90). Fish were sampled with 2 to 8 multi-mesh gillnets, varying their number depending on lake size, deployed at different depths during 20-24h and thereafter captures were counted. Amphibians were counted by visual inspection in five different transects around the lake. For a wider subset of lakes we had presence/absence data from visual inspections. (Miro et al. 2018).

Water samples were taken from the lake exit or in the surface in the middle of the lake for the chemical analyses. They were performed following the methodologies described in Ventura et al. (2000). Chemicals analysed were pH,
alkalinity (Alk), conductivity (Cond), total phosphorous (TP), total nitrogen (TN) and dissolved inorganic nitrogen (DIN). Chlorophyll a (Chla) was analysed from samples taken at 1 the depth of the Secchi disk or at 1 to 2 m over the lake bottom if not possible. It was analysed with a stratigraphic column UPLC ACQUITY (Waters Corporation USA) and ACQUITY UPLC C18 SB 1.8 µm HSS (2.1 mm x 100 mm). Methodologies are detailed in (Buchaca et al. 2005). Light extinction constant (Kd) was calculated from the Secchi disk depth taken from the boat. When the disk arrived at the bottom of the lake it was estimated to be $K_d=0.02$ m$^{-1}$ (Buchaca et al. 2005). Cattle pressure was determined in the field counting the number of excrements around the lakes’ perimeter (ca. 20 m). Macrophytes submerged species were identified by visual inspections and diving (Further details of the variables are in Appendix 13).

The percentage of the coverage was determined by visual inspection in the field and they were only available for the reduced set of samples. Lake depth was measured with an echo sounder (Plastimo). Other morphological variables, such as lake area and catchment area, were derived from GIS for all the sampled lakes in the Pyrenees (Casals-Carrasco et al. 2009). Radiation variables were also derived from the Cartography. Climatic variables of the lake, such as mean and maximum temperature, accumulated degree-days (ADD), diel temperature range (DTR) the date of ice-off or the ice-cover duration were predicted following the models described in chapters 2 and 3. Air temperatures used in this model were observations from the Catalan Meteorological Office from 2000 to 2018, and past air temperature reconstructions from Agusti-Panareda and Thompson (2002). Julian date and the date from predicted ice-off were also calculated.

Spatial variables used were Moran Eigenvector Maps (MEM), which are eigenvectors selected maximizing Moran index of autocorrelation after the diagonalization of a spatial weighted matrix (Dray et al. 2006). To define neighbouring among lakes we used relative neighbours distance in this analysis. From all the eigenvectors, we retained the ones with significant positive Moran Index. Eigenvectors with high values represented local patterns
while low values represented regional ones. All the analysis were done using the package “spacemakerR” (Dray 2010) in R language (R Core Team 2019).

Statistical analyses

Redundancy analyses

To analyse how predators, climatic and environmental factors explained the zooplankton composition, we used redundancy analyses (RDA). We conducted three different RDA analyses in order to unveil 1) Biogeographic variation of zooplankton communities (RDA_PYR), 2) Fish predation effects on zooplankton communities (RDA_FISH) and 3) Change in zooplankton communities during lake restoration (RDA_RES). The main morphological characteristics of the study sites of the three subsets are detailed in Table 4.1. Before the analyses, some variables were normalised by transforming them with the logarithm or logarithm+1 and later standardized (They are detailed in Appendix 14). Abundance variables were also transformed with the logarithm+1. Analyses were performed in R language (R Core Team 2019). First, variables were selected among the following categories: Geomorphological, chemical, radiation, climatic and predators (variables included in the initial models are detailed in Appendixes 13 and 14). Variables were selected by stepwise forward selection using the package “vegan” (Oksanen et al. 2019) following the method described in (Blanchet et al. 2008). The variables selected in each group were grouped together and selected again with the same methodology. We checked that the covariance among resulting variables was low by proving that VIFs values were lower than ten (Borcard et al. 2011). Significance of the RDAs and their axis were tested with permutation test (n=1999). From the resulting RDA we performed a partition of the variance of the variables grouped in 6 variables groups to know the relative importance of each one and their shared variance. The variables were grouped as geomorphological, spatial, chemical/phytoplankton, climatic, radiation and predators (The variables included in each group are described in Appendix 14).
Structural Equation Model

In order to more specifically analyse the relations among predators, climatic variables, resources and zooplankton, we performed a Structural Equation Model (SEM). These models are a series of linear regression models, which are fitted by assimilating model covariance to that of the observed values. They have been used in ecology to find direct and indirect effects among habitats and food webs (e.g. Strauss et al. 2016). We conducted the SEM using the “sem” package (Fox 2006) in R language (R Core Team 2019). First, we transformed the variables with the logarithm+1 to linearize the data (Maurer et al. 2014), and we calculated the covariance matrix, then we defined an initial model (Variables are specified in Appendix 14, and the initial model in Appendix 16) from which we dropped non-significant variables sequentially until all remaining variables resulted significant. This analysis was conducted with the subset of 132 lakes sampled within the LIFE LIMNOPIRINEUS project (both the FISH and RES dataset), since for these lakes we had quantitative information for the predators’ abundances. We included here the samples from the restoration lakes to see both the effects of temperatures and predators on the zooplankton community.

Analyses of variance

In order to study the effects of predation on the proportion of rotifers to crustacean abundance, the size of the crustacean species and on fecundity we divided the lakes into different categories depending on the fish predators present in the lakes. We separated between lakes with trout (SALM) or minnow (PHOX), with both fish (SALM+PHOX), lakes without fish (NAT), and restoration lakes (RES). Data were normalised as detailed above. Then we conducted analyses of the variance (ANOVAs) to seek differences among the crustacean taxa for which we had sufficient data. Then we performed Tuckey post-hoc tests to inspect one-to-one differences. To see differences in fecundity, we used *Daphnia longispina* females, for which we had sufficient data, and we analysed the different relations between clutch size and egg size in relation to the females’ size for the different predator categories of the lakes.
Results

Zooplankton in Pyrenean lakes

Among the sampled Pyrenean lakes and ponds we found 25 different crustacean species and 15 different rotifer genera. They belonged to 207 crustacean plankton and 125 rotifers samples of lakes and ponds. Diversity was low, as mean richness was 2.5 both for crustacean species and rotifer genera. The maximum number of species we found in a lake was 6 crustacean species and 6 rotifer genera. The most ubiquitous crustacean species were *Cyclops abyssorum*, *Daphnia longispina*, *Chydorus sphaericus* and *Eudiaptomus vulgaris* (occurrences of 150, 127, 66 and 50 respectively), especially *C. abyssorum* and *D. longispina*, which appeared in more than half of the lakes and ponds. However, *C. sphaericus* was not very abundant (Fig. 4.2). As for the mean abundance, *C. abyssorum* and *D. longispina* were also the two species with the higher abundance. *Mixodiaptomus laciniatus*, which was only present in 15 water bodies, showed high mean abundances, even more than *E. vulgaris*, which was quite ubiquitous. The remaining species showed low abundances, but we could also find other calanoids as *Diaptomus cyaneus* and *Diaptomus castaneti*, cyclopoids as *Eucyclops serrulatus* and cladocerans as *Ceriodaphnia quadrangula*, *Alona affinis*, *Daphnia pulicaria*, *Eurycercus lamellatus* or *Scapholeberis mucronata* in more than ten water bodies. We could also find in less than ten localities species like the cyclopoids *Acanthocyclops vernalis*, *Paracyclops fimbriatus* and *Macrocyclops fuscus*, cladocerans as *Simocephalus vetulus*, *Daphnia obtusa*, *Alona intermedia*, *Acropaerus harpae*, *Polyphemus pediculus* and *Daphnia parvula*, as well as the amphipod *Gammarus cf. lacustris*.

Regarding the rotifers, the genus *Kellicottia* was present in the majority of lakes, 96 out of 126 lakes (Fig. 4.3b), therefore, it was the most abundant rotifer genus in the Pyrenees (Fig. 4.3a). Other ubiquitous genera were *Asplanchna*, *Keratella*, and *Polyarthra*, which were present in more than 50 lakes. Of these three, both *Keratella* and *Polyarthra* showed high abundances, of more than 100 individuals per cubic meter, whereas *Asplanchna* was less
abundant. The genera *Conochilus* and *Lecane* were also ubiquitous, as they were present in more than 25 lakes. *Conochilus* was also abundant with a mean abundance of more than 100 ind. m\(^{-3}\). *Flinia* and *Colurella* appeared in more than ten lakes where *Flinia* was highly abundant. Other genera such as *Proales*, *Pleosoma*, *Euchlanis*, *Cephalodella*, *Trichocerca*, *Trichotria* and *Brachionus* were rare, only present in less than five localities. Of these genera *Brachionus* showed high abundances when present.

Among the Pyrenean zooplankton, size varied between few micrometers of crustacean embryos or nauplii and small rotifers to around 3 mm of the biggest crustacean species (Fig. 4.4). The biggest species were the calanoids *D. cyaneus* and *D. castaneti*, followed by the cladocerans *E. lamellatus*, *D. pulicaria* and *D. obtusa*. Both *D. cyaneus* and *D. pulicaria* showed wide range of sizes with some individuals of 3 mm length. *M. laciniatus*, *D. longispina*, *E. vulgaris* and *C.
Abyssorum were medium-sized. Even so, the last three species could grow over 2 mm, and *D. longispina* could reach the size of bigger *D. pulicaria*. Small crustaceans included small cladocerans such as the Chydoridae *A. affinis*, *A. harpae* and *C. sphaericus* or cyclopoids as *E. serrulatus* and *P. fimbriatus*. As for the rotifers, they did not differ much in their sizes (Fig. 4.4b), they were normally between 100 and 200 μm. The only genus which achieved bigger sizes was *Asplanchna*, which could grow over 600 μm. *Conochilus* also showed some individuals which could grow over 300 μm.

![Figure 4.3: Mean rotifers genera abundance a) and presence b) in the samples Pyrenean lakes (n=125).](image)

**Factors explaining zooplankton composition**

Geomorphology, space, chemical, radiation, climatic and predators variables together could explain a big proportion of the composition of the zooplankton in Pyrenean high mountain lakes. The variance explained by the three subsets of samples analysed was considerable, $R^2=0.25$ for RDA_PYR, $R^2=0.23$ for the RDA_FISH and $R^2=0.47$ for RDA_RES. These three analyses sought different purposes. RDA_PYR was intended to unveil biogeographic factors in the whole region, whereas RDA_FISH analysed the effects of predators’ abundance and RDA_RES accounted for changes due to restoration. The shared variance among variables groups was high, especially for the RDA_RES analysis; even
when the variance inflation factors (VIFs) were lower than 10. The groups of variables which explained more variance were geomorphology and chemistry/phytoplankton, followed by climate and predators (Table 4.1). The ones which explained less variation in the zooplankton composition were radiation and space. There were some differences among the three subsets analysed. For the whole Pyrenees region (RDA_PYR) geomorphological, chemical and climatic variables explained a similar proportion of variance. In the analysis of the fish predation effects (RDA_FISH) we found the greater geomorphological effects. Also, the variability explained by predators was the highest among the analyses, as we included abundance data of the predators. In addition, we found significant climatic and spatial effects. Finally, for the analysis of the restoration lakes (RDA_RES) most of the variability was explained by chemical variables followed by geomorphological variables and predators. Also radiation and climatic variables were significant in the final models.

