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ZOOPLANKTONIC POPULATION DYNAMICS AND PELAGIC FOOD WEBS IN THE DEEPEST SUBALPINE LAKES RELATED TO ANTHROPOGENIC PRESSURE AND CLIMATE CHANGE

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ABSTRACT

Climate change is considered to be one of the most severe threats to earth and aquatic ecosystems and the concern about the effects that global warming can have on biological communities is growing. Even if the number of studies concerning global warming is worldwide increasing the effects of climate change are challenging to monitor and understand because of the multitude of responses within an ecosystem. In the last years, analyses of long-term data sets provide increasing evidence on the sensitivity of water bodies to climatic fluctuation. Small size and fast reproducing zooplankton organisms are sensitive to environmental change. Moreover, they represent key components of aquatic food webs, connecting primary producers to big secondary consumers as fishes. The purpose of the present PhD research is to expand the knowledge on the zooplankton communities of the large and deep lakes south of the Alps, detecting trends and significant change or shift in the zooplanktonic food webs structure due to global or local impacts. The analysis of long-term series of limnological observations and a paleolimnological study on lake sediment have been combined in order to disentangle the effects of climate change from that due to more local and anthropic stressor as eutrophication. The obtained results suggested that, in deep subalpine lakes, the effect of climate change on pelagic food webs components is complex, including several direct and indirect effects, and it is mediated by nutrient availability. In particular, in deep lakes winter climatic variability triggers a cascading effect that involves the entire lake ecosystems, modifying lake physical-chemical and biological characteristic. The effects of warmer temperature in winter can reverberate on zooplankton seasonal patterns until summer, affecting the food webs functioning. The paleolimnological investigation, through the combined study of biotic and abiotic factor, allowed clarifying the synergic effects of the most important driver of change in lake ecosystems, suggesting that climatic factors should be considered with nutrient availability as a determinant element in controlling the temporal development of plankton communities and pelagic food web structure.

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1 INTRODUCTION

Freshwater is a fundamental resource, essential to all the natural and human activities. Lakes are indispensable freshwater resources in earth ecosystems, they store tons of water and provide a multitude of different ecosystem services, which are essential to maintain biodiversity and human life (Gleick & Miller, 2003). Freshwater bodies are important habitats rich in biodiversity; moreover, they provide food, water for agriculture and energy and they are also sources of drinkable water.

At present, climate change is considered to be one of the most severe threats to ecosystems around the globe (Adrian *et al.*, 2009). Even if the number of studies concerning climate change is increasing in the last decades (Vadadi-Fülöp & Hufnagel, 2014; O'Reilly *et al.*, 2015; Lepori, Roberts & Schmidt, 2018b), its effects are challenging to monitor and understand because of the multitude of responses within the ecosystems. Several studies demonstrated the sensitivity of lakes to climate and showed that physical, chemical and biological lake properties respond to climate-related changes (Williamson *et al.*, 2008; Adrian *et al.*, 2009). Lakes are sensitive to environmental changes and can integrate environmental variation from the atmosphere and catchment area (Adrian *et al.*, 2009).

Worldwide, several researchers have highlighted visible and easily measurable changes in lakes. An example of these evident variations is the alteration of lake water levels or the variation in the timing of ice formation reported by Williamson *et al.* (2008) and Magnuson *et al.* (2000). Other climate change impacts may be more complex to detect and measure and the use of some ecological indicators of climate forcing can be necessary. Some good indicators of environmental change have been identified in measurable variables as water temperature, dissolved organic carbon (DOC), chemical-physical parameters, plankton composition and phenology.

In the last years have emerged evidences that highlight some climate-related responses of lake biota. The effects of climate on lake biota can be divided into two main categories. The first one includes the direct effects of warming on the phenology of organisms, the second is the indirect influence of warming that can reinforce the effects of other anthropogenic pressures, such as eutrophication or the spread of alien species (Lepori *et al.*, 2018a). This interaction between climate variation and anthropogenic pressure makes more difficult to interpret and understand the effect of climate change on lakes and on their food webs.

One of the most common and well-studied environmental problems that affect lakes all around the word is eutrophication (i.e. an enrichment in the concentration of nutrients)(Smith, 2003; Deng *et al.*, 2016). In particular, in Europe this phenomenon generally had reached a maximum during the second half of the twentieth century, caused by the increased human activity and urbanization. In the last decades, some management strategies aimed to reduce the nutrients loads (mainly phosphorus) lead to the restoration of some lakes water quality and the decrease in nutrients concentration (Jeppesen *et al.*, 2005; Schindler *et al.*, 2016). However, some studies reported that climate warming appears to reinforce some of the symptoms of eutrophication (Lepori *et al.*, 2018b). Therefore, disentangle the effects of the two problems and actuate efficient management strategies become more difficult.

Planktonic organisms have been widely used as indicators of ecosystem changes, in fact they respond rapidly to environmental variations because of their relatively short life cycle (Vadadi-Fülöp & Hufnagel, 2014). Moreover, phytoplankton and zooplankton communities in many lakes have been relatively well documented for a long period of time and these records can be crucial to understand the changes taking place in lake ecosystems. Indeed, long-term series of plankton analyses allowed characterizing reliable seasonal pattern, multi-annual trends and shifts of populations and communities in response to global or local impacts (Morabito *et al.*, 2018a).

Zooplanktonic organisms are a key component of lakes pelagic food webs. They occupy a central position, consuming primary producers and being consumed by predators, including other invertebrates and fishes. Indeed, they are the center of bottom-up (nutrients and phytoplankton) and top-down factors (fish and invertebrate predators) that regulate pelagic food webs structure (Lampert, 2006). This central position makes them ideal objects in order to study interactions between several trophic levels, clarifying the predator-prey interactions and seasonal variations in the transfer of matter and energy from the bottom to the top of the food web (Piscia *et al.*, 2018). Moreover, zooplankton organisms are geographically widespread and distributed worldwide and that makes them particularly helpful in the development of synoptic studies, comparing lakes with different ecological characteristics.

It should be noted that zooplankton have also a fundamental role in controlling algal blooms with the grazing activity. In particular, in many temperate lakes, the most conspicuous event during seasonal succession of the phytoplankton community is the Clear Water Phase, i.e. a period of low phytoplankton biovolume (Lampert *et al.*, 1986; Sommer *et al.*, 2012). Even if in some cases it can be explained by the exhaustion of a pool of limiting nutrients as phosphorus, it is usually attributed to intense grazing from a growing population of crustacean zooplankton (Berger *et al.*, 2007).

In recent years several studies focused their attention on the effects of climate variability on zooplankton dynamics and on the possible mismatch resulting in food webs functioning (Straile *et al.*, 2003; Straile, Kerimoglu & Peeters, 2015). Some authors observed that changes in zooplankton phenology can provide a good reflection of climate shifts (Straile, 2000; Manca, Portogallo & Brown, 2007). Climate can induce alterations in growth rates, abundance and species composition. Such changes can create a mismatch between the consumers and the food resources affecting the entire pelagic food webs. Some researchers highlighted a connection between the climate variations and important ecological events as the Clear Water Phase (Scheffer *et al.*, 2001), demonstrating that climate change can affect not only individual species, but also the dynamics of the entire food webs. Long-term observations suggested asynchronous shifts in zooplankton phenology caused by global warming and they highlighted that changes in phenology between interacting species may disturb feeding interactions, with negative consequences for the entire ecosystem functioning (Straile *et al.*, 2015).

Most of the long-term studies about zooplankton phenology in relation to climate change has been conducted in North America and in Central Europe (Vadadi-Fülöp & Hufnagel, 2014). In Central Europe several studies about the complex effect of climate and its interaction have been conducted in the lakes of the north alpine region (Straile & Geller, 1998b; Straile *et al.*, 2007; Straile, 2015), conversely in deep south alpine lakes only few long term investigation have approached this issue and mainly at a regional scale. In particular, they focused on the effects that climate may have on the prevalence of some zooplanktonic species, as the predators *Bythotrephes longimanus* (Manca *et al.*, 2007; Manca, 2011), which showed higher abundances at high water temperatures. Moreover, some studies investigated the effects that climatic variability can have on vertical mixing regime and nutrient availability in epilimnion and on the consequence for *Daphnia* population dynamics in spring (Leoni, Garibaldi & Gulati, 2014; Manca, Rogora & Salmaso, 2015).

In general, in the perialpine region it has been demonstrated that zooplankton biomass and species composition strongly depends by lakes trophic condition, but also that meteo-climatic factors have a strong influence on zooplankton community (Straile & Geller, 1998b). The majority of the studies regarding the interaction between climatic factors and trophic levels regarded the well-studied *Daphnia* spp. that it is considered a model organisms in ecology (Gleik, 2012). Meteorological factors may alter the abundance of organisms in different ways: directly, affecting metabolic rates, or indirectly, influencing the abundance of potential prey or food availability (Straile & Geller, 1998a). In particular, emerging evidences have highlighted the relation between winter air temperature and the period of development of daphnids in spring. Cold winters slowed down the growth rate of *Daphnia* population in spring and stabilize their reproductive potential in successive months (Straile, 2000). Moreover, winter and spring temperatures have been pointed out as important drivers in *Daphnia* phenology. In some researches on shallow and hypertrophic reservoirs, seasonal air temperatures have been indicated as the principal drivers in determining the *Daphnia* midsummer decline, i.e. a period of low *Daphnia* abundance after the maximum density peak in spring (Benndorf *et al.*, 2001; Wagner & Benndorf, 2007).

In order to detect the relationship between climatic variability and lake limnological characteristics a useful tool has been individuated in the teleconnection indices, i.e. large-scale circulation patterns that provide conceptual tools to understand the impact of climatic fluctuations on aquatic and terrestrial ecosystems at different levels of complexity (Dokulil *et al.*, 2006). Among the highly cited and well-known indices there are the North Atlantic Oscillation (NAO), El Niño Southern Oscillation (ENSO), the East Atlantic pattern (EA) (Straile *et al.*, 2003; Stenseth *et al.*, 2003) and, relevant for the Mediterranean area, the Eastern Mediterranean Pattern (EMP)(Hatzaki *et al.*, 2007). All of them well represent seasonal climatic variability and they can affect the physical, chemical and biological characteristics of lake ecosystems (Dokulil *et al.*, 2006; Salmaso, 2012). Between the biological characteristics influenced by teleconnection indices there are plankton population dynamics and community structure (Straile *et al.*, 2003). In particular some authors highlighted that zooplankton is affected both directly, by warmer temperatures that modify the timing of population development, and indirectly, by the modification of nutrient availability and food exploitation (Seebens *et al.*, 2007).