The explicative variables selected for the regional dataset RDA_PYR were lake area (Larea) and altitude for geomorphology; dissolved inorganic nitrogen (DIN), Chlorophyll a (Chla), Alkalinity (Alk) and dissolved inorganic nitrogen (DIN) for chemistry/phytoplankton variables; climatic variables were represented by ice-off and ADD4. The predator variables resulting in the model was salmonids presence. For the analysis of the fish predation effects, RDA_FISH, the variables selected were maximum depth (Depth) as geomorphological variable; minnow abundance (Phox_cpue) as predator variable, ADD7.6 representing the climate and MEM 4 as spatial variable. Regarding the restoration effects (RDA_RES) we found alkalinity, the light extinction coefficient (Kd), pH and DIN constituted the chemical/phytoplankton variability, geomorphological variables included maximum depth and total catchment area (Tcatchment). Predators included both minnows and salmonids abundances. The climatic variable selected was the date since ice-off (Fdate). Radiation variables were also selected, both sun hours (Sun) and direct radiation (Dradiation) (Fig. 4.5).
The analysis of the whole Pyrenees region (Fig. 4.5a) showed a clear differentiation between natural lakes and lakes with salmonids presence, which were related to the first axis together with lake area and Chlorophyll a, on the opposite direction were altitude and ice-off. The second axis was related to chemical variables, as the DIN, also related to higher altitudes and ice-off dates in opposition to the thermic variable ADD4. The species D. longispina (Dapl), C. abyssorum (Cyca), the rotifers genera Kellicottia and Asplanchna were related to lakes with salmonids, Chlorophyll and bigger areas, whereas E. vulgaris (Evul) and the rotifers Polyarthra, Keratella, Lecane and Conochilus were related to warm lakes with low DIN concentrations. Big calanoids were related to high DIN concentrations, higher and colder lakes, with D. cyaneus (Diacy) and M. laciniatus (Mlac) related to lakes with bigger surface area.

The analysis of the fish predation effects (RDA_FISH; Fig. 4.5b) showed a first axis partially related to maximum depth and a spatial pattern (MEM 4), as opposed to warm lakes, with high accumulated degree-days (ADD7.6) and with minnow abundance. In the second axis we found minnows and depth as opposed to the warm lakes. The big copepod D. cyaneus and the abundant C. abyssorum and D. longispina were related to deep lakes, whereas the also dominant calanoid E. vulgaris and the smaller C. quadrangula were related to warm and shallow lakes and a spatial pattern. Lakes with abundant minnows were clearly related to rotifers and opposed to planktonic crustaceans. It was also clear the separation between lakes with minnows alone and the lakes with both minnows and salmonids.
Figure 4.4: Size of the measured planktonic crustacean species a) and rotifers genera b). Boxplots show the mean, first and third quartile.
Figure 4.5: Redundancy analyses (RDAs) of the zooplankton taxa as a function of environmental variables and predators. a) RDA_PYR: Analysis of the whole region spatial variability in zooplankton communities (n=156). b) RDA_FISH: Analysis for the spatial effect of predators (n=44). c) RDA_RES: Analysis of the effects of lake restoration (n=81). Zooplankton taxa are drawn in blue and environmental variables and predators in red. Samples are painted in green for natural lakes, yellow for lakes introduced with salmonids, orange for lakes introduced with minnows and violet for lakes with both salmonids and minnows. The lakes in process of restoration are painted in their original status colour and circled in black, and the Vilac pond is depicted in dark blue. Each axis includes its explained variance.
In the restoration analysis (RDA_RES), we could see a differentiation in the first axis of a group related to minnows abundance, and partly to lake depth and another group related to sun hours and the date since ice-off, represented by the Vilac pond, which is a shallow warm pond at 1,600 masl. The second axis was related to big lakes with high alkalinity and salmonids abundance. Minnows were related to some rotifers genera such as *Conochilus* and *Keratella*. The calanoid *E. vulgaris* was especially related to the warm conditions that characterize the Vilac pond. *D. longispina* was related to high alkalinity and lakes with salmonids abundance. *C. abyssorum*, *D. cyaneus* and rotifers, as *Kellicottia*, *Polyarthra* and *Asplanchna*, were related to deeper lakes. In this analysis, the differentiation between groups was not that clear. Whereas Vilac pond and minnow lakes were separated, the other group was mixed. Restoration lakes were not grouped together but they were not close to minnow lakes. Some of these lakes showed close values to either *D. longispina* and *C. abyssorum*, whereas in other cases they were more related to *C. sphaericus* abundance.

*How the effect of different predators cascade down the food web*

Since RDAs suggested that rotifer abundance was related to the abundance of minnows, we tested whether there were significant differences in the proportion of rotifers to crustaceans amongst the different fish presence categories (Fig. 4.6). We found that for the FISH and RES datasets minnow lakes had a significantly higher proportion of rotifers than the rest of the lake types. While there were no significant differences in the PYR dataset.
Figure 4.6: Rotifers versus crustacean ratio logarithmically transformed classified in categories by introduced fish presence. NAT: Natural, without fish, RES: Restoration lakes, without fish after restoration, PHOX: With introduced Phoxinus sp., SALM: With introduced salmonids, SALM+PHOX: With both salmonids and Phoxinus sp. VILAC: Vilac pond. Letters represent categories significantly different (p<0.05) in Tuckey tests.

With a structural equation model, we could see the interactions among predators, crustaceans, climate and resources. The initial model with the hypothesized relations is detailed in Appendix 16. Introduced fish, both salmonids and minnows had a clear negative effect on newts (-0.44 and -0.59 for salmonids and minnows respectively), corixids (-0.46; -0.63), calanoids (-
0.34; -0.54) and daphnids (-0.24; -0.26; Fig. 4.7). The effects were more intense with minnows and on bigger organisms (newts and corixids), than on both crustacean groups. On the other hand, salmonids were positively related to cyclopoids (0.20). Newts had a negative effect on calanoids (-0.34), daphnids (-0.26) and small cladocerans (-0.18), affecting positively rotifers (0.42). Corixids presence was positively related to cyclopoids (0.43), daphnids (0.30) and small cladocerans (0.61), and negatively to rotifers (-0.61). These were not density dependent effects, just co-occurrence with this group as we had not enough data of corixids abundance for this analysis. Among zooplankton groups, we only found one significant relation between cyclopoids and daphnids, which was positive (0.43). As for the relations with climate, the analysis showed a negative effect of water mean temperature on cyclopoids (-0.35) and small cladocers (-0.22), whereas it affected positively Chla concentration (0.24). A later date since ice-off affected positively small cladocerans (0.31) and calanoids (0.21) and negatively rotifers (-0.22). Chlorophyll a had a contrasting relation with cladocerans, it had a positive relation with daphnids (0.22), while it had a negative correlation with the other cladocerans (-0.55).

Effects of predation on zooplankton size structure

We found significant differences (p<0.05) in the size of some crustacean planktonic species depending on the fish introduced in the lakes according to ANOVAs. These species were the biggest calanoid: *D. cyaneus*, the most abundant species: *C. abyssorum, D. longispina* and *E. vulgaris*, the ubiquitous small cladoceran *C. sphaericus* and three other cladocerans *A. affinis, C. quadrangula* and *E. lamellatus* (Fig. 4.8). For some species, we had scarce measures for some categories, as their abundance was low, so it was not possible to do the analysis or it should be interpreted with caution. In the case of the biggest crustacean species, we found that they were bigger in natural lakes than in lakes with fishes, in fact, they were not present in lakes with only minnows. For the most abundant species we found that *C. abyssorum* was bigger in fishless lakes or lakes with both salmonids and minnows, whereas it was smaller in lakes with minnows alone. For *D. longispina*, we found the same pattern but they were smaller in lakes with both minnows and salmonids. In the case of *E. vulgaris*, the overall population mean size was smaller in natural
lakes than on the other categories. However, we could find bigger individuals in natural lakes, as also in restored lakes in comparison to lakes with fish, where maximum sizes were constrained. *C. quadrangula* was bigger in lakes with both salmonids and minnows, whereas it had some big individuals in natural lakes. The smallest crustacean *C. sphaericus* was bigger in natural lakes than in the other categories.

Figure 4.7: Path diagram of the structural equation model (n=112) of the zooplankton groups (Cyc: Cyclopoids, Dph: Daphnids, Cla: Smaller cladocerans, Cal: Calanoids, Rot: Rotifers) as function of potential predators (Salm: Salmonids cpue, Phox: Minnows cpue, Newt: Newt abundance, Corix: Corixids presence/absence), lake climate (Tmean: mean temperature, Fdate: Date of the sampling since ice-off) and resources (Chla: Chlorophyll a).
Figure 4.8: Sizes of the planktonic crustacean species classified in categories by introduced fish presence. See Fig. 4.6 for category types. Only adult stages are represented distinguishing between females (f) and males (m), with the exception of *C. quadrangula* and *C. sphaericus*, for which all individuals have been represented without distinction. Only species with significant differences among categories (p<0.05) are represented. Letters represent categories significantly different (p<0.05) in Tuckey tests.
Effect of predation on zooplankton fecundity

We observed that the size of reproductive *Daphnia longispina* carrying eggs ranged between 768 and 2,472 µm. Ranges in reproductive *D. longispina* sizes varied depending on the fishes present in the lakes (Fig. 4.9). They were limited to maximum sizes of 2,088 µm in lakes with salmonids, and 1,920 µm in lakes with minnows, and even less in lakes with both minnows and salmonids, 1,776 µm. The maximum clutch size increased with female size but only in lakes without minnows (fishless and lakes with salmonids). *D. longispina* egg size ranged between 91 µm and 560 µm. Egg sizes increased with female size, and the increase was steeper in lakes with introduced minnows and after restoration (Fig. 4.9).