More specifically, for the Mediterranean area Salmaso *et al.* (2012) showed that East Atlantic pattern (EA) and Eastern Mediterranean Pattern (EMP) have a strong and significant link with the air and water temperatures and can be suitable proxies of the climatic variability in the region of the deep subalpine lakes, that are the focus of these PhD project. In the oligomictic deep subalpine lakes, winter climatic variability, well represented by EA and EMP, affected *Daphnia* phenology both altering directly the timing of population development and indirectly affecting the nutrient availability for phytoplankton and thus *Daphnia* population density (Manca *et al.*, 2015). However, the effects of seasonal climatic variability on secondary consumers and on lakes with different trophic levels and nutrient concentration have never been tested before. Moreover, in deep subalpine lakes the propagation of the effect of winter and spring climatic variability on seasonal zooplankton phenology in summer has never been investigated.

Climate change affects plankton community not only through the cascading effect triggered by winter climatic variability, but also altering catchment hydrological dynamics that reverberate on chemical and biological characteristics of lake waters. Indeed, in the last decades several researches verified that climate change may increase the frequency of extreme wheatear events such as heavy rainfall and floods event across Europe and North America (Graham & Vinebrooke, 2009; Kundzewicz, Hirabayashi & Kanae, 2010). These events contribute in increasing the runoff of terrestrial particulates and dissolved substances into lakes from their catchment area (Graham & Vinebrooke, 2009). Recent studies reported that episodes of intense rainfall may affect phytoplankton community composition increasing flushing (Srifa *et al.*, 2016), nutrient loading (Jeppesen *et al.*, 2009), water turbidity (Richardson *et al.*, 2019). Indeed, large lakes worldwide resulted susceptible to anthropogenic effects (nutrient and sediments loading) and sediment resuspension that can alter the quality and quantity of light available to phytoplankton (Graham & Vinebrooke, 2009). Alteration in phytoplankton seasonal succession, as well as a depression in the primary production can have an impact on the whole-lake metabolism (Morabito *et al.*, 2018b). However, the understanding of how the effects of such events carry through to plankton communities is limited.

Even if the number of evidences reporting climate-induced changes in zooplankton phenology is increased in the last decades, in particular in marine environments (Hays, Richardson & Robinson, 2005; Richardson, 2008), there is still a lack of comprehensive understanding about the effects that climate change can have on lake pelagic food webs and how these variations can affect water quality and management (Vadadi-Fülöp & Hufnagel, 2014). Synoptic studies that analyze in detail the synergic effects of multiple environmental factors on zooplankton populations are still rare and it became crucial to investigate in detail how climate interacts with other local stressors, as eutrophication. Furthermore, understanding the underlying mechanisms in shaping food webs and disentangle the effects caused by climate from that caused by other important anthropogenic stressors, as eutrophication, can be essential to realize some efficient management strategies and to conserve lake ecosystems and biodiversity. Although most physical responses of lakes to climate change are consistent among different climate scenarios, biological responses resulted more variable because of the complex nature of ecological interactions in lakes (Wagner & Benndorf, 2007). The evidence of climate change effects is clearer for lakes in remote regions, where warming issues are most pronounced and where the effects are not masked by the impact of human activity such as nutrient pollution or land-use changes (Battarbee *et al.*, 2012). On contrary, in most urbanized area, identify the exact causes of changes in plankton dynamics can be problematic because typically there is not a single environmental driver but multiple factors occurring simultaneously (O'Neil *et al.*, 2012).

Paleolimnology has been indicated as a powerful tool to reconstruct the effects of climate and other anthropogenic stressors because it allows to study period of time in which their impact did not overlap (Perga *et al.*, 2015). Using paleolimnological data combined with long-term observation may offer the best opportunity of generating datasets of sufficient length and quality. These datasets can be used to study ecosystems changes in period affected by different global and local stressors (Battarbee *et al.*, 2012). Indeed, sediment records do not possess the precision and accuracy of observational data, but they provide an integrated record of lake history and evolution. Therefore, sediment records, in particular subfossil Cladocera (Crustacea, Branchiopoda), can be used to extend the record back in time, allowing the definition of lakes status before the overlapping of local (e.g. eutrophication) and global (i.e climate change) stressors.

Aims of the project

Based on the increased interest in the effects of climate change on lakes biological communities and the possible interaction with other anthropogenic stressors such as eutrophication, the purpose of the present PhD project is to clarify the answers of zooplanktonic communities to the synergic impact of global warming and eutrophication in deep subalpine lakes. The investigation of long-term dataset has been combined with a paleolimnological study in order to achieve a detailed comprehension of plankton dynamics and to detect trends and significant changes or shifts in zooplankton phenology. Modifications that take place in deep subalpine lakes, due to synergic effects of meteoclimatic factors and eutrophication, can alter food web functioning and lake water quality.

Zooplankton are key components of pelagic food webs and a detailed comprehension of the shifts in population seasonal patterns is useful not only as a support to the management of aquatic resources, but also to predict and tackle future environmental changes.

The main activities of this PhD work were focused in achieve generalized information about the effects of climate on zooplankton community composition and abundance in deep oligomictic lakes and in particular in the eutrophic Lake Iseo, clarifying the synergic effects of climate change and eutrophication. Indeed, the seasonal zooplankton patterns are the result of multiple processes interplaying at different spatial and time scale, and sometimes climate driven modification can be masked by different factors that affects both population variability and the coupling between predator and prey. In order to define generalized trends and identify the causes of possible variation in the expected patterns, zooplankton population dynamics have been analyzed and compared in analogous periods in different lakes. We chose lakes characterized by different anthropogenic pressure but similar morphometry and climatic position in order to understand the impact of external variables on lakes population dynamics and food web functioning. The present PhD research was focused on three main aspects concerning the answer of zooplankton population dynamics to climate, and the present work follow this division (Fig. 1):

1- The answer of pelagic food webs to climatic variation in relation to lakes trophic level: investigating how winter climate variability affects zooplankton phenology, directly and indirectly, in a deep eutrophic lake, comparing the results with those reported for other deep oligotrophic lakes.

2- Climate induced changes in primary consumers population structure during summer: in this part, the cascading effect triggered by seasonal temperatures will be extended to zooplankton summer phenology, in order to investigate the underling mechanism of *Daphnia* midsummer decline.

3- A paleolimnological study of a deep subalpine lake (Lake Iseo) in relation to climate change and trophic evolution: paleolimnology and in particular subfossil Cladocera remains will be used in order to understand the changes taking place in lake ecosystems in period affected by different global and local stressors.

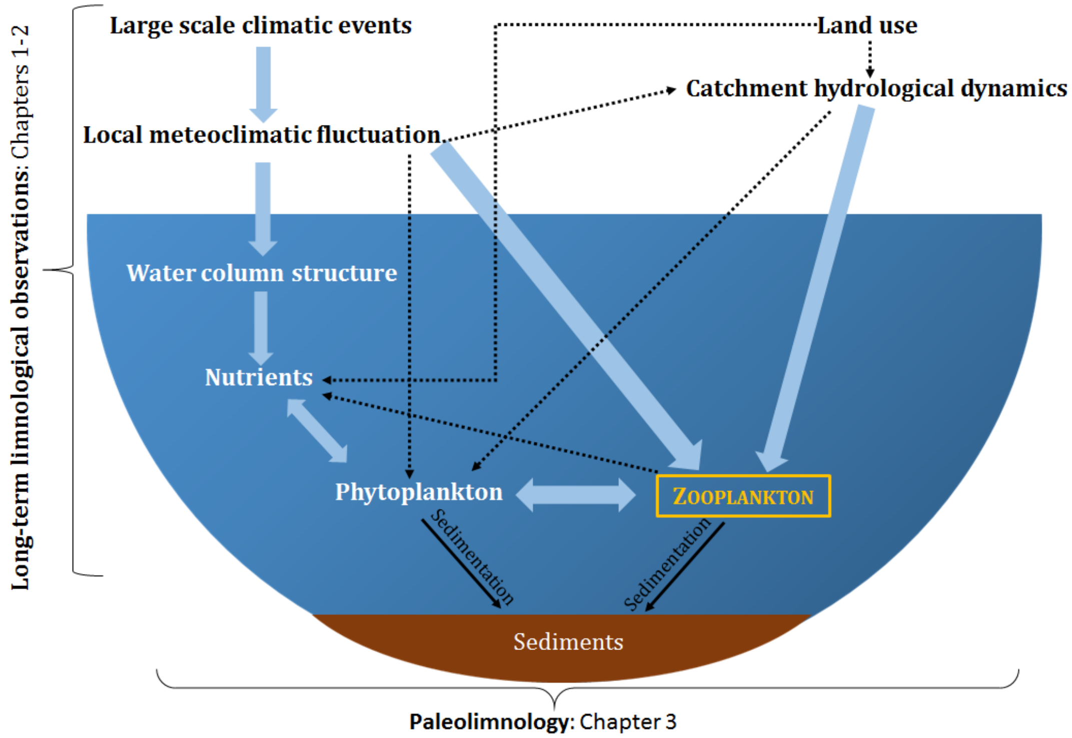

Figure 1- Conceptual diagram illustrating the main natural and anthropogenic factors affecting zooplankton community in lake ecosystems. The continued arrows represented the relation considered in this thesis, while the dotted arrows represented the relation reported in literature but not tested in the project.