![Graph showing clutch and egg sizes for each *Daphnia longispina* female size by fish introduced categories. Dashed lines represent the maximum clutch sizes delimited by the female with greater clutch size and the smallest. When it was significant, the relation between egg and female sizes linear regression is plotted by continuous lines. See Fig. 4.6 for category types.](image)

Figure 4.9: Clutch and egg sizes for each *Daphnia longispina* female size by fish introduced categories. Dashed lines represent the maximum clutch sizes delimited by the female with greater clutch size and the smallest. When it was significant, the relation between egg and female sizes linear regression is plotted by continuous lines. See Fig. 4.6 for category types.
Discussion

Zooplankton diversity in the Pyrenees

We found that both crustacean plankton and rotifers diversity in Pyrenean lakes was low. It was 2.5 crustacean plankton species and 2.5 rotifers genera. Past studies in the Pyrenees also gave similar results as the mode was 2 crustacean species in both lakes and ponds, and maximum number of species was 5 and 6 species for lakes and ponds respectively (Buñay 2017). Of the Pyrenean crustacean species, *D. longispina*, *C. abyssorum* and *C. sphaericus* were present in Southern and Central Europe high mountain lakes and differentiated them from Northern lakes in Europe, on the contrary Rotifers assemblages were mainly explained by a longitudinal gradient (Kernan et al. 2009). Of the zooplankton species found in the Pyrenees, only few species were dominant, whereas most of the species showed low ubiquity and abundance among the Pyrenees. Nevertheless, these species could be dominant when present. This is noticeable in the case of *M. laciniatus*, which showed low occurrence but high abundances (Fig. 4.2). The most dominant species among crustacean plankton were *D. longispina*, *C. abyssorum* and *E. vulgaris*, which were medium-sized species. Following the size-effective theory (Brooks and Dodson 1965), bigger zooplankton species would have competitive advantage, thus displacing smaller species by competitive exclusion (Hardin 1960). However, we claim that fish predation would affect bigger species, extirpating them, and therefore, allowing higher densities of smaller competitors (Knapp et al. 2001b; Schabetsberger et al. 2006; Tiberti et al. 2014b). This mechanism may explain the high abundances of some rotifers genera (Fig. 4.2) and the high ubiquity of the mostly benthonic *C. sphaericus*, which may take advantage in the absence of bigger pelagic competitors.

Factors explaining zooplankton composition

We found that combined abiotic and biotic factors explained zooplankton composition in Pyrenean lakes through RDA analyses. We developed three different RDA analyses to find which variables explained the biogeographic
factors among the Pyrenean region in RDA_PYR, the effects of predation by fish (principally) on RDA_FISH and the effects of lakes restoration by fish removal on RDA_RES. The greater effects on zooplankton communities on these different scales were those of geomorphological variables followed by chemical/phytoplankton variables. Compared to these categories, climatic and predation variables explained less variance (Table 4.2) However, they were important to explain the abundances of species (Fig. 4.5). Moreover, predation variables had higher effects using predators’ abundance data than with just presence data on the whole region. The effect of radiation and spatial variables was lower. Shared variance was high only in the analysis of the restoration, probably because variables as morphological and radiation (in the way used in this work) are invariant in time (Table 4.2). It must be taken into account that variables among the same categories may be related, but also variables can be related among groups. Then, the variables represented in the RDAs can be related to other effects not present in these analyses. So for example, climatic variables were explained in a great proportion by geomorphological variables, and therefore, they were highly correlated (Appendix 15). Altitude was not only related to climate variables, but it is also known that fish were introduced mostly in lower lakes of the Pyrenees (Miro and Ventura 2013; Miró and Ventura 2015). Vegetation coverage in lake catchments is also positively related to nitrate (NO$_3^-$ and negatively related to dissolved organic carbon (DOC) (Camarero et al. 2009).

The variables which explained greater variability in zooplankton composition were included amongst geomorphological and chemical variables. The relative importance of these variables varied depending on the dataset. In the regional scale, they were equilibrated, whereas in the fish effect analysis (RDA_FISH), geomorphological influence was higher. Chemical effects were more important for the restoration analysis (RDA_RES), as they can vary in time, and therefore, they can better explain zooplankton composition. Geomorphological variables selected in the RDA models were altitude, total catchment, maximum depth and lake area. These variables were somehow positively related to DIN and DIN/TP (Fig. 4.5), as it was demonstrated that Pyrenean lakes (>0.5 ha) had high DIN and DIN/TP ratios, being phosphorous limited, in contraposition to ponds (<0.5 ha), which were mostly nitrogen limited (Buñay 2017), and DIN
can be retained in bigger catchments (Camarero et al. 2009). Chemical composition, mainly TP and DIN/TP explained primary producers in Pyrenean lakes, represented by Chl a (µm l⁻¹) (Buñay 2017). Also, light attenuation coefficient (Kd) is a variable related to Chlorophyll a, as this pigment increases light absorption. Nutrients were not the only chemical variables explaining zooplankton composition.

Spatial effects were also relevant to explain zooplankton composition as shown in the RDA_FISH model, where it could explain the 2.03 percent of the variance (Table 4.2; Fig. 4.5), for the other analyses, spatial variables were not selected. Recent studies showed how spatial filtering, in addition to the environment, could explain zooplankton assemblage in coastal and freshwater lagoons in the northeast of the Iberian Peninsula (Gascon et al. 2016) and also in the Pyrenees (Buñay 2017). In this study, we have shown that some patterns are both related to the abundance of some species, and to some environmental variables, when the coordinates of the RDAs point in the same direction. In our study the MEM selected pointed mostly towards shallow warm lakes, opposed to lakes with minnows abundance, related to E. vulgaris and C. quadrangula abundance and opposed to rotifers (Fig. 4.5).

**Predation pressures change zooplankton abundance**

Predators alone could explain a considerable amount of variance in the zooplankton composition in Pyrenean lakes (Table 4.1). In the three RDA analyses that we conducted, we found an effect of introduced fish, but none of the natural predators (corixids nor newts) were selected in the final models (Fig. 4.5). In those analyses, we could explain more variability when we used abundance instead of presence data, in the analysis of fish predation effects (RDA_FISH), and also in the analysis of the restoration effects (Table 4.1), therefore, measuring predators abundance, although costly, is more informative. Effects of introduced fish on zooplankton of lakes have been described in many regions as the Alps (Schabetsberger et al. 2006; Schabetsberger et al. 2009; Tiberti et al. 2014b; Tiberti et al. 2019) Sierra Nevada in California (Knapp et al. 2001b), and the Tatras (Gliwicz 1994).
Generally, big zooplankton species abundance decreased due to predation and small species, mainly rotifers, increased as consequence of the reduction in competence (Knapp et al. 2001b; Tiberti et al. 2014b). We found that the effects of salmonids and minnows were different for the different scales, in the regional scale, salmonids explained zooplankton composition (Fig. 4.5a), this can be explained by the great extension of lakes introduced with salmonids, in comparison to the number of lakes introduced with minnows. Minnows in turn, explained the zooplankton composition in the analysis of fish predators effects, considering their abundance. Here, we put in context the relative importance of introduced fish predation among environmental variability to explain zooplankton composition, and we further analysed the effects of fish predation in a regional scale, considering fish abundances and taking into account the changes after restoration (Fig. 4.5).

We also found differences in predation pressure among predators. Minnows had a greater negative effect than salmonids (Fig. 4.6; 4.7), and both introduced fish had more impact than newt abundances. Effects of introduced fish on both newts and corixids were also stronger than on zooplankton. Negative effects on swimmer insects (Knapp et al. 2001b) and amphibians (Knapp et al. 2001b; Orizaola and Brana 2006; Miro et al. 2018) have been reported. We found that impacts on these organisms can further affect zooplankton in a cascading effect that can reduce abundances of some zooplankton groups of natural or restored lakes when there are natural predators, which can exert a predation pressure slighter than that of fishes. Differences in the effects of different predators were observed in lakes of the Alps, where minnows had a great impact on big cyclopoids and cladocerans, whereas these groups were adapted to newt predation. Moreover, the introduction of trout in a lake with minnows resulted in an increase in cladoceran abundance (Schabetsberger et al. 2006), this shows the different effect of predators, as we have found. Differential predation pressure poses some important conservation concerns. The importance of knowing the impacts of the different species and their interactions arises to prioritize restoration actions. Moreover, knowing the effects of natural predators of zooplankton is fundamental to better understand how lakes can evolve after the eradication of introduced fish species.
Changes in size of the zooplankton

Our results showed significant differences in crustacean planktonic species sizes (Fig. 4.7). We did not even find big species in lakes with minnows. Similar effects were found in lakes with brook trout, where species with a mean size bigger than 1 – 1.2 mm disappeared from the lakes (Knapp et al. 2001b; Tiberti et al. 2014a). However, in our case, only minnow had these effects, and in lakes dominated by salmonids, the effect was of size reduction, especially noticeable for the biggest species *D. cyaneus* and *D. pulicaria*, which are clearly above this threshold, but also medium sized *D. longispina*, *E. vulgaris* and *C. abyssorum*, which approximate this threshold, were also affected. It appears that there is a limitation in species maximum sizes (Fig. 4.8) which depend on the crustacean species, but also in predator size, and, possibly, the species. It is known that younger and smaller fish individuals can predate on smaller plankton, but they are less effective than bigger individuals, as shown with brook trout (Tiberti et al. 2014a). Also, differences in the predation pressure was shown among different fish species (Schabetsberger et al. 2009). Moreover, predation may affect more conspicuous and abundant species (Hairston 1979; Gliwicz 2001). Therefore, depending on the predator, zooplankton can be limited in a higher or lower size, producing the smaller predators a lower boundary.

How fecundity is affected by the predation

We found reduced fecundity in lakes with introduced minnows, compared to lakes with salmonids or natural lakes. This is probably the result of the reduction in size of *D. longispina* reproductive females with eggs. In lakes with introduced fish they were smaller than 1,872 µm, and we did not find a positive relation between clutch size and female size in lakes with minnows. In natural and restored lakes, *D. longispina* females were bigger than in lakes with fish and we found a positive relation between female size and clutch size. From these results it might seem that *D. longispina* females with eggs would be a more visible prey for both minnows and salmonids. In restored lakes, bigger *Daphnia* females scarcely grew more than 2,000 µm, whereas in natural lakes, they could grow close to 2,500 µm. In spite of the relative smaller size of *Daphnia* females in restored lakes, they could reach maximum clutch size.
similar to those of natural lakes, indicating a recovery of fecundity during restoration. We can also see that some females carry fewer eggs than they could by their body size (Fig. 9). This could be due to different effects of predation in the different lakes, as we mixed data from diverse lakes for each category, to egg predation by copepodites or to nutrients limitations (Gliwicz and Stibor 1993; Gliwicz and Lampert 1994). Further research should be conducted to differentiate these effects.