In particular, this work is focused on Lake Iseo and on the other deep subalpine lakes (mainly Lake Lugano), that constitute the most important lake district in Italy. They are located south of the Alps, in one of the most densely populated and highly productive area of the country, and represent an essential strategic water supply for agriculture, industry, fishing and drinking. These lakes are all located in the same climatic region but have different trophic evolution and different current trophic levels (Salmaso *et al.*, 2003, 2013; Rogora *et al.*, 2018). They can be suitable to a comparative study in order to detect the effects of climate and anthropogenic pressure on the zooplanktonic food webs of deep oligomictic lakes.

Specifically, Lake Iseo appeared to be an ideal site to conduct this kind of investigations. Indeed, it is located at the end of a big and strongly populated valley (Val Camonica, 80 km long). Lake Iseo is a naturally oligotrophic water body; however, over the past 40 years, the increase of nutrient loadings brought it to a meso-eutrophic condition (Salmaso *et al.*, 2013; Leoni *et al.*, 2014). In addition, it is characterized by a big ratio between the lake catchment and the lake surface area (28.49) and that strongly influenced lake characteristics and its sedimentation rate. Considering its geographical position and its features, Lake Iseo resulted strongly influenced by the increasing anthropogenic pressure and the changes in meteo-climatic conditions (Leoni, Nava & Patelli, 2018). Moreover, on Lake Iseo a monitoring campaign is being carried out since the beginning of 1990s and this allows the interpretation and comparison of paleolimnological results in the light of those provided by the decadal freshwater investigations.

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2 THE ANSWER OF PELAGIC FOOD WEB TO CLIMATIC VARIATION IN RELATION TO LAKES TROPHIC LEVEL

Climate is considered to be one of the major drivers of changes in zooplankton population phenology, affecting both directly and indirectly the timing of population development and food availability (Manca, Rogora & Salmaso, 2015). In particular, some researches have observed that temperature variation promotes temporary modifications in biotic interactions and in the relation between predator and prey (Manca, Portogallo & Brown, 2007; Vadadi-Fülöp & Hufnagel, 2014; Straile, Kerimoglu & Peeters, 2015). Shifts among the several levels of pelagic food webs may result in changes in the ecosystem structure and functioning (Moss *et al.*, 2009; Shurin *et al.*, 2010; Manca *et al.*, 2015). Zooplankton is a key component of the pelagic food webs, as the grazing activity can regulate phytoplankton growth and it is a crucial food resource for predators such as the zooplanktivorous fishes (Pascariello *et al.*, 2019). For this reason, a modification in plankton phenology can reverberate on the upper levels of the pelagic food webs affecting the entire lake ecosystem and the water quality.

In deep oligomictic lakes the effect of climate is particularly complex because it is usually mediated by nutrient availability in surface levels, where zooplankton population lives (Lepori, Roberts & Schmidt, 2018). Therefore, there is a growing need for study concerning the effect of climate variability on pelagic food webs considering lake trophic levels. Indeed, the abundance of nutrients and in particular phosphorus is a key factor in determining phytoplankton development and as consequence, food availability for primary consumers. In particular, it has been verified that in deep lakes, the increase of water temperatures and the consequently enhanced stratification period cause a reduction in the vertical extension of the late winter water turnover (Salmaso *et al.*, 2013). The reduction in the extent of the mixed layer may have a strong impact on the oxygenation of hypolimnetic waters and on the nutrient replenishment of the epilimnetic layers, in particular phosphorus (Rogora *et al.*, 2018; Salmaso *et al.*, 2018). In this context resulted of high scientific and economic value to achieve a clearer knowledge about the role of climate change in modifying lake limnological characteristics and zooplanktonic population phenology. In fact, a clearer comprehension of the underling mechanisms of changes taking place in pelagic food webs will be crucial to predict future modifications in lake ecosystems functioning.

In deep subalpine lakes winter climatic variability, well represented by the mean winter value of East Atlantic pattern (EA) and Eastern Mediterranean Pattern (EMP) strongly influences deep and oligomictic lakes ecosystems, acting through direct, indirect and integrated effects on the limnological processes (Manca *et al.*, 2015). Therefore, the first part of this PhD work is aimed to clarify the effects of inter-annual climatic variations, represented by teleconnection indices, on physical–chemical features, phytoplankton and pelagic Cladocera phenology, including several levels of lake pelagic food webs (primary and secondary consumers). Moreover, in order to detect the answer of pelagic food webs to climate in relation to eutrophication problems, the data about the deep and eutrophic Lake Iseo were compared with that concerning other deep subalpine lakes with lower trophic levels.

The results have been collected, presented and discussed in the paper: "Leoni B., Nava V. & Patelli M. (2018) Relationships among climate variability, Cladocera phenology and the pelagic food web in deep lakes in different trophic states. Marine and Freshwater Research 69, 1534–1550." In particular, in order to realize the following study I have contributed in zooplankton classification and count, in long-term data analysis, in results elaboration and interpretation and in paper writing and editing.

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Relationships among climate variability, Cladocera phenology and the pelagic food web in deep lakes in different trophic states

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Abstract

Achieving a better understanding of the role of climate change in altering population phenology, seasonal cycles in freshwater organisms, and ecosystem structure and function is of high scientific and economic value. The present paper has demonstrated the different food-web responses to teleconnection indices, which are proxy of climate fluctuations, in lakes characterised by different trophic levels. We analysed an 18-year long-term dataset (1998–2015) recorded in the deep eutrophic Lake Iseo and we compared our results to those concerning deep southern-Alpine oligotrophic lakes. Our results confirmed that winter large-scale circulation patterns (for Mediterranean area: East Atlantic Pattern and Eastern Mediterranean Pattern) control a chain of linked causal factors, affecting the winter air temperature, spring water temperature, the resulting water vertical-mixing depth and epilimnetic concentration of total phosphorus. We highlighted that in a lake, characterised by high phosphorus concentration, the spring enrichment in nutrients did not result in either a considerable increase of phytoplankton growth, nor, consequently, in a zooplankton density peak. Whereas in oligotrophic lakes, cascading effects influenced the algal carrying capacity and *Daphnia* population density. We observed that climatic fluctuations, mediated by, for example, colder water temperature in spring, postponed the timing of population recovery after diapause of both primary consumers (*Daphnia* and *Eubosmina*) and secondary consumers (*Bythotrephes* and *Leptodora*). The latter being verified for the first time in a eutrophic lake.

Keywords: Cascading effects, East Atlantic Pattern, Eastern Mediterranean Pattern, phytoplankton, primary consumers, secondary consumers, trophy.

Introduction

In the past few years, studies on the impact of climate change on aquatic ecosystems have intensified. A growing body of evidence has highlighted a strong impact on lakes of interannual climatic variability, with direct and indirect effects, as altered air and water temperatures cause variations in the thermal structure, in chemical and physical characteristics, in food web, and in features of the biocenosis, such as phytoplankton and zooplankton (Dukes and Mooney 1999; Sala *et al.* 2000; Walther *et al.* 2002; Parmesan and Yohe 2003; Root *et al.* 2003; Thomas *et al.* 2004; Rusak *et al.* 2008; Adrian *et al.* 2009; Manca and DeMott 2009; Winder and Sommer 2012; Vadadi-Fu¨lo¨p and Hufnagel 2014; Leoni *et al.* 2018; Morabito *et al.* 2018).

Owing to their short life cycle and sensitivity to changes in water temperatures, zooplankton species are particularly responsive to climate change. Comprehensive conceptual models of the direct and indirect effects of climatic variability on the freshwater zooplanktonic community have shown a variation in distribution, abundance and phenology (Vadadi- Fulop *et al.* 2012).

In this context, the large-scale climatic indices represent a useful tool in ecology, providing a framework for the interpretation of community- and species-level responses to altered climatic conditions (Salmaso 2012; Vadadi-Fu¨lo¨p *et al.* 2012).

Teleconnection indices have been widely used to study climate patterns and anomalies over continental scales (Soulard and Lin 2017), representing proxies for the overall climate conditions and fluctuations, because they integrate covariability patterns between multiple climate variables at different time ranges (Stenseth *et al.* 2003). They refer to recurring and persistent circulation anomalies between two distant geographical areas, indicating their time variations and implications for regional climate (Hatzaki *et al.* 2007). They integrate several weather components both in space and time, and, in this way, they have a greater explanatory power than do local weather variables (Vadadi-Fulop *et al.* 2012).

In the Mediterranean area, climate variability has been shown to be strongly influenced by two large-scale atmospheric circulation patterns, namely the East Atlantic Pattern (EA) and the Eastern Mediterranean Pattern (EMP), particularly in the winter period (deCastro *et al.* 2008; Schroeder *et al.* 2010; Toreti *et al.* 2010; Josey *et al.* 2011; Salmaso 2012). EA and EMP indices have been recognised as driving forces behind variation in limnological processes acting through direct, indirect, and integrated effects in warm holo-oligomictic lakes of the southern-Alpine domain. They have been linked to cascading effects on physical, hydrological and chemical water features in five deep, large lakes, with the impact depending on the lake trophic level (Salmaso and Cerasino 2012; Salmaso *et al.* 2014). The cascading effects of negative winter values of EA (or positive of EMP) have involved the following, in sequence: low winter air temperature, low winter water temperature, low lake stability, great vertical water mixing, uniform distribution in water column of chemical compounds accumulated in hypolimnetic layers and the resulting increase of nutrients (e.g. phosphorus) in the surface layer (Salmaso *et al.* 2014; Rogora *et al.* 2018). The cascading effects could be extended to the influence on the development of the *Daphnia* population in spring, probably being mediated by an increase of phytoplankton biomass as discussed in Manca *et al.* (2015).

However, in meso-eutrophic ecosystems with high concentration of nutrients, a further increase of phosphorus after full mixing events seemed to affect more the quality than the quantity of phytoplankton, influencing differently the phenology of *Daphnia* (Leoni *et al.* 2014*b*; Leoni 2017). For this reason, we hypothesise the existence of different pelagic food-web responses to climate variation (i.e. teleconnection indices) in lakes characterised by different trophic levels. The majority of studies on the relationship between teleconnection indices and zooplankton have focused on primary consumers, in particular, on *Daphnia*, a key component of the zooplankton community (Wojtal-Frankiewicz 2012), whereas, to the best of our knowledge, their relations with zooplanktonic secondary consumers have not been tested. However, some investigations have hypothesised that changes in the life cycles of zooplankton secondary consumers could be a result of climatic change. The analysis performed by Manca *et al.*

(2007) on the long-term data series of Lake Maggiore zooplankton suggested that changes in density and phenology of *Bythotrephes* were probably connected to climatic variations, in particular to the rise of lake temperature, which could have altered the reproductive cycle of the species.

The aim of the present paper was to contribute to clarifying the effects of inter-annual climatic variations on physical–chemical features, phytoplankton and pelagic Cladocera phenology, analysing an 18-year long-term dataset (from 1998 to 2015) from the deep perialpine and eutrophic Lake Iseo. Particular emphasis has been placed on the relationships between mean winter values of EA and EMP and three phenological parameters of zooplanktonic Cladocera in spring, including both primary (*Daphnia longispina* group and *Eubosmina longicornis*) and secondary (*Bythotrephes longimanus* and *Leptodora kindtii*) consumers. Our purpose was to compare the cascading effects on pelagic food web illustrated by Salmaso *et al.* (2014) and Manca *et al.* (2015) in deep oligotrophic lakes with the ones in a meso-eutrophic lake, hypothesising the existence of different food-web responses to climate variations in lakes characterized by higher trophic levels.

Materials and methods

Study site

Lake Iseo is a deep perialpine lake with a surface of 61.8 km^2, a maximum depth of 258 m and an average depth of 124 m. The lake, included in the LTER network (http://www.lter-europe.net/lter-europe/infrastructure/networks/italy, accessed 7 June2018), is situated in northern Italy at the end of a pre-Alpine valley, Val Camonica. River Oglio, that crosses the valley, is the principal tributary and emissary of the lake (see fig. 1 in Leoni *et al.* 2014*a*). Lake Iseo should be classified as ‘warm monomictic’, as water temperatures never drop below 48C. However, owing to the great depth, winter mixing occurs only during harsh, windy winters. Over the past 20 years, complete winter mixing, in fact, occurred only in 2005 and 2006 (Leoni *et al.* 2014*b*). Lake Iseo is a naturally oligotrophic water body; however, over the past 40 years, the increase in nutrient loadings brought the lake to a meso-eutrophic condition, with average concentration of total phosphorus,80–90 mgPL_1 (Leoni *et al.* 2014*b*; Salmaso *et al.* 2014).

Teleconnection indices

Mean winter values (December, January and February, $_{DJF}$) of two teleconnection indices, East Atlantic Pattern (EA_{DJF}) and Eastern Mediterranean Pattern (EMP_{DJF}), have been applied to analyse the relationships among the climate variability and some selected phenological parameters of plankton (see below). These indices were selected, as the role of the NAO, largely applied in Europe, in the control of atmospheric temperatures in the Italian peninsula and in the large region south of the Alps was proved to be quite weak (Toreti *et al.* 2010; Salmaso 2012; Manca *et al.* 2015).

The mean monthly EA values were calculated by the NOAA–CPC, using rotated principal-component analysis (RPCA) applied to standardised 500-hPa height anomalies (www.cpc.ncep.noaa.gov, accessed 27 April 2018). Values of EMP (see Salmaso 2012) were obtained computing the differences in the mean daily geopotential height between two fixed centres located at 52.58N, 258W, and 32.58N, 22.58E (Hatzaki *et al.* 2007). The two considered teleconnection indices, EA and EMP, describe climate circulation patterns over Europe, on the basis of the anomaly dipole in North Atlantic and North Africa. They have opposite signs; negative (positive) values of the EA_{DJF} (EMP_{DJF}) are linked with the occurrence of cold winters over the Mediterranean area. This condition is reversed with a progressive change of the EA_{DJF} (EMP_{DJF}) towards positive (negative) values.

Sampling, field measurements and laboratory analyses

Samples of water, phytoplankton and zooplankton were taken in the deepest point of Lake Iseo (4584301100N, 1080304600E, LTER sampling station), with a frequency variable between 25 days and 1 month, with some missing sampling dates. Measurements of temperature in the water column of the lake were taken using an underwater multiparameter probe (WTW multi 3432, Xylem Analytics Germany Sales GmbH & Co. KG, WTW, Weilheim, Germany). The extent of the deep-water mixing events during the spring months was estimated by analysing the sequences of depth profiles of the physical and chemical data (Leoni *et al.*

2014*a*; Salmaso *et al.* 2014). Water samples for total phosphorus (TP) concentration analysis were collected using a Niskin bottle (Idromarambiente, Genova, Italy) at 10 different depths (0, 10, 20, 30, 50, 75, 100, 150, 200 and 245 m) and the analytical procedures followed standard methods (Leoni *et al.* 2014*a*). Integrated samples of phytoplankton from 0 to 20 m were collected using a Zullig's bottle (Idromarambiente). Density and biovolume assessment of phytoplanktonic taxa were performed on Lugol's solution-preserved subsamples (see Leoni *et al.* 2014*b*; Marti *et al.* 2016).

Cladocera were mostly collected using a net of 200-mm-mesh size; they have been sampled from 1998 to 2015 by vertical tows from 20-m depth to surface. The samples were immediately fixed in formalin 4% and, by optical microscope; organisms have been classified to genus or species level and counted to estimate the taxon density and biovolume: *Daphnia* gr. *Longispina* (hereafter as *Daphnia*), *Eubosmina longicornis* (as *Eubosmina*), *Bythotrephes longimanus* (as *Bythotrephes*) and *Leptodora kindtii* (as *Leptodora*).

We evaluated, for each year (1) the day of population growth after winter pause (INCREASE), selecting the first day, from March to June, showing an increase in population abundance, (2) the maximum population density value reached in spring–early summer (MAXDENS) and (3) the day in which this value has been reached (DAYMAXDENS). In warm-monomictic lakes, phenological parameters have to be evaluated when they are mainly affected by large-scale climatic winter fluctuations, that is, in spring immediately after vertical mixing, winter taxa diapause and food web awakening (Salmaso *et al.* 2014; Manca *et al.* 2015). The pelagic taxa responded to environmental drivers with a different lag, depending on the species behavior and ecology. INCREASE and DAYMAXDENS have been reported according to the Julian calendar.

Mean daily air temperatures, provided by ARPA Lombardia, have been calculated by data measured at the meteorological station of Sarnico (197 m ASL),,1 km away from the southern border of the lake. In our study we considered, as predictor, the air mean temperature from December to February ($T\text{air}_{\text{DJF}}$) because it strongly affected the thermal structure of the lake in late winter, acting on the heat-exchange processes between water and atmosphere. The convective movements, due to heat loss, and wind shear destroyed the thermal stability and, in late winter before the increase of air temperature, the lake underwent vertical mixing. The occurrence of overturn episodes and the determination of maximum mixing depth were estimated by analysing, along the water column, the homogenisation of several physical and chemical parameter values, such as temperature, pH, conductivity and oxygen (Salmaso 2012; Salmaso *et al.* 2014).

Data analysis

To reconstruct the cascade effects of interannual climate fluctuations (using teleconnection indices as proxies) on pelagic food web, several relationships between an ordered series of linked variables were analysed by linear regression analysis, including the following: $\mathrm{EA}_{\mathrm{DJF}}$ or $\mathrm{EMP}_{\mathrm{DJF}}$, winter air temperature ($T\mathrm{air}_{\mathrm{DJF}}$); minimum water temperature in late winter–early spring from 0- to 50-m depth ($T\mathrm{water}_{\mathrm{SPR}}$); water vertical-mixing depth in late winter–early spring ($\mathrm{Mix}_{\mathrm{SPR}}$); maximum concentration of total phosphorus at late winter–early spring water overturn from 0- to 50-m depth (TPSPR); spring phytoplankton biovolume (mean and maximum values from March to June); zooplankton primary consumer phenology (from March to June); and zooplankton secondary consumer phenology (from March to June). Further relationships between the phenology of zooplanktonic consumers (see below: INCREASE, MAXDENS and DAYMAXDENS) and environmental variables ($T\mathrm{water}_{\mathrm{SPR}}$, $\mathrm{TP}_{\mathrm{SPR}}$, $\mathrm{Mix}_{\mathrm{SPR}}$, $\mathrm{EA}_{\mathrm{DJF}}$ or $\mathrm{EMP}_{\mathrm{DJF}}$) were also considered. A few data have not been included in the analyses because they were identified as outliers after inspecting relationships; these included *Bythotrephes* INCREASE in 1998, secondaryconsumer phenological parameters in 2006, *Eubosmina* phenological parameters and $\mathrm{EMP}_{\mathrm{DJF}}$ in 2015.

Data, non-normally distributed, concerning phytoplankton mean biovolume (PHYMEANBIO), phytoplankton maximum biovolume (PHYMAXBIO) and the maximum density values of Cladocera taxa were transformed by double square roots before the analyses. Values of mixing depth were transformed by log10. Statistical analyses were performed with IBM SPSS Statistics (ver. 23.0, IBM Corp., New York, NY, USA).

Results

Physical and chemical variables and planktonic features (Fig. 1)

In the whole analysed period (1998–2015), EA_{DJF} and EMP_{DJF} showed a significant opposite temporal pattern (R2 ¼ _0.83, P , 0.01). Mean air temperatures between December and February ranged from 3 to 88C. The minimum late winter–early spring water temperatures between 0 and 50mwere ranged 6.0–7.38C, with cooling of the water column in 2000, 2005, 2006 and 2010 (Fig. 1*a*).

Despite several years of low winter air temperature occurring, only in 2005 and 2006 was there the concurrence of isothermal conditions in the water column, weak lake stability and strong wind needed to trigger convective movements until the lake bottom. The full mixing was indirectly observed by the complete homogenisation in the physical and chemical characteristics of the water column down to 258-m depth. During other years (1998–2004 and 2007–2015), the mixing depths in Lake Iseo ranged from 30 to 150 m (Fig. 1*b*).