**Temperature effects on the composition, abundance, sizes of the zooplankton**

Water temperature and the ice-cover were important to explain zooplankton composition (Table 4.1, Fig. 4.5). A later ice-off was related to higher *D. castaneti* abundance, as this calanoid would be more abundant in higher ponds (Fig. 4.5a). Also, the date of ice-off marked zooplankton phenology, as the date since ice-off (Fdate) was related to *E. vulgaris* abundance (Fig. 4.5c), and also to calanoids in general, and cladocerans (Fig. 4.7), so their abundances would increase by the end of the ice-free season and by the advance of ice-off date, on the other hand, rotifers were negatively related to this timing. It has already been observed an advance in fast-growing plankton phenology related to an earlier ice-off (Adrian et al. 1999), and as ice-off models for the Pyrenees project future ice-off decrease (chapter 3), we may expect earlier rotifers abundance, and a decrease in their abundances throughout the summer season owing to the advance in ice-off, if the relation between ice-off and rotifers abundance is maintained in the future. Cladocerans and calanoids were more abundant later on the ice-free period, they could show earlier high abundances caused by increasing water temperatures. Some studies showed an advance in daphnids species summer peaks, but not for other crustacean species (Adrian et al. 1999; Adrian et al. 2006). Nevertheless, the ice-free period is expected to increase in Pyrenean lakes (chapter 3), so further research on phenological changes of plankton is needed. In addition to the effects of the ice-cover, both accumulated degree-days (ADD4 and ADD7.6) were related to *E. vulgaris* and *C quadrangula* abundance and to the abundance of other rotifers, such as *Polyarthra*, *Lecane* and *Keratella* (Fig. 4.5), whereas cyclopoids density was negatively related to mean water temperature (Fig. 4.7). This might be due to a thermal niche
differentiation between those taxa, were *E. vulgaris* preferred warm small ponds and cyclopoids were more abundant in cold lakes. Future temperature increases would increase the habitats suitable for *E. vulgaris*, though in smaller ponds warming could lead to decreased performance and enhanced mortality of this species, whereas cyclopoids, inhabiting bigger lakes, could compensate epilimnion warming through vertical migrations (Lampert 2005), even increasing their growth because of the accelerating effect of temperature on metabolism (Honsey et al. 2018). Therefore, studying lakes thermal stratification under increasing temperatures is of great importance to a better assessment of possible effects of warming on zooplankton. Mean water temperature also increased the primary producers abundance (Chla), as it increases photosynthesis and primary production (Allen et al. 2005). In the Pyrenees, accumulated degree-days also explained the composition of primary producers, being positively related to diatoms and negatively to cyanobacteria, chrysophytes and cryptophytes (Buñay 2017). An increase in primary producers might also increase the abundance of zooplankton, specifically due to the increase in *Daphnia* as can be seen in the SEM (Fig. 4.7).

**Conclusions**

In this study, we have found some insights of the effects of both climatic variables and invasive fish on zooplankton. However, research is still needed on how warming of lakes produces changes in lakes’ stratification and how zooplankton acclimates to these changes. Also, it is of great interest to analyse how zooplankton structure themselves through predation and competition. Moreover, temporal changes in the communities have to be analysed to assess the effects of climate warming together with predation pressure and also restoration effects. And last but not least, it is of great interest to explain fecundity variability and its relation with *Daphnia* abundances.
5. General Discussion
Thermal and ice-cover characteristics of Pyrenean lakes

We have described the variability and importance of the main ice-free water surface thermal variables (chapter 2) and ice phenology (chapter 3) variables in Pyrenean high mountain lakes. One of the most characteristic features of Pyrenean lakes was that they always froze in winter, showing a continuous ice-cover and they were free of ice during summer. The fact of being at high altitudes, creates the cold conditions in winter that allow the ice-formation in spite of being in a latitude closer to the equator than lowland Northern lakes, which show winters without ice-covers or with intermittent ice-cover (Livingstone and Adrian 2009). Ice-cover duration in the Pyrenees was lower than in Rila, Retezat and Scotland lakes but shorter than Norway and Alps’ lakes (Thompson et al. 2009). Pyrenean lakes and ponds showed high variability in their summer surface temperatures and ice-cover duration. Maximum temperatures were high in the Pyrenees, as we recorded maximum temperatures as high as 27.3 °C. High temperatures as this can have negative effects on some of the species of the lakes (Butterwick et al. 2005).

Explicative variables of surface water temperature and ice-cover

Altitude was the variable which explained more variability in summer surface water temperatures (chapter 2) and on ice phenology (chapter 3), as it was included in all the models and was the most important effect on ADD, mean and maximum air temperatures, ice-on, ice-off and ice-cover duration, and it was the second in importance for diel temperature range and three-day temperature oscillation. The effect of altitude was negative in all variables but ice-off and ice-cover duration, since altitude explains the variation of air temperature in space, as air temperature decreases with altitude due to the decrease in pressure, and water temperature is related to air temperature owing to the heat exchange between them (Edinger et al. 1968; Livingstone et al. 1999).
Lake area was the most explanatory variable of diel temperature range (DTR) and three days oscillation amongst all variables (chapter 2). Lake area is related to lake depth and mixing depth, which affect the heat a lake can store, and it increases their thermic inertia. Therefore, it can reduce the diel temperature range and oscillation; this effect was also found by Woolway et al. (2015; 2016). Lake area was also important for explaining ADD, mean and maximum temperature. In this sense, Kettle et al. (2004) found a smoothing effect of lake area in the relation between air and water temperature. A greater lake area also retarded ice-on, thus decreasing ice-cover, whereas we found no effect on ice-off (chapter 3).

We also found a relevant effect of the catchment geomorphology on ADD, mean and maximum temperature (chapter 3), and also on ice-on (chapter 4). The effect of catchment has been incorporated in physical models of lake temperatures by adding the heat balances of the inlet and outlet streams (Vinna et al. 2017). However, they are not commonly used in empirical models (e.j. Sharma et al. 2008; O'Reilly et al. 2015). Here we used a simple but significant approach using the ratio between total catchment and lake area, which is a proxy of water renewal time in the lake. This ratio reduced ADD, mean and maximum temperature and ice-on. We also included the ratio between direct catchment and total catchment, which indicates the proportion of the inlet water that passes through an upper lake (and therefore, buffering the inlet water temperature). It reduces mean and maximum temperature as a higher flushing reduces the temperature.

Sun hours in the lakes had a positive effect on ADD, mean, maximum temperature, diel temperature range and three-day oscillation (chapter 2), it reduced also ice-off date and ice-cover duration, but not the ice-on date (chapter 3). The approximation we used here was to model spatial variation in incoming radiation due to the shading by topography using GIS modelling derived from digital elevation models, which showed a significant result. The further inclusion of cloud cover could improve the models, and might possibly explain the interannual differences in lake thermic and ice-cover variables and also to better explain spatial differences not explained by our models.
However, as cloud cover is less spatially coherent than air temperatures, care should be taken when adding it to the models. Some approximations consist in considering theoretical clear-sky radiation, which do not consider either topographical shading or cloud cover (Kettle et al. 2004). Also, regionalization from GCMs has been used in mechanistic models (Winslow et al. 2017). Moreover, using satellite data to measure incoming radiation and cloud-cover is also a possibility (O’Reilly et al. 2015). Nevertheless, these approximations have spatial precision limitations. Therefore, integrating both topography and cloud cover effects would be of outmost interest to improve ice and temperature modelling.

As we have mentioned before, spatial variation in air temperature along the Pyrenees in summer depended mostly on altitude, in fact, temporal variation in air temperature was highly coherent, changing in the same way along all the mountain range and at different altitudes. Therefore, in our models, we used seasonal and interannual temperature data from a single automatic weather station, Lake Redon (2,240m), which represents interannual variation in air temperatures. Interannual air variability was positively related to ADD and mean temperature, and in the case of maximum temperature, it mainly affected high altitude lakes. Spring air temperature was more important for explaining these variables than that of summer, possibly because it also influences the ice-off date (chapter 2). In fact, interannual variation in air temperature explained the ice-off date but not the ice-on date. Also ice-off was more variable in time than ice-on (chapter 3). This difference is due to ice-on being more dependent on lake heat storage than on external forcing. Higher variability in ice-off was also found in Benson et al. (2012) and a higher sensitivity of ice-off than of ice-on in Vavrus et al. (1996). Furthermore, it has been described that ice-on is more unpredictable (Sporka et al. 2006; Arp et al. 2010; Weyhenmeyer et al. 2011).
In the models, there was a proportion of the interannual variability, in addition to that of air temperature, which was included in the random part of the models. This random variance could be attributed to other meteorological variables such as wind, cloud cover or precipitation which have not been included in the model and can vary from year to year. The year as random variability explained ADD, maximum temperature and three-day oscillation, but it did not explain mean temperature or diel temperature range (chapter 2). Random variability also explained ice-on and ice-off date and ice-cover (chapter 3). Considering the year as a random variable, allowed us to know how the different years included in the models influenced temperature and ice variables, and outside these years, it informed us about interannual variability out of the air temperature variation, which was included in the models as fixed term. There are examples of empirical models where precipitation, cloud cover and wind speed were not significant (Sharma et al. 2008), or just including cloud cover (O’Reilly et al. 2015), which also gave good results. Whether these meteorological variables may add extra explained variability, air temperatures together with geomorphological variables explained a great proportion, as also shown in (O’Reilly et al. 2015). Further studies in Pyrenean lakes could test the improvement of the models developed here with these meteorological variables.

In addition to lake area effects on water temperature variables and on ice cover, we also considered a differentiation between lakes and ponds. Differences among lakes and ponds were found in their biochemical composition and zooplankton community in the Pyrenees (Buñay 2017). Closely related to the thresholds found in previous studies we took a threshold of 0.5 ha to differentiate lakes and ponds. Differences between these two categories were found for ADD and mean temperature (chapter 2) but not on ice phenology.

We also found some significant interactions among variables. This was the case of the negative interaction between interannual air temperature and altitude on ADD7.6. Contrarily, there was a positive interaction between interannual air temperature and altitude on maximum temperature. Moreover, there was a
positive interaction between lake area and altitude for ADD7.6, mean and maximum temperature and diel temperature range (chapter 2). The interaction between interannual air temperature and altitude may arise due to an earlier ice-off in lower lakes and thus a greater increase in temperatures in warmer years, whereas higher lakes would remain disconnected of air temperatures due to the insulating effect of the ice-cover (Sporka et al. 2006). In contrast, maximum temperatures would increase more at high altitudes in warm years. A higher warming in altitude has also been reported in other studies (O'Reilly et al. 2015; Pepin et al. 2015). The positive interaction between altitude and lake area implies a steeper decreasing slope of ponds in comparison to bigger lakes, which can be due to their higher response to external forcing, because of their relative shallower mixing depth. Differences between small and big lakes have also been found by Winslow et al. (2015).