Epilimnetic phosphorus concentration was characterised by significant fluctuations from 21.6 to 74.8 mg P L^{-1} (Fig. 1*c*), owing to the alternation between shallower or deeper mixing events. The extension of convective circulation depths in different years, triggered by winter atmospheric condition, affected the vertical nutrient transport to the epilimnion from deeper lake layers.

During the 18 study years, the phytoplankton mean biovolume in spring (March–June) ranged between 541 and 6942 mm^3 m^{-3} (Fig. 1*d*), whereas the maximum biovolume ranged from 948 to 10 047 mm^3 m^{-3}.

The four zooplanktonic taxa of Lake Iseo showed strong differences in the timing of initial growth and maximum population density, both among species and among years. The day in which the *Daphnia* population started to growth ranged between 42 and 108 Julian days, with a mean ± s.d. value of 67 ±20 days. In the other species, corresponding values ranged between 16 and 122 Julian days, with a mean value of 75 ± 34 (*Eubosmina*), between 90 and 222 Julian days, with a mean value of 145 ±33 (*Leptodora*), and between 72 and 202 Julian days, with a mean value of 136 ± 37 (*Bythotrephes*).

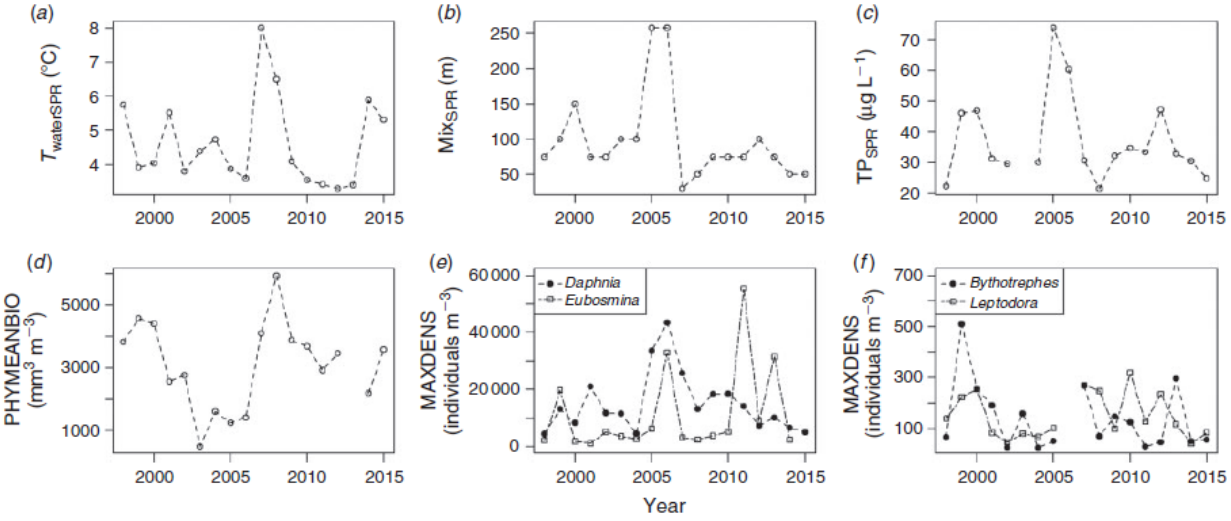


Figure 1 - Temporal variations (from 1998 to 2015) of (*a*) minimum water temperature in spring in the layer from 0- to 50-m depth (*T*water$_{SPR}$); (*b*) spring water vertical-mixing depth (Mix$_{SPR}$); (*c*) maximum concentration of total phosphorus at spring water overturn in the layer from 0- to 50-m depth (TP$_{SPR}$); (*d*) phytoplankton mean biovolume from March to June (PHYMEANBIO); (*e*) maximum density values, from March to June, of primary consumers *Daphnia* and *Eubosmina* (MAXDENS); (*f*) maximum density values, from March to June, of secondary consumers *Bythotrephes* and *Leptodora* (MAXDENS).

The day of maximum population density ranged between 91 and 175 Julian days, with a mean value of 132 ±23 (*Daphnia*), between 73 and 175 Julian days, with a mean value of 135 ± 30 (*Eubosmina*), between 129 and 257 Julian days, with a mean value of 208 ± 41 (*Leptodora*), and between 92 and 286 Julian days, with a mean value of 190 ± 46. A huge variability was observed in population maximum density during spring in different years, ranging for *Daphnia* between 4329 and 43 474 individuals m^{-3} (mean ± s.d.: 15 009 ± 10 534 individuals m^{-3}; Fig. 1*e*). Corresponding values in the other species were between 1055 and 55 530 individuals m^{-3} (mean ± s.d.: 11 030 ± 15 218 individuals m^{-3}; *Eubosmina*; Fig. 1*e*), between 42 and 318 individuals m^{-3} (mean ±s.d.: 149 ± 88 individuals m^{-3}; *Leptodora*; Fig. 1*f*) and between 26 and 509 individuals m^{-3} (mean ± s.d.: 139 ± 131 individuals m^{-3}; *Bythotrephes*; Fig. 1*f*).

Cascading effects of EA_{DJF} and EMP_{DJF} on the pelagic food web

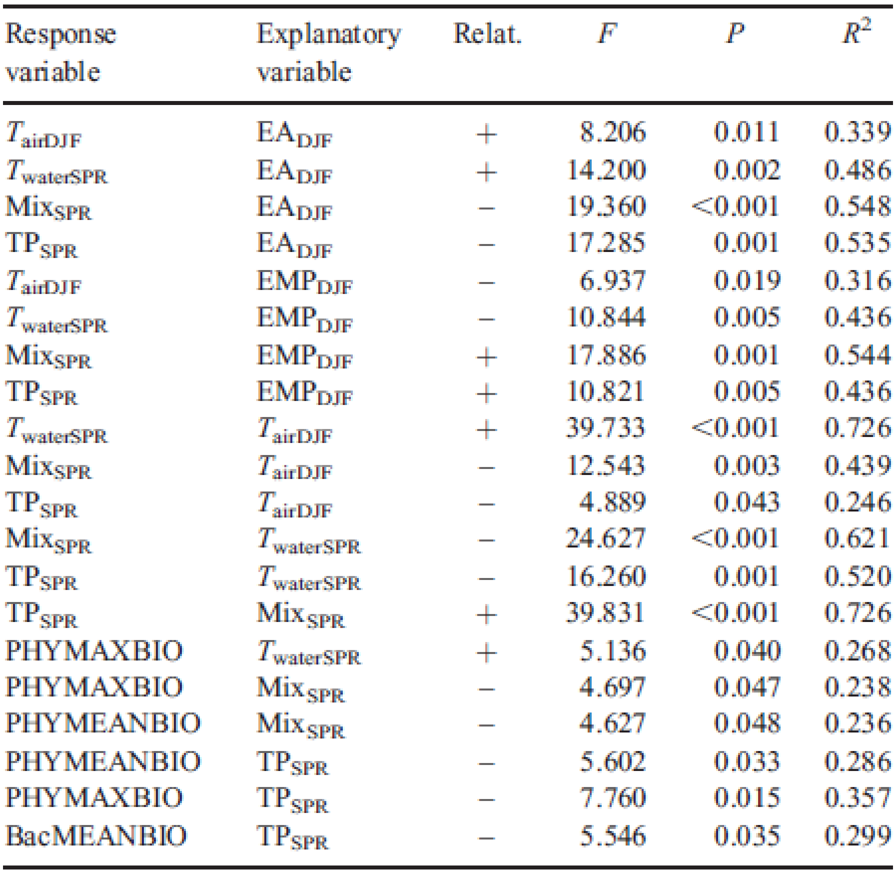
Overall, statistically significant results of the linear regression analysis are reported in Tables 1, 2 and in Fig. 2, 3. The linear regression analyses highlighted that the two teleconnection indices significantly affected the winter air temperatures, with a significant positive effect for EA_{DJF} (Fig. 2a) and negative effect for EMP_{DJF} on the annual $Tair_{DJF}$ values. Furthermore, EA_{DJF} and EMP_{DJF} had a similar effect on the late winter–early–spring water temperature between 0 and 50 m, that showed a significant and positive relationship with $Tair_{DJF}$ (Table 1, Fig. 2b).

The two teleconnection indices showed a strong control on the convective circulation depth of Lake Iseo, evidenced by the linear regressions linking Mix_{SPR} with EA_{DJF}, and EMP_{DJF}. Mixing depth was also negatively related to $Twater_{SPR}$ (Fig. 2c) and $Tair_{DJF}$. Similarly, EA_{DJF} and EMP_{DJF} had strong effects on the epilimnetic concentrations of TP_{SPR} measured at spring overturn and TP_{SPR} was also related to Mix_{SPR}, $Twater_{SPR}$ (Fig. 2d) and $Tair_{DJF}$ (Table 1).

The mean and the maximum biovolume of phytoplankton did not show a significant relationship with EA_{DJF} and EMP_{DJF}; however, significant and negative links with total phosphorus in the epilimnetic layers during the water overturn (Fig. 2*e*) and with mixing depth were observed (Table 1). However, the phytoplankton maximum biovolume showed a positive relation with $Twater_{SPR}$ (Table 1). Moreover, we verified significant but negative relationships between TP_{SPR} enrichment and Bacillariophyceae mean biovolume in spring (Table 1).

Focusing on primary consumers (Table 2), the day of Daphnia increase after winter diapause showed a significant and positive relationship with EMP_{DJF}, but a significant and negative relationship with EADJF (Fig. 3a), $Twater_{SPR}$, the mean and the maximum biovolume of phytoplankton (Fig. 2f). The maximum density of Daphnia had a positive significant link only with total phosphorus in spring. Eubosmina increase day showed a significant relationship with EMP_{DJF} (Fig. 3b) and $Twater_{SPR}$. Eubosmina maximum density values had links with $Twater_{SPR}$ and $Tair_{DJF}$ (Table 2), whereas the day in which Eubosmina reached the maximum population density showed a significant relationship with EA_{DJF} (Fig. 3c), EMP_{DJF}, $Twater_{SPR}$ and $Tair_{DJF}$ (Table 2).

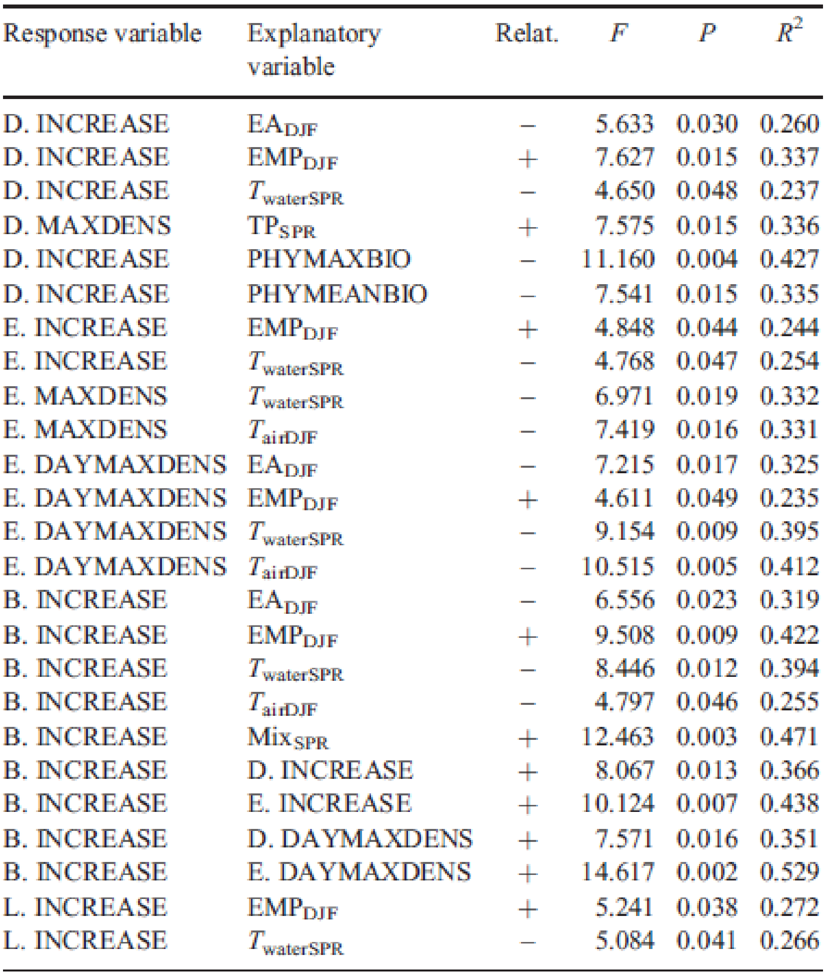
Table 1- Results of linear regression analyses among winter values of teleconnection indices (East Atlantic Pattern (EA_{DJF}) and Eastern Mediterranean Pattern (EMP_{DJF})), winter air temperature ($Tair_{DJF}$) and chemical–physical parameters ($Twater_{SPR}$, Mix_{SPR}, TP_{SPR}, PHYMEANBIO and PHYMAXBIO). $Twater_{SPR}$, minimum water temperature in spring in the layer from 0- to 50-m depth; Mix_{SPR}, spring water vertical-mixing depth; TP_{SPR}, maximum concentration of total phosphorus at spring water overturn in the layer from 0- to 50-m depth); PHYMEANBIO, mean biovolume from March to June; and PHYMAXBIO, maximum biovolume from March to June. Relat., positive (+) or negative (-) relationship



Response variable	Explanatory variable	Relat.	*F*	*P*	R^2
T_{airDJF}	EA_{DJF}	+	8.206	0.011	0.339
$T_{waterSPR}$	EA_{DJF}	+	14.200	0.002	0.486
Mix_{SPR}	EA_{DJF}	−	19.360	<0.001	0.548
TP_{SPR}	EA_{DJF}	−	17.285	0.001	0.535
T_{airDJF}	EMP_{DJF}	−	6.937	0.019	0.316
$T_{waterSPR}$	EMP_{DJF}	−	10.844	0.005	0.436
Mix_{SPR}	EMP_{DJF}	+	17.886	0.001	0.544
TP_{SPR}	EMP_{DJF}	+	10.821	0.005	0.436
$T_{waterSPR}$	T_{airDJF}	+	39.733	<0.001	0.726
Mix_{SPR}	T_{airDJF}	−	12.543	0.003	0.439
TP_{SPR}	T_{airDJF}	−	4.889	0.043	0.246
Mix_{SPR}	$T_{waterSPR}$	−	24.627	<0.001	0.621
TP_{SPR}	$T_{waterSPR}$	−	16.260	0.001	0.520
TP_{SPR}	Mix_{SPR}	+	39.831	<0.001	0.726
PHYMAXBIO	$T_{waterSPR}$	+	5.136	0.040	0.268
PHYMAXBIO	Mix_{SPR}	−	4.697	0.047	0.238
PHYMEANBIO	Mix_{SPR}	−	4.627	0.048	0.236
PHYMEANBIO	TP_{SPR}	−	5.602	0.033	0.286
PHYMAXBIO	TP_{SPR}	−	7.760	0.015	0.357
BacMEANBIO	TP_{SPR}	−	5.546	0.035	0.299

.

Table 2- Result of linear regression analyses among winter values of East Atlantic Pattern (EA_{DJF}) and Eastern Mediterranean Pattern (EMP_{DJF}), air temperature, water temperature, vertical-mixing depth, total phosphorus (TP_{SPR}), and PHYMEANBIO and PHYMAXBIO (see Table 1 for explanation of abbreviations) as explanatory variables and phenology of zooplankton, as response variables (mean and maximum taxon density values from March to June, MEANDENS and MAXDENS respectively). D., *Daphnia*, E., *Eubosmina*, B., *Bythotrephes* and L., *Leptodora*. Relat., positive (+) or negative (-) relationship.



Response variable	Explanatory variable	Relat.	F	P	R^2
D. INCREASE	EA_{DJF}	−	5.633	0.030	0.260
D. INCREASE	EMP_{DJF}	+	7.627	0.015	0.337
D. INCREASE	$T_{waterSPR}$	−	4.650	0.048	0.237
D. MAXDENS	TP_{SPR}	+	7.575	0.015	0.336
D. INCREASE	PHYMAXBIO	−	11.160	0.004	0.427
D. INCREASE	PHYMEANBIO	−	7.541	0.015	0.335
E. INCREASE	EMP_{DJF}	+	4.848	0.044	0.244
E. INCREASE	$T_{waterSPR}$	−	4.768	0.047	0.254
E. MAXDENS	$T_{waterSPR}$	−	6.971	0.019	0.332
E. MAXDENS	T_{airDJF}	−	7.419	0.016	0.331
E. DAYMAXDENS	EA_{DJF}	−	7.215	0.017	0.325
E. DAYMAXDENS	EMP_{DJF}	+	4.611	0.049	0.235
E. DAYMAXDENS	$T_{waterSPR}$	−	9.154	0.009	0.395
E. DAYMAXDENS	T_{airDJF}	−	10.515	0.005	0.412
B. INCREASE	EA_{DJF}	−	6.556	0.023	0.319
B. INCREASE	EMP_{DJF}	+	9.508	0.009	0.422
B. INCREASE	$T_{waterSPR}$	−	8.446	0.012	0.394
B. INCREASE	T_{airDJF}	−	4.797	0.046	0.255
B. INCREASE	Mix_{SPR}	+	12.463	0.003	0.471
B. INCREASE	D. INCREASE	+	8.067	0.013	0.366
B. INCREASE	E. INCREASE	+	10.124	0.007	0.438
B. INCREASE	D. DAYMAXDENS	+	7.571	0.016	0.351
B. INCREASE	E. DAYMAXDENS	+	14.617	0.002	0.529
L. INCREASE	EMP_{DJF}	+	5.241	0.038	0.272
L. INCREASE	$T_{waterSPR}$	−	5.084	0.041	0.266

As for secondary consumers, the day in which it could be detected, the increase of *Bythotrephes* after winter diapause was significantly related to EA_{DJF} (Fig. 3*d*), EMP_{DJF}, Mix_{SPR}, $Twater_{SPR}$ and $Tair_{DJF}$. Furthermore, this parameter showed significant links with increase days for *Daphnia* (Fig. 2*g*) and *Eubosmina* and with the timing of maximum density of *Daphnia* and *Eubosmina.* Finally, *Leptodora* increase days showed a significant link with EMP_{DJF} (Fig. 3*e*) and $Twater_{SPR}$ (Table 2).

Discussion

Climate is considered one of the major drivers of change in population phenology, directly and indirectly influencing seasonal cycles in marine and freshwater organisms (Straile 2002; Winder and Schindler 2004).A better understanding of the role of climate change in altering zooplankton dynamics, structure and function is of high scientific and economic value (Vadadi-Fulop and Hufnagel 2014). In the present study, we focused on population- and community-level responses of zooplankton Cladocera to teleconnection indices (EA and EMP) in the first part of the year (i.e. February–June), when in warm-monomictic lakes the limnological features and planktonic communities are strongly affected by large-scale climatic winter fluctuations, as demonstrated in many studies (e.g. Salmaso *et al.* 2014; Manca *et al.* 2015). The winter large-scale circulation patterns control a long chain of linked causal factors, affecting the winter air temperature, spring water temperature, the resulting depth of water vertical mixing and epilimnetic concentration of total phosphorus in late winter–early spring. In summer and fall, instead, the internal lake food-web dynamics prevail to control the nutrient distribution in the water column and phytoplankton growth.

We demonstrated the influence of two teleconnection indices (winter values of EA and EMP) on two interdependent trophic levels of the pelagic food web, primary and secondary zooplanktonic consumers (Fig. 2, 3). Furthermore, our results supported our hypothesis that the food web in lakes characterized by different trophic levels responded differently to climate variation. Large-scale climatic indices can have a higher explanatory power than do local weather variables both in space and in time (Hallett *et al.* 2004) and they can improve our understanding of which weather features may be more important in the study area (e.g. air temperature).