**Relationship among air temperature, summer surface water temperature and ice-cover**

Interannual variability of air temperature influenced both ice phenology and summer water temperature. Air temperature during spring affected both ice-off and summer air and water temperatures. The effect of summer temperatures and lake size had a greater effect on ice-on than air temperatures. The date of ice-on was also related to an earlier ice-off. Thus, these effects indicate a certain thermal inertia of the lakes through the year and from one year to another. This was tested by previous studies with temporal autocorrelation models but no relation was found (Sharma et al. 2013). Our results are somehow in contradiction with them, so it would be of interest to test temporal autocorrelation in ice and thermic models.
Future projections of ice-cover and water surface temperature

Future projections of our models gave a temperature increase between 0.06 and 0.46 °C per decade in this century, depending on the warming scenario (chapter 2). Previous studies on past decades in European lakes reported higher trends than the ones of our projections, between 0.49 and 0.66 °C per decade (Dokulil 2014). However, differences in warming trends have been found for different lakes and regions (O'Reilly et al. 2015). For the Pyrenees, there was still no information about warming trends, but recent trends for the Alps show more similar results to our future projections, being one of the world’s regions with lower trends, 0.12 °C per decade, with some lakes with higher warming trends (O'Reilly et al. 2015). Therefore, the Pyrenees would have a closer trend to that of the Alps than to the higher trends of other European lakes. As for the ice-cover, our projections estimated a decrease in its duration between 20 and 32 days mainly owing to the advance in ice-off date, whereas ice-on date is not expected to be retarded but to vary randomly from year to year (chapter 3). Our projections are in agreement with the decrease in other regions but with a lower decrease, compared to the 5-75 days in Fennoscandia (Lepparanta 2010), and 3-43 days in North America (Hewitt et al. 2018). The reduction of ice-cover duration seems also lower than in other regions, which is in agreement with the lower water temperature trends in the Pyrenees.

Effects of biotic and abiotic factors on zooplankton composition and structure

Both biotic and abiotic factors explained zooplankton composition. According to RDAs, most of the explained variability was due to geomorphology, basically related to lake morphometry, chemistry, which included both nutrients and pH. It was followed by climatic and predators variables. Radiation and spatial
variables represented by MEMs explained the lowest variation (chapter 5). Lake morphology can be closely related to its chemistry as phosphorous and nutrient loads differed between lakes and ponds and it influenced primary producers abundance and composition (Buñay 2017) and that may also affect zooplankton composition. The significant spatial patterns of species distribution can be due either to spatial patterns of environmental variables or to species dispersion (Gascon et al. 2016; Buñay 2017). The spatial pattern we found was related to environmental variables, specifically to warm shallow lakes with abundant *E. vulgaris* and *C. quadrangula*.

**Invasive fish and temperature implications for zooplankton composition**

Both invasive fish and climatic variables had an effect on zooplankton composition in Pyrenean lakes. Salmonids and minnows had a differentiated effect. Regarding climate, both summer temperatures and ice phenology explained part of the composition of the assemblages (chapter 4). In comparison to geomorphological, chemical and spatial variables, it may seem that the effects of introduced fish species and climate, through water temperatures and ice phenology, are smaller. However, the fact that these stressors have the capability of modifying the community structure, given all the variability of the zooplankton and their ecosystem, is yet an important effect. Not to mention the possible interactions between invasive fish, climate and other variables. Invasive fish can increase the recycling of nutrients (Schindler et al. 2001), therefore, affecting the community in an indirect way. In the case of climate, CO₂ increases could cause increased acidification in addition to the deposition of nitrates and sulfates. Recently, increasing dissolved organic carbon trends associated to climate have been described, which could be attributed to nitrates and sulfates deposition decrease, increased CO₂ concentrations or temperature rise (Evans et al. 2005). In addition, temperature increases and ice-cover reduction could cause changes in the processing of nutrients, due to a reduction of the anoxic conditions.
below the ice-cover or to a more durable and stable thermoclines, due to lakes warming.

We found different predation pressures between introduced fish; salmonids and minnows, the latter having a stronger impact. We also found a negative effect of natural predators, such as newts, on bigger planktonic crustaceans. In the case of corixids, we did not have enough data to test these effects quantitatively. We also found a negative effect of fishes on both newts and corixids. This can produce a cascading effect through the food web, which could lead even to an increase of planktonic crustaceans because of the elimination of their natural predators, mostly in the presence of trout, which is less effective in predation than minnows (chapter 4). Whether there are clues of different predation effects depending on the species (Schabetsberger et al. 2009) and size (Tiberti et al. 2014a), still more research is needed to disentangle the different species effect on zooplankton and how their interactions affect predation on species. It is also fundamental to know the effects of invasive species through the food webs, as their impacts on species can be indirect. Moreover, it is also of paramount importance to include other biotic and abiotic factors when analyzing the impacts of invasive species, as they may also affect the communities.

The models developed in chapter 2 and chapter 3 were necessary to obtain the variables of temperature and ice phenology respectively, to evaluate the effects of climate on the zooplankton community, as we had data on a limited number of lakes or data on punctual temperature measurements, which are not representative of the whole ice-free season. These variables were modelled using morphologic variables such as altitude, lake or catchment area and also radiation and air temperature variables, therefore, they may be related to these variables depending on the weight they have in the models. Moreover, a phenological date was calculated considering the time of the sampling since the ice-off date. These variables were important to explain the composition of the zooplankton and the abundance of the primary producers (chapter 4). We found that rotifers decreased in their abundance by the end of the ice-free season, while calanoids and cladocers showed higher abundances
later. We expect an advance in the phenology of zooplankton due to the advance in ice-off, this advance is expected for rotifers and daphnids as shown in Adrian et al. (1999), whereas other species may react more slowly to these changes. Moreover, the growing season will last longer with unknown effects. We also found a thermal niche differentiation between calanoids and cyclopoids. As E. vulgaris showed a preference towards warmer ponds and C. abyssorum was related to cold lakes. We argue that C. abyssorum could compensate or even benefit of lakes warming through vertical migrations. Whereas E. vulgaris would see their niche expanded, high temperatures could also have negative effects on pond species.

**Effects of invasive fish on planktonic crustacean size and fecundity**

We found a negative impact of fish on bigger zooplankton species (chapter 4). Where minnows were present we could not find big species as D. pulicaria or D. cyaneus, and they were also reduced in size in lakes with trout. Other studies suggested that species above a threshold of 1 to 1.2 mm would disappear in lakes with introduced fish (Knapp et al. 2001a; Tiberti et al. 2014a). Other medium species would be affected, and so their sizes reduced. Our results suggest that crustacean species would be affected more in their maximum sizes that on their mean size.

In the case of D. longispina, for which we had enough data on fecundity, we saw that egg carrying females had lower sizes in lakes with fish, and consequently, lower fecundity. As commonly, bigger females had more numerous clutches. Restoration of lakes by fish removal did not recover D. longispina sizes, but recovered their fecundity. We also found that there were females which carried fewer eggs than their capacity by their size. This could be due to cyclops copepodites predation or to lower nutrients loads in the lakes of those females as described in (Gliwicz and Stibor 1993; Gliwicz and Lampert 1994).
Future perspectives

Whereas the impacts of fish invasions in the Pyrenees affect a great proportion of the lakes with a clear negative effect on bigger zooplankton species, the effects of climate change are noticeable in the change of water temperatures and ice cover, but yet they have to be clarified regarding the effects on zooplankton. Although we expect that fish banning in protected areas, education and restoration projects can prevent further extension of invasive fish, water temperatures and ice-cover are expected to continue changing, so further monitoring of water temperatures, ice-cover and zooplankton would be needed. Moreover, other groups, such as amphibians, which include some endangered species in the Pyrenees are especially vulnerable to fish predation and warming. Another effect of climate change is a reduction in precipitation in the Mediterranean basin; this effect is not so certain as temperature rise, but it could also affect water temperature and ice-cover, and furthermore, a reduction in precipitation could lead to the disappearance of smaller ponds. Also, the interactions between lakes warming and introduced fish could be clarified in the Pyrenees, in addition to the effects that those variables could cause on chemical variables as phosphate and water transparency and physical structure of the lakes. These and more questions that have been emerging in this thesis are yet to be resolved.
6. General Conclusions

Part I Modelling thermal and ice phenology in the Pyrenees

1. The most remarkable thermal characteristics of monitored lakes in the Pyrenees were a mean surface ice-free period temperature of 11.0 °C along the whole region, while mean temperatures ranged between 5.4 and 15.9 °C among lakes. Maximum registered temperature of 27.3 °C, and a mean diel temperature range of 1.5 °C.

2. The mean ice-cover duration in monitored lakes was of eight months (ranging from 4 to 11 months). On average, they froze on 12th November and melted on the 5th June.

3. We have developed empirical models of thermal and ice phenology variables of the lakes. Altitude was the variable which better explained accumulated degree-days, mean and maximum temperatures and ice phenology variables, as air temperature decreases in altitude, and water and air temperatures are in equilibrium.

4. Lake area explained more variability in diel temperature range and three-day temperature oscillation.

5. We found a significant effect of the morphology of the catchment on ADD, mean and maximum temperature and ice-off.

6. Solar radiation had a positive effect on ADD, mean and maximum temperatures, and it reduced ice-off date and ice-cover duration.

7. Seasonal air temperatures were positively related to ADD, mean temperatures and maximum temperatures in high altitudes and ice-off dates. Spring temperatures explained more variability than those of summer.

8. Introducing random variability we could increase the models performance. Interannual variability increased ADD, maximum temperature and ice phenology models performance. Also, considering differences between lakes and ponds served to improve the models of ADD and mean temperature.
9. We found significant interactions among explicative variables, which cause a higher proportional warming at low altitude lakes, whereas maximum temperatures would increase more at high altitudes. A steeper decrease of temperatures in altitude was found for smaller lakes and ponds than for big lakes.

10. Spring air temperature had a great effect on summer water temperatures, partly through the advance in ice-off. Summer water temperature had more influence on the ice-on date than autumn air temperatures. The date of ice-off was related to that of the previous ice-on date.

11. Future summer water surface temperatures warming trends expectations are between 0.06 and 0.25 °C in Pyrenean lakes, reaching an average increase of 2 °C by 2090 from present mean temperatures of 11.3 °C in the warmer scenario.

12. Ice-off dates are foreseen to advance between 23 and 32 days by 2090, whereas we found no trend on ice-on but interannual differences. In spite of the ice-cover duration reduction, all Pyrenean lakes and ponds are expected to continue freezing in winter until the end of the century.

Part II Relative effects of temperature and predation on zooplankton

13. Both biotic and abiotic factors explained zooplankton communities’ composition in Pyrenean lakes. Geomorphological and chemical/phytoplankton variables were those which explained higher variability including lake and catchment sizes and nutrients. They were followed by predators and temperature variables, which were basically related to introduced fish, to ice phenology and summer surface water temperature. Radiation and spatial patterns represented by MEMs had a slighter effect on zooplankton.

14. Introduced fish had a higher impact on zooplankton than natural predators, minnows having a greater impact than salmonids. The
zooplankton groups more affected by predation were cladocerans and calanoid copepods.

15. Predation on zooplankton was size-selective. Bigger planktonic crustaceans were not present in lakes with minnows alone, and had smaller sizes in lakes with salmonids. Medium-sized planktonic crustaceans showed reduced sizes in lakes with fishes, and especially in lakes with minnows.

16. *Daphnia* fecundities were reduced in lakes with fish, which were related to a reduced size of *Daphnia* females, as fecundity had a positive relation with size. Lakes restoration recovered *Daphnia* fecundity. We found that *Daphnia* carried fewer eggs than their maximum capacity.

17. Rotifers were negatively related to the date since ice-off, whereas calanoids and cladocerans had a positive relation. Mean water temperatures had a positive effect on Chlorophyll a and a negative relation with cyclopoids and small cladocerans. *E. vulgaris* was also positively related to ADD4 and mean temperatures.
Bibliography


Austin, J. A., and S. M. Colman. 2007. Lake Superior summer water temperatures are increasing more rapidly than regional air

Balcells, E. 1956. Estudio morfologico, biologico y ecologico de Rana temporaria, L. Boletin del Instituto de Biologa Aplicada 24: 81-103. doi:


Balcells, E. 1975. Observaciones en el ciclo biológico de anfibios de alta montaña y su interés en la detección del inicio de la estación vegetativa. P. Cent. pir. Biol. exp. 7: 55-153. doi:


Buchaca, T. 2009. Pigments indicadors: estudi del senyal en estanys dels Pirineus i de la seva aplicació en paleolimnologia. Institut d'Estudis Catalans. doi:


Catalan, J. and others 2006. High mountain lakes: Extreme habitats and witnesses of environmental change. Limnetica 25: 551-584. doi:


de Mendoza, G., E. Rico, and J. Catalan. 2012. Predation by introduced fish constrains the thermal distribution of aquatic Coleoptera in mountain


Dray, S. 2010. SpacemakeR: Spatialmodelling. R package version 0.0-5/r101. doi:


ICGC. 2012c. Topographic base of Catalonia.


IPCC. 2018a. Global warming of 1.5°C. An IPCC Special Report on the impacts of global warming of 1.5°C above pre-industrial levels and related global greenhouse gas emission pathways, in the context of strengthening the global response to the threat of climate change, sustainable development, and efforts to eradicate poverty World Meteorological Organization.

global greenhouse gas emission pathways, in the context of strengthening the global response to the threat of climate change, sustainable development, and efforts to eradicate poverty. World Meteorological Organization.


Palecki, M. A., and R. G. Barry. 1986. Freeze-up and break-up of lakes as an index of temperature changes during the transition seasons: A case-


Rahel, F. J. 1997. From Johnny Appleseed to Dr Frankenstein: Changing values and the legacy of fisheries management. Fisheries 22: 8-9. doi:


Tiberti, R., S. Brighenti, R. Iacobuzio, G. Pasquini, and M. Rolla. 2014a. Behind the impact of introduced trout in high altitude lakes: adult, not juvenile
fish are responsible of the selective predation on crustacean zooplankton. J. Limnol. 73: 593-597. doi: 10.4081/jlimnol.2014.969


Appendix

Appendix 1: Summary of the morphological, radiation and thermic variables in the Pyrenees and sampled water bodies. For the global of the Pyrenean water bodies, there is information from 3,909 lakes and ponds, for which 2,630 had data for their catchments, while the sample lakes include 59 lakes (See Table 2.1 for variables description, here the variables are not transformed).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Pyrenees maximum</th>
<th>mean</th>
<th>minimum</th>
<th>Studied lakes maximum</th>
<th>mean</th>
<th>minimum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Altitude (m)</td>
<td>2978</td>
<td>2248</td>
<td>1053</td>
<td>2880</td>
<td>2315</td>
<td>1561</td>
</tr>
<tr>
<td>Larea (ha)</td>
<td>160.46</td>
<td>1.32</td>
<td>0.00011</td>
<td>24.16</td>
<td>3.03</td>
<td>0.015</td>
</tr>
<tr>
<td>Dcatchment (ha)</td>
<td>2929.92</td>
<td>48.69</td>
<td>0.0068</td>
<td>2887.58</td>
<td>88.68</td>
<td>0.20</td>
</tr>
<tr>
<td>Tcatchment (ha)</td>
<td>5392.01</td>
<td>99.93</td>
<td>0.0068</td>
<td>5392.01</td>
<td>226.79</td>
<td>0.35</td>
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<tr>
<td>Radiation (h)</td>
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<td>9.86</td>
<td>5.66</td>
<td>10.99</td>
<td>9.77</td>
<td>7.68</td>
</tr>
<tr>
<td>Tspring ( °C)</td>
<td>2.17</td>
<td>0.53</td>
<td>-1.61</td>
<td>7.80</td>
<td>4.58</td>
<td>2.58</td>
</tr>
<tr>
<td>Tsummer ( °C)</td>
<td>11.32</td>
<td>10.15</td>
<td>8.52</td>
<td>7.80</td>
<td>4.58</td>
<td>2.58</td>
</tr>
<tr>
<td>Tautumn ( °C)</td>
<td>7.80</td>
<td>4.58</td>
<td>2.58</td>
<td>-1.86</td>
<td>-3.72</td>
<td>-4.77</td>
</tr>
<tr>
<td>Twinter ( °C)r</td>
<td>2126.37</td>
<td>702.49</td>
<td>18.08</td>
<td>2931.47</td>
<td>1169.31</td>
<td>41.56</td>
</tr>
<tr>
<td>ADD 7.6 ( °C day)</td>
<td>15.31</td>
<td>11.04</td>
<td>5.91</td>
<td>15.31</td>
<td>11.04</td>
<td>5.91</td>
</tr>
<tr>
<td>ADD4 ( °C day)</td>
<td>27.30</td>
<td>17.90</td>
<td>8.40</td>
<td>27.30</td>
<td>17.90</td>
<td>8.40</td>
</tr>
<tr>
<td>DTR ( °C)</td>
<td>4.60</td>
<td>1.53</td>
<td>0.60</td>
<td>4.60</td>
<td>1.53</td>
<td>0.60</td>
</tr>
<tr>
<td>Tosc ( °C)</td>
<td>2.80</td>
<td>1.10</td>
<td>0.50</td>
<td>2.80</td>
<td>1.10</td>
<td>0.50</td>
</tr>
<tr>
<td>Ice-on (day)</td>
<td>354</td>
<td>316</td>
<td>286</td>
<td>354</td>
<td>316</td>
<td>286</td>
</tr>
<tr>
<td>Ice-off (day)</td>
<td>242</td>
<td>156</td>
<td>61</td>
<td>242</td>
<td>156</td>
<td>61</td>
</tr>
<tr>
<td>Ice-cover (day)</td>
<td>308</td>
<td>210</td>
<td>98</td>
<td>308</td>
<td>210</td>
<td>98</td>
</tr>
</tbody>
</table>
Appendix 2: Description of the automatic weather stations (AWS). The geographic location (ETRS89), the altitude and the date of functioning start of the AWS are detailed.

<table>
<thead>
<tr>
<th>Weather station</th>
<th>Latitude (º)</th>
<th>Longitude (º)</th>
<th>Altitude (m)</th>
<th>Function date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vielha</td>
<td>42.7</td>
<td>0.79</td>
<td>1002</td>
<td>15/02/1996</td>
</tr>
<tr>
<td>Das</td>
<td>42.39</td>
<td>1.87</td>
<td>1097</td>
<td>22/05/2001</td>
</tr>
<tr>
<td>Gisclareny</td>
<td>42.26</td>
<td>1.76</td>
<td>1386</td>
<td>12/03/1999</td>
</tr>
<tr>
<td>Núria</td>
<td>42.4</td>
<td>2.16</td>
<td>1971</td>
<td>15/05/1998</td>
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<tr>
<td>Cadí</td>
<td>42.29</td>
<td>1.71</td>
<td>2143</td>
<td>06/11/2003</td>
</tr>
<tr>
<td>Sasseuva</td>
<td>42.77</td>
<td>0.73</td>
<td>2228</td>
<td>20/09/2001</td>
</tr>
<tr>
<td>Malniu</td>
<td>42.47</td>
<td>1.78</td>
<td>2230</td>
<td>03/11/1999</td>
</tr>
<tr>
<td>Lac Redon</td>
<td>42.64</td>
<td>0.78</td>
<td>2247</td>
<td>15/07/1999</td>
</tr>
<tr>
<td>Bonaigua</td>
<td>42.65</td>
<td>0.98</td>
<td>2266</td>
<td>06/11/1997</td>
</tr>
<tr>
<td>Certascan</td>
<td>42.7</td>
<td>1.27</td>
<td>2400</td>
<td>11/11/2000</td>
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<td>Ulldeter</td>
<td>42.42</td>
<td>2.25</td>
<td>2410</td>
<td>28/09/2011</td>
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<tr>
<td>Salòria</td>
<td>42.52</td>
<td>1.37</td>
<td>2451</td>
<td>30/09/2004</td>
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<td>Espot</td>
<td>42.53</td>
<td>1.05</td>
<td>2519</td>
<td>01/03/2002</td>
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<td>Boí</td>
<td>42.47</td>
<td>0.88</td>
<td>2535</td>
<td>13/11/1998</td>
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</table>
Appendix 3: Summary table of the adjustment indicators of the temperature models using different selection processes. In these cases the resulting models are multiple regression models without random structure, and therefore, $R^2$ ordinary and $R^2$ adjusted are represented a (See Table 3.1 for variables description)