Our results showed a link between $\mathrm{EMP}_{\mathrm{DJF}}$ (or $\mathrm{EA}_{\mathrm{DJF}}$) and zooplanktonic primary consumers increasing after winter diapause (for both *Daphnia* and *Eubosmina*), and the timing of maximum population density (for *Eubosmina*) in the spring period. In response to positive (negative) phase of the EA (EMP), after weakening winter stress, the spring breakup dates of primary consumers were shifted earlier, in some cases by more than 30 days (Fig. 3). Similarly, secondary consumers showed an earlier increase as a result of milder winters (Fig. 3).

Climate warming-related change in habitat structure, e.g. a longer duration of water stratification period, can induce considerable cascading effects in pelagic food webs (see Vadadi-Fulop and Hufnagel 2014, and references therein). In Lake Iseo, for all the considered Cladocera taxa, their increase after winter diapause could be related to spring water temperature resulting from winter air temperature, as reported in Manca et al. (2015) for Daphnia in Lakes Garda and Maggiore.

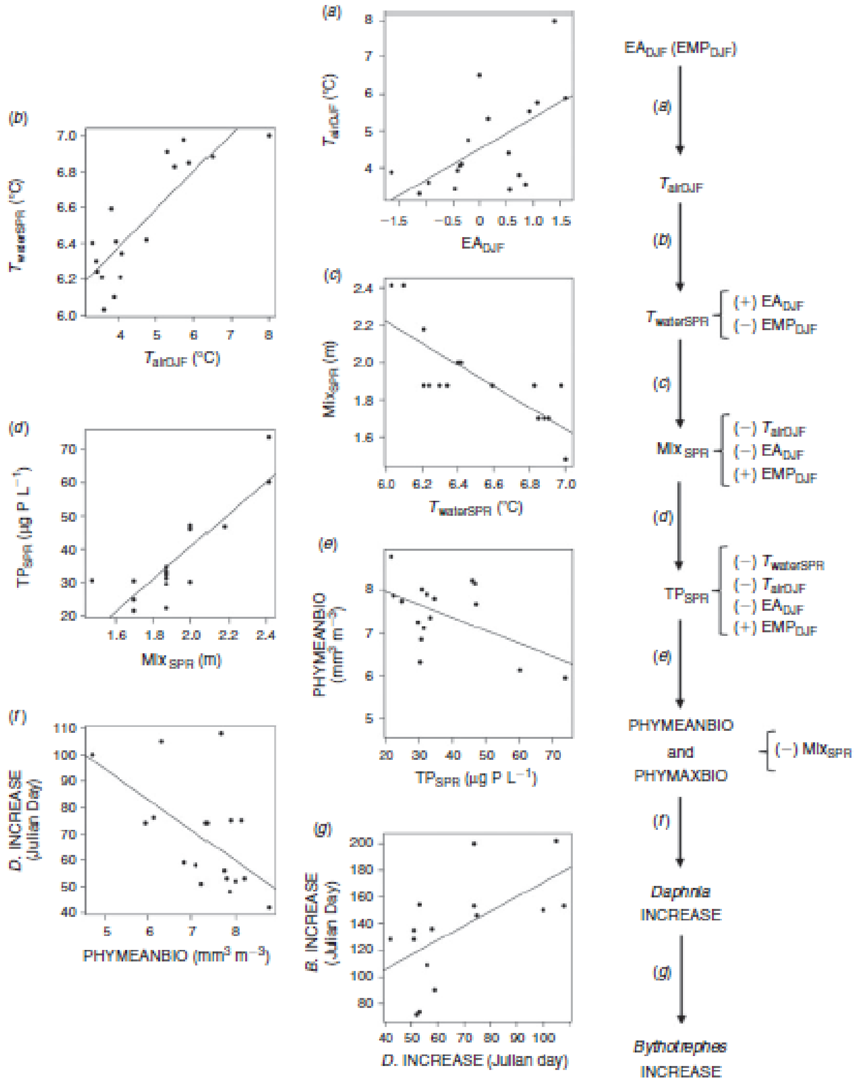


Figure 2- Cascading effects of East Atlantic Pattern (EA_{DJF}) and Eastern Mediterranean Pattern (EMP_{DJF}) on limnological characteristics of Lake Iseo and planktonic food web. All relationships are statistically significant. In the curly brackets are shown the other significant relationships pointed out by the linear regression analyses. *P*-values are reported in Tab. 1 and 2.

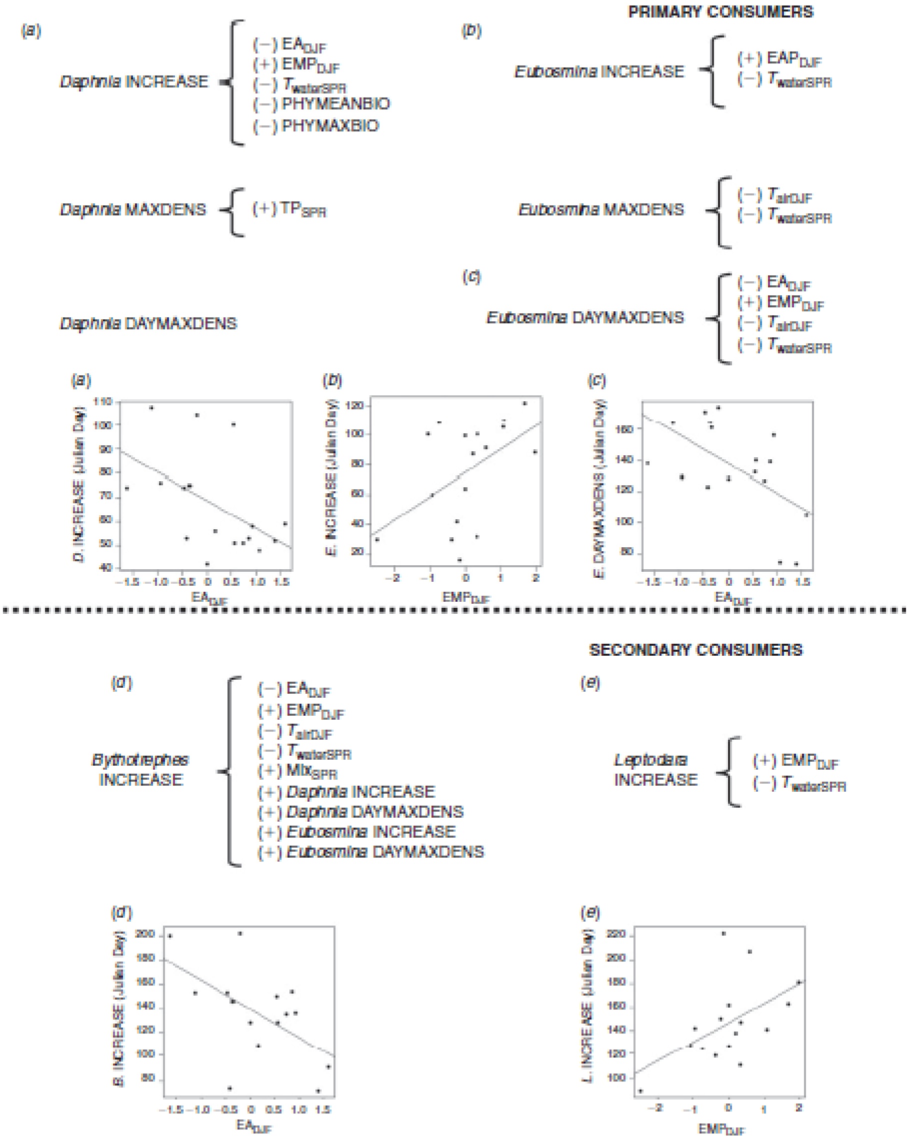


Figure 3- Significant relationships shown by the linear regression analysis among zooplanktonic primary consumers, zooplanktonic secondary consumers, limnological features, winter air temperature and teleconnection indices. *P*-values are reported in Table 2. Charts show significant relationships between zooplanktonic taxa and teleconnection indices.

Thermal stratification and related depth of vertical convective circulation strongly affected nutrient availability for phytoplankton in the euphotic zone and food amount for zooplankton (Salmaso *et al.* 2014). Holo-oligotrophic deep lakes, usually, experience a strong increase of nutrients and phytoplankton during the full mixing periods (Straile 2000, 2002) and that could positively affect the primary consumer abundances of zooplanktonic taxa (Manca *et al.* 2015).

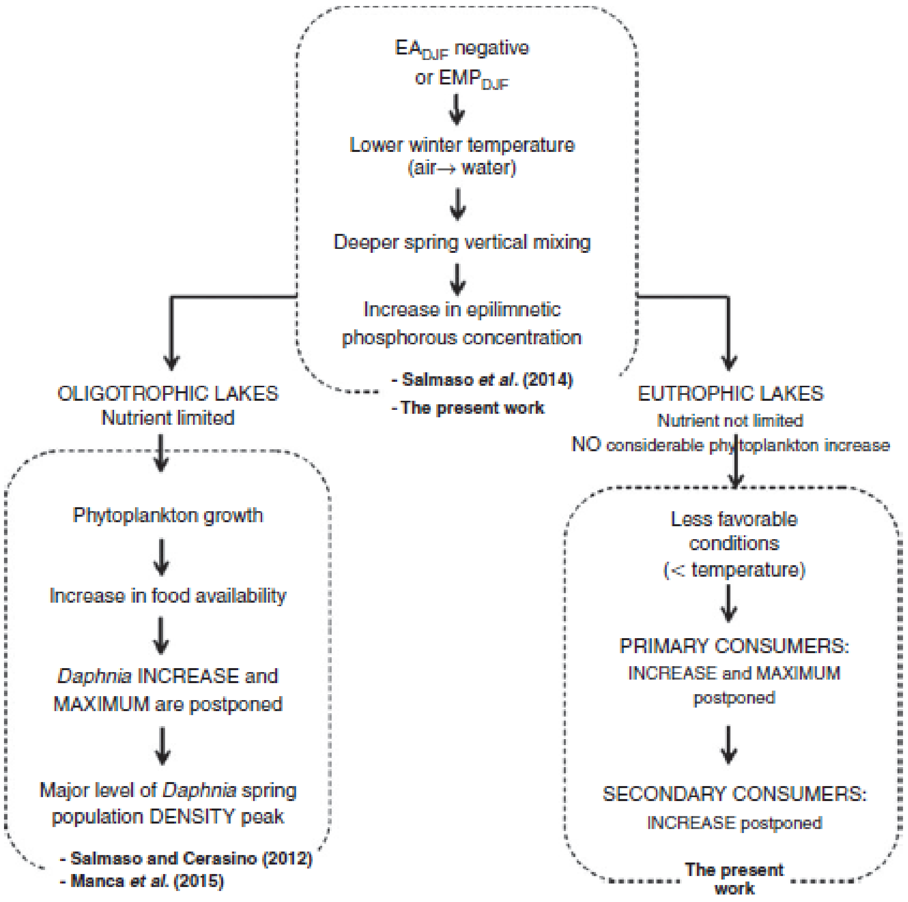


Figure 4- Diagram summarising the cascading effect on the phenology of zooplanktonic Cladocera by the interannual fluctuation of the East Atlantic Pattern (EA$_{DFJ}$) and Eastern Mediterranean Pattern and (EMP$_{DJF}$). The diagram is an extension of that reported by Salmaso *et al.* (2014) on the limnological characteristics of the largest lake south of the Alps and in Manca *et al.* (2015).

We hypothesised that in eutrophic lakes, such as Lake Iseo, the cascading effects of inter-annual climatic variability on trophic web could be different and the epilimnetic enrichment of phosphorus, after deep mixing events, could be a less important driver of the planktonic food web than in oligotrophic systems, as total phosphorus concentration is always consistent and allows a great development of phytoplankton (Leoni *et al.* 2014*b*). In fact, during the deeper mixing events of Lake Iseo, the enrichment of total phosphorus did not promote a significant increase of the primary productivity; by contrast, the low temperature and high turbulence seemed to limit phytoplankton development, as showed by significant and negative relationship among mixing depth and the mean and maximum biovolume of phytoplankton reached in spring (Table 1).

Our results highlighted low and not significant relationships among climatic indices and Cladocera taxon value. The maximum density of *Daphnia* showed a positive and significant relationship only with total phosphorus. In fact, after deeper mixing circulation, the increase in phosphorus concentration can affect the phytoplankton quality, even if not the quantity, and promote the development of particular zooplanktonic species (e.g. *Daphnia*; Leoni *et al.* 2014*b*).

According to our results, we can visualise the cascading effects of climate variability on the pelagic food web, interpreted through meteoclimatic forcing and planktonic spring population phenology, reported in Salmaso *et al.* (2014) and Manca *et al.* (2015). In the proposed diagram (Fig. 2), the cascading effects were expanded, to the best of our knowledge, across secondary consumers and were compared among deep southern-Alpine lakes with a low and high trophic level.

Manca *et al.* (2015) demonstrated that the level of density peaks reached by *Daphnia* in spring are indirectly mediated by winter water temperature, resulting in the degree of water mixing and influencing the algal carrying capacity (Fig. 4, left-hand side).

In eutrophic lakes, the time in which primary and secondary consumers start their increase in spring and the time in which primary consumers reach maximum density were directlymediated by winter water temperature, relating to harsh winters. Low winter air and water temperature caused deepest vertical mixing of water and, in turn, the early spring increase of phosphorus into the upper water layers. However, in eutrophic lakes, not limited by phosphorus, the early spring enrichment in nutrients resulted in neither a considerable phytoplankton growth nor, consequently, in a peak of zooplankton density. Nevertheless, the higher concentration of phosphorus could affect the algal food quality (i.e. presence of particular phytoplankton taxa, as diatoms and green algae) and, thus, the positive response of *Daphnia*, which has fairly high phosphorus requirements among the planktonic cladoceran taxa and is apparently more sensitive (Sterner 2008; Leoni *et al.* 2014*b*).

The cascading effect owing to climatic fluctuations on lake dynamics did not seem to involve the studied phenological responses of *Eubosmina* (primary consumer) because it did not show any significant relationship with phytoplankton biovolume changes. However, important factors according to our results were meteo-climatic indices, controlling water temperature, and the effect on timing of onset and of maximum *Eubosmina* population density growth in spring. Such a relationship was also found for initial population growth of *Leptodora* (predatory zooplankter) and EMPDJF; in accordance with its autoecology, it started developing with upper-middle water temperature (Branstrator 2005).

In conclusion, the present study showed for the first time, to the best of our knowledge, how climatic fluctuations, well represented in the Mediterranean area by the East Atlantic pattern and Eastern Mediterranean Patterns, can have a different effect on deep south alpine lakes with different trophic levels (Fig. 4). In oligotrophic lakes, the climate variability induced a cascading effect, from chemical–physical features until primary and secondary production, alike to the eutrophication impacts on lentic ecosystems. Whereas in eutrophic lakes, the cascading effect of climate fluctuation did not result in a substantial increase in phytoplankton biovolume, as nutrients were always in high concentrations.
Regardless of lake trophic levels, climatic fluctuations, mediated by water temperature in spring, affected the timing of recovery after winter diapause of primary consumers and, verified for the first time in a eutrophic lake, of secondary consumers.

Finally, this work affirmed how climate forcing and impact predictors of climate change on the functioning of lake ecosystems can be usefully represented by meteo-climatic indices. Their application showed important perspectives in the research of climate change and long-term climatic fluctuation on food web dynamics and on freshwater ecology.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Acknowledgements

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3 CLIMATE INDUCED CHANGE IN PRIMARY CONSUMERS POPULATION STRUCTURE DURING SUMMER

Climate has a great influence on zooplankton population dynamics; indeed, in the previous chapter has been verified and discussed how it triggers a cascading effect involving lake limnological characteristic and phyto- and zoo- plankton dynamics. Climate change can have great repercussions for lakes ecosystems, because temperature influences water column stability, nutrient enrichment, and the degree of new production, and thus the abundance, size composition, diversity, and trophic efficiency of zooplankton (Richardson, 2008; Manca, Rogora & Salmaso, 2015; Leoni, Nava & Patelli, 2018). Change in the structure of the zooplanktonic population composition and in species abundance can have repercussions on the entire functioning of lake pelagic food webs. For example, it has been observed that a shift in the timing of daphnid development may result in a temporal mismatch between the maximum demand of fish larvae and the maximum of daphnid biomass with negative effects on year-class strength of fish (Straile & Geller, 1998). Moreover a shift in zooplankton primary consumers development in spring can bring to modification in the intensity of the cleat water phase event (Schindler, 2009)

Daphnia spp. are considered to be particularly useful for answering questions regarding the effects of climatic on plankton communities and on lake pelagic food webs (Wojtal-Frankiewicz & Frankiewicz, 2011). Actually, they had a major role as grazer, feeding on algae, and as food resources for predators (zooplankton secondary consumers and fish), contributing to transfer energy from the primary producers to consumers at higher trophic levels and reflecting the influence of both bottom-up and top-down processes (Lampert, 2006). Winder and Schindler (2004) observed that climate-induced change in lakes ecosystems can lead to long-term decline in *Daphnia* population having a critical impact on predator-prey interactions and possible severe consequences for the upper trophic levels.

In many lakes, populations of *Daphnia* follow regular seasonal patterns, where density starts to increase during late winter, reaches a maximum density peak during spring, decreases during summer, and finally rises again at the end of summer (Benndorf *et al.*, 2001; Wojtal-Frankiewicz & Frankiewicz, 2011). However, the summer decline of *Daphnia* population abundance, commonly referred in literature as midsummer decline (MSD), seems to have high inter-annual variability, resulting particularly prolonged and pronounced in some year (Wojtal-Frankiewicz, 2012). Several studies have focused on the causes that control the midsummer decline of *Daphnia*, in particular in shallow and hypertrophic reservoirs (Mehner *et al.*, 1998; Hülsmann & Voigt, 2002; Hulsmann, 2003; Wojtal-Frankiewicz & Frankiewicz, 2011). These studies highlight several factors that can influence *Daphnia* summer phenology and the most important resulted spring and summer temperatures (Benndorf *et al.*, 2001). Temperature seems to influence *Daphnia* MSD through both direct and indirect effects, controlling food availability during the clear water phase and predation rate by young fish of the years (Mehner *et al.*, 1998; Wagner & Benndorf, 2007).

At the best of our knowledge, at the moment, no studies about the midsummer decline in deep subalpine lakes have been conducted, and the influence of the cascading effects triggered by winter climate variability on plankton phenology has never been tested until the summer.

This part of this PhD work has been developed in order to disentangle the factors that may lead to *Daphnia* midsummer decline in deep oligomictic lakes. In particular, has been performed a statistical investigation of the main factors that may affect *Daphnia* summer abundance on the long-term limnological dataset of two subalpine lakes (Lake Iseo and Lake Lugano). These lakes were chosen because they seemed particularly suitable for this kind of study, in fact, they are characterized by an enhanced water stratification period in the last decades, with phosphorus accumulation in the hypolimnion and similar current trophic status, but in the past, they had different trophic evolution.

In particular, in order to realize the present study I have contributed in Lake Iseo zooplankton classification and count, in long-term data analysis, in results elaboration and interpretation and in paper writing and editing. The results have been collected, presented and discussed in the paper "Patelli M., Leoni B., Lepori

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Causes of *Daphnia* midsummer decline in two deep meromictic subalpine lakes.

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Summary:

1 *Daphnia* are a key organisms in pelagic food webs, acting as a food resource for fish and predatory zooplankton and regulating phytoplankton through grazing. Its population dynamics follows regular seasonal patterns, with spring peaks followed by summer population declines (midsummer declines, MSDs). MSDs show high inter-annual variation, which has been attributed to different causes. However, the mechanisms controlling the MSD remain poorly understood, especially in deep stratified lakes.

2 We tried to disentangle the factors causing *Daphnia* MDSs in Lake Lugano and Lake Iseo (in Switzerland and Italy), two