<table>
<thead>
<tr>
<th>Model</th>
<th>AIC</th>
<th>BIC</th>
<th>RMSE</th>
<th>$R^2$ marginal/ordinary</th>
<th>$R^2$ conditional/adjusted</th>
<th>Fixed structure</th>
</tr>
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<tbody>
<tr>
<td>ADD7.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>forward</td>
<td>318</td>
<td>352</td>
<td>179.3</td>
<td>0.74</td>
<td>0.83</td>
<td>water Radiation+Tcatchment/Larea+Larea+Altitude+Tspring+Larea:Altitude</td>
</tr>
<tr>
<td>forward no interaction</td>
<td>331</td>
<td>362</td>
<td>190.9</td>
<td>0.71</td>
<td>0.82</td>
<td>water Radiation+Tcatchment/Larea+Larea+Altitude+Tspring</td>
</tr>
<tr>
<td>backward no interaction</td>
<td>331</td>
<td>362</td>
<td>190.9</td>
<td>0.71</td>
<td>0.82</td>
<td>water Radiation+Tcatchment/Larea+Larea+Altitude+Tspring</td>
</tr>
<tr>
<td>backward interaction</td>
<td>322</td>
<td>363</td>
<td>175.2</td>
<td>0.75</td>
<td>0.84</td>
<td>water Radiation+Tcatchment/Larea+X+Larea+Altitude+Tspring+Larea:Altitude:Tspring</td>
</tr>
<tr>
<td>ADD4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>forward interaction</td>
<td>300</td>
<td>336</td>
<td>232.6</td>
<td>0.75</td>
<td>0.82</td>
<td>water Radiation+Tcatchment/Larea+Larea+Altitude+Tspring+Larea:Altitude</td>
</tr>
<tr>
<td>forward no interaction</td>
<td>300</td>
<td>330</td>
<td>248.2</td>
<td>0.71</td>
<td>0.82</td>
<td>water Radiation+Tcatchment/Larea+Larea+Altitude+Tspring</td>
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<tr>
<td>backward no interaction</td>
<td>302</td>
<td>335</td>
<td>244.9</td>
<td>0.72</td>
<td>0.82</td>
<td>water Radiation+Tcatchment/Larea+X+Larea+Altitude+Tspring</td>
</tr>
<tr>
<td>backward interaction</td>
<td>301</td>
<td>334</td>
<td>234.9</td>
<td>0.72</td>
<td>0.83</td>
<td>year Radiation+Tcatchment/Larea+Y+Larea+Altitude+Tspring+Larea:Altitude</td>
</tr>
<tr>
<td>Tmean</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>forward</td>
<td>493</td>
<td>530</td>
<td>1.1</td>
<td>0.52</td>
<td>0.63</td>
<td>water Radiation+Dcatchment/Tcatchment+</td>
</tr>
<tr>
<td>Variable</td>
<td>Interaction Type</td>
<td>Coefficient Values</td>
<td>Significance</td>
<td>Model Formulation</td>
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<td>------------------</td>
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<tr>
<td><strong>Tmean</strong></td>
<td>forward no interaction</td>
<td>504 545 1.3 0.46 0.66</td>
<td>water body</td>
<td>Tcatchment/Larea+Tsummer+Tspring+Larea+Altitude+Larea:Altitude + Radiation+Dcatchment/Tcatchment+X+Y+Larea+Larea:Altitude+Tcatchment/Larea+Tspring+Tsummer</td>
<td></td>
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<tr>
<td><strong>Tmean</strong></td>
<td>backward no interaction</td>
<td>504 545 1.3 0.46 0.66</td>
<td>water body</td>
<td>Radiation+Dcatchment/Tcatchment+Tcatchment/Larea+X+Y+Larea+Altitude+Tspring+Tsummer</td>
<td></td>
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</tr>
<tr>
<td><strong>Tmean</strong></td>
<td>backward interaction</td>
<td>493 530 1.1 0.52 0.63</td>
<td>water body</td>
<td>Tcatchment/Larea+Tsummer+Tspring+Larea+Altitude+Larea:Altitude</td>
<td></td>
<td></td>
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<tr>
<td><strong>Tmax</strong></td>
<td>forward interaction</td>
<td>395 425 1.9 0.58 0.77</td>
<td>year</td>
<td>Radiation+Tcatchment/Larea+Larea+Altitude+Larea:Altitude+Altitude:Tspring</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Tmax</strong></td>
<td>forward no interaction</td>
<td>407 431 2.0 0.55 0.74</td>
<td>year</td>
<td>Radiation+Tcatchment/Larea+Larea+Altitude</td>
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<td></td>
</tr>
<tr>
<td><strong>Tmax</strong></td>
<td>backward no interaction</td>
<td>409 436 2.0 0.55 0.75</td>
<td>year</td>
<td>Radiation+Dcatchment/Tcatchment+Tcatchment/Larea+Larea+Altitude</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Tmax</strong></td>
<td>backward interaction</td>
<td>399 437 1.9 0.60 0.78</td>
<td>year</td>
<td>Radiation+Dcatchment/Tcatchment+Tcatchment/Larea+Altitude+Larea:Altitude+X+Larea+Altitude+Larea:Altitude+Altitude:Tspring</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>DTR</strong></td>
<td>forward interaction</td>
<td>542 563 0.55 0.45ₐ 0.44ₐ</td>
<td>-</td>
<td>Radiation + X + Larea + Altitude + Larea:Altitude</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>DTR</strong></td>
<td>forward no interaction</td>
<td>544 568 0.56 0.43ₐ 0.42ₐ</td>
<td>-</td>
<td>Radiation + X + Larea + Altitude</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>DTR</strong></td>
<td>backward no interaction</td>
<td>523 544 0.56 0.43ₐ 0.42ₐ</td>
<td>-</td>
<td>Radiation + X + Larea + Altitude</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>DTR</strong></td>
<td>backward</td>
<td>523 544 0.55 0.45ₐ 0.44ₐ</td>
<td>-</td>
<td>Radiation + X + Larea + Altitude + Larea:Altitude</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interaction</td>
<td>Forward Interaction</td>
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<td>528</td>
<td>0.26</td>
<td>0.50</td>
<td>0.53</td>
</tr>
<tr>
<td>-------------</td>
<td>---------------------</td>
<td>-----</td>
<td>-----</td>
<td>------</td>
<td>------</td>
<td>------</td>
</tr>
<tr>
<td>Tosc</td>
<td>Forward no interaction</td>
<td>508</td>
<td>528</td>
<td>0.26</td>
<td>0.50</td>
<td>0.53</td>
</tr>
<tr>
<td>Tosc</td>
<td>Backward no interaction</td>
<td>508</td>
<td>528</td>
<td>0.26</td>
<td>0.50</td>
<td>0.53</td>
</tr>
<tr>
<td>Tosc</td>
<td>Backward no interaction</td>
<td>512</td>
<td>542</td>
<td>0.26</td>
<td>0.47</td>
<td>0.54</td>
</tr>
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</table>
Appendix 4: Description GCMs of the CMIP5 used to calculate future air temperature projections at Lake Redon.

<table>
<thead>
<tr>
<th>Institution</th>
<th>Model</th>
<th>Project</th>
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<tr>
<td>CNRM-CERFACS</td>
<td>CNRM-CM5</td>
<td>CMIP5</td>
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<td>ICHEC</td>
<td>EC-EARTH</td>
<td>CMIP5</td>
</tr>
<tr>
<td>MOHC</td>
<td>Had GEM 2-ES</td>
<td>CMIP5</td>
</tr>
<tr>
<td>IPSL</td>
<td>IPSL - CM5A - MR</td>
<td>CMIP5</td>
</tr>
<tr>
<td>MPI-M</td>
<td>MPI - ESM - LR</td>
<td>CMIP5</td>
</tr>
<tr>
<td>NCC</td>
<td>NorESM1 - ME</td>
<td>CMIP5</td>
</tr>
</tbody>
</table>

Appendix 5: Variables distribution in the Pyrenees (grey) and the studied lakes for temperature and ice (black) for morphological and radiation variables in the water bodies. For the global of the Pyrenean water bodies, there is information from 3,909 lakes and ponds, for which 2,630 had data for their catchments, while the sample lakes include 59 lakes (See Table 2.1 for variables description, here the variables are not transformed).
Appendix 6: Spring air temperatures in 14 Central and Eastern Pyrenean AWS from 2000 to 2015 given by Servei Meteorològic de Catalunya (Location of the AWS can be seen in Fig. 2.1 and their information in Appendix 2). a) temporal series b) correlogram of the series.
Appendix 7: Correlation between radiation variables. Global solar radiation (S), direct radiation (Dir), diffuse (Dif) and sun hours (Sun) are calculated for the lake (l), direct catchment (d) and total catchment (t).

Appendix 8: Pearson correlations between morphologic, radiation, and thermic variables of water bodies in the Pyrenees (See Table 2.1 for variables description).
Appendix 9: Summary table of the adjustment indicators of the ice phenology models using different selection methods (See Table 3.1 for variables description).

<table>
<thead>
<tr>
<th>Model</th>
<th>AIC</th>
<th>BIC</th>
<th>RMSE</th>
<th>$R^2$ marginal</th>
<th>$R^2$ conditional</th>
<th>Random structure</th>
<th>Model final</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ice-on</td>
<td>554.07</td>
<td>574.67</td>
<td>9.82</td>
<td>0.30</td>
<td>0.47</td>
<td>year</td>
<td>Tcatchment/Larea+Larea+Altitude</td>
</tr>
<tr>
<td>Ice-on</td>
<td>558.65</td>
<td>586.05</td>
<td>9.72</td>
<td>0.31</td>
<td>0.49</td>
<td>year</td>
<td>Tcatchment/Larea+X+Y+Larea+Altitude</td>
</tr>
<tr>
<td>Ice-off</td>
<td>354.19</td>
<td>378.28</td>
<td>17.45</td>
<td>0.62</td>
<td>0.80</td>
<td>year</td>
<td>Radiation+Y+Altitude+Tspring</td>
</tr>
<tr>
<td>Ice-off</td>
<td>354.19</td>
<td>378.28</td>
<td>17.45</td>
<td>0.62</td>
<td>0.80</td>
<td>year</td>
<td>Radiation+Y+Altitude+Tspring</td>
</tr>
<tr>
<td>Ice-cover</td>
<td>345.0</td>
<td>372.4</td>
<td>18.67</td>
<td>0.69</td>
<td>0.81</td>
<td>year</td>
<td>Radiation+Y+Larea+Altitude+Tspring</td>
</tr>
<tr>
<td>Ice-cover</td>
<td>345.0</td>
<td>372.4</td>
<td>18.67</td>
<td>0.69</td>
<td>0.81</td>
<td>year</td>
<td>Radiation+Y+Larea+Altitude+Tspring</td>
</tr>
</tbody>
</table>
Appendix 10: Parameters of the structural equation models of ice phenology, air and water temperature and $R^2$ for the endogenous variables of the models.

<table>
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<tr>
<th>Model parameters</th>
<th></th>
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<td>Chi$^2$</td>
<td>495.07</td>
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<tr>
<td>Df</td>
<td>32</td>
</tr>
<tr>
<td>Pr(&gt;Chisq)</td>
<td>2.05E-84</td>
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<tr>
<td>AIC</td>
<td>541.07</td>
</tr>
<tr>
<td>BIC</td>
<td>331.10</td>
</tr>
</tbody>
</table>

$R^2$ for endogenous variables

- Tspr: 0.32
- Ice-off: 0.73
- ADD4: 0.71
- Ice-on1: 0.27
- Tsum: 0.16
- Ice-on: 0.24

Appendix 11: Correlations between morphological, radiation, air temperature and ice phenology and water thermic variables (See Table 3.1 for variables description).
Appendix 12: Initial structural equation model of the ice phenology and air and water temperatures, where air temperatures are supposed to affect the lake during its season and the coming, and Altitude and Larea all ice and lake temperature variables (See Table 3.1 for variables description).

Appendix 13: Summary of the morphological, chemical, radiation, climatic and predator variables in the sampled lakes for zooplankton.

<table>
<thead>
<tr>
<th>Variables</th>
<th>max</th>
<th>mean</th>
<th>min</th>
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</thead>
<tbody>
<tr>
<td>Altitude (m)</td>
<td>2880</td>
<td>2249</td>
<td>1618</td>
</tr>
<tr>
<td>Larea (ha)</td>
<td>53.18</td>
<td>3.42</td>
<td>0.0015</td>
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<tr>
<td>Dcatchment (ha)</td>
<td>2887.58</td>
<td>76.10</td>
<td>0.021</td>
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<tr>
<td>Tcatchment (ha)</td>
<td>5392.01</td>
<td>164.09</td>
<td>0.021</td>
</tr>
<tr>
<td>Depth (m)</td>
<td>123</td>
<td>12.78</td>
<td>0.1</td>
</tr>
<tr>
<td>Ice (%)</td>
<td>8</td>
<td>0.68</td>
<td>0</td>
</tr>
<tr>
<td>Bedrock (%)</td>
<td>89</td>
<td>41.53</td>
<td>0</td>
</tr>
<tr>
<td>Swamps (%)</td>
<td>6</td>
<td>0.97</td>
<td>0</td>
</tr>
<tr>
<td>Prairies (%)</td>
<td>90</td>
<td>29.46</td>
<td>1</td>
</tr>
<tr>
<td>Bushes (%)</td>
<td>50</td>
<td>16.14</td>
<td>0</td>
</tr>
<tr>
<td>Trees (%)</td>
<td>20</td>
<td>8.74</td>
<td>0</td>
</tr>
<tr>
<td>Debris (%)</td>
<td>50</td>
<td>2.97</td>
<td>0</td>
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<tr>
<td>Parameter</td>
<td>Value1</td>
<td>Value2</td>
<td>Value3</td>
</tr>
<tr>
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<td>Alk (µeq l⁻¹)</td>
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<td>Cond (µS l⁻¹)</td>
<td>190.8</td>
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<td>3.2</td>
</tr>
<tr>
<td>pH</td>
<td>9.82</td>
<td>7.15</td>
<td>4.5</td>
</tr>
<tr>
<td>TN (µg l⁻¹)</td>
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<td>296.64</td>
<td>43.9</td>
</tr>
<tr>
<td>DIN (µg l⁻¹)</td>
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<td>69.15</td>
<td>1.3</td>
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<td>TP (µg l⁻¹)</td>
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<td>Kd</td>
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<td>Chla (µg l⁻¹)</td>
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<td>Cattle (ºn)</td>
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<td>Msp (ºn)</td>
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<td>Radiation (h)</td>
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<td>Dradiation (W m⁻²)</td>
<td>4260.21</td>
<td>3725.39</td>
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<td>Tradiation (W m⁻²)</td>
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<td>ADD7.6 ( °C day)</td>
<td>1906.82</td>
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<td>ADD4 ( °C day)</td>
<td>2662.67</td>
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<td>Tmean ( °C)</td>
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<td>MaxTa ( °C)</td>
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<td>DTR ( °C)</td>
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<td>Toscillation ( °C)</td>
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<td>Ice-on (day)</td>
<td>331.20</td>
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<td>Ice-off (day)</td>
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<td>Ice-cover (day)</td>
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<td>Jdate (day)</td>
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<tr>
<td>Fdate (day)</td>
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<td>Fish_cpue (ºn)</td>
<td>200.33</td>
<td>35.73</td>
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<td>Phox_cpue (ºn)</td>
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<td>Salm_cpue (ºn)</td>
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<td>Corix_n (ºn)</td>
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<td>Newt_n (ºn)</td>
<td>1.8</td>
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<td>Amphibian_n (ºn)</td>
<td>162</td>
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</table>
Appendix 14: Table of the explicative variables included in the zooplankton analyses. Each variable is briefly described and it is indicated in which analyses are included: RDA1 for the RDA including predators abundances, RDA2 for the RDA with presence absence data of predators and SEM for the Structural Equation Models.

<table>
<thead>
<tr>
<th>Category</th>
<th>Variable</th>
<th>Description</th>
<th>Analyses</th>
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<tbody>
<tr>
<td>Geomorphological</td>
<td>Altitude</td>
<td>Altitude (m).</td>
<td>RDA1, RDA2</td>
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<tr>
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<td>Larea</td>
<td>Lake area (ha). Log transform.</td>
<td>RDA1, RDA2</td>
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<td>Dcatchment</td>
<td>Direct catchment area (ha). Log transform.</td>
<td>RDA1, RDA2</td>
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<td></td>
<td>Tcatchment</td>
<td>Total catchment area (ha). Log transform.</td>
<td>RDA1, RDA2</td>
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<td>Tcatchment/Larea</td>
<td>Ratio between total catchment and lake area. Log transform.</td>
<td>RDA1, RDA2</td>
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<td>Dcatchment/Tcatchment</td>
<td>Ratio between the direct catchment and the total catchment. Log transform.</td>
<td>RDA1, RDA2</td>
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<tr>
<td></td>
<td>Depth</td>
<td>Maximum lake depth (m). Log transform.</td>
<td>RDA1</td>
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<tr>
<td></td>
<td>Ice</td>
<td>Percentage area of the catchment covered with ice or snow (%). Log+1 transform.</td>
<td>RDA1</td>
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<td>Bedrock</td>
<td>Percentage area of the catchment transform. covered with bedrock (%). Log+1 transform.</td>
<td>RDA1</td>
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<tr>
<td></td>
<td>Swamps</td>
<td>Percentage area of the catchment</td>
<td>RDA1</td>
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<tr>
<td>Component</td>
<td>Description</td>
<td>Transformations</td>
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<tr>
<td>Prairies</td>
<td>Percentage area of the catchment covered with prairies (%)</td>
<td>Log+1, RDA1</td>
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<tr>
<td>Bushes</td>
<td>Percentage area of the catchment covered with bushes (%)</td>
<td>Log+1, RDA1</td>
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<tr>
<td>Trees</td>
<td>Percentage area of the catchment covered with coniferous trees (%)</td>
<td>Log+1, RDA1</td>
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<tr>
<td>Debris</td>
<td>Percentage area of the catchment covered with debris cones (%)</td>
<td>Log+1, RDA1</td>
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<tr>
<td>Chemical</td>
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<td>RDA1, RDA2</td>
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<tr>
<td>Alk</td>
<td>Alkalinity ($\mu$eq l$^{-1}$). Log transform.</td>
<td>RDA1, RDA2</td>
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<tr>
<td>Cond</td>
<td>Conductivity ($\mu$S). Log transform.</td>
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<td>pH</td>
<td>pH. Log transform</td>
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<td>TN</td>
<td>Total nitrogen ($\mu$g l$^{-1}$). Log transform.</td>
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<td>DIN</td>
<td>Dissolved inorganic nitrogen ($\mu$g l$^{-1}$) Log transform.</td>
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<td>TP</td>
<td>Total phosphorous ($\mu$g l$^{-1}$). Log transform.</td>
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<td>DIN/TP</td>
<td>Ratio between DIN and TP. Log transform.</td>
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<tr>
<td>Variable</td>
<td>Description</td>
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<td>----------</td>
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<td>Kd</td>
<td>Light extinction constant calculated from Secchi disk depth.</td>
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<td>Chla</td>
<td>Chlorophyll a (µg l⁻¹).</td>
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<td>Cattle</td>
<td>Cattle pressure estimated by animal excrements (n°).</td>
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<td>Msp</td>
<td>Submerged macrophytes species richness (ºn).</td>
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<td>Radiation</td>
<td>Duration of incoming solar radiation (h)</td>
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<td>Dradiation</td>
<td>Direct incoming solar radiation (W m⁻²)</td>
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<tr>
<td>Tradiation</td>
<td>Total incoming solar radiation (W m⁻²)</td>
<td>RDA1, RDA2</td>
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<tr>
<td>Climate</td>
<td>Accumulated degree-days over 7.6 °C ( °C day⁻¹)</td>
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<td>ADD4</td>
<td>Accumulated degree-days over 4 °C ( °C day⁻¹)</td>
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<td>Tmax</td>
<td>Maximum temperatures of the lake surface ( °C)</td>
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<td>Tmean</td>
<td>Mean temperature of the lake surface during the ice-free period ( °C)</td>
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<td>DTR</td>
<td>Diel temperature range of the lake surface during the</td>
<td>RDA1, RDA2</td>
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<tr>
<td>Parameter</td>
<td>Description</td>
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<td>Icecover</td>
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<td>RDA1, RDA2</td>
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<td>Ice-off</td>
<td>Date of ice cover melting (d)</td>
<td>RDA1, RDA2</td>
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<tr>
<td>Ice-on</td>
<td>Date of ice cover</td>
<td>RDA1, RDA2</td>
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<td>Jdate</td>
<td>Julian date of the sampling day (d)</td>
<td>RDA1, RDA2</td>
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<td>Fdate</td>
<td>Phenology date, sampling date since the ice-off date (d)</td>
<td>RDA1, RDA2, SEM</td>
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<td>Spatial</td>
<td>MEM</td>
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<td>Moran eigenvectors map. They are numerated.</td>
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<td>Predators</td>
<td>Fish_cpue</td>
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<td>Catchment for unit effort of all fishes (ºn). Log +1 transform.</td>
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<td>Phox_cpue</td>
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<td>Minnow catchments per unit effort (ºn). Log +1 transform.</td>
<td>RDA1, SEM</td>
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<td>Salm_cpue</td>
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<td>Salmonids catchments per unit effort (ºn). Log +1 transform.</td>
<td>RDA1, SEM</td>
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<td>Corix_n</td>
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<td>Corixidae abundance (ºn). Log +1 transform.</td>
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<td>Newt_n</td>
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<td>Newt abundance (ºn)</td>
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<td>Amphibian_n</td>
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<td>Amphibian abundance (ºn). Log +1 transform.</td>
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<td>Fish presence/absence.</td>
<td>RDA2</td>
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<td>Salmonids</td>
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Appendix 15: Pearson correlations among environmental (morphologic, chemical, radiation, climatic) and predators variables of Pyrenean lakes and ponds.
Appendix 16: Path diagram of the initial model hypothesised for the Structural Equation Model of zooplankton, with all defined relations drawn with grey lines. Variables are: Cyc: Cyclopoids, Dph: Daphnids, Cla: Smaller cladocerans, Cal: calanoids, Rot: Rotifers, Salm: Salmonids cpue, Phox: Minnows cpue, Newt: Newt abundance, Corix: Corixids presence/absence, Tmean: mean temperature, Fdate: Date of the sampling since ice-off, Chla: Chlorophyll a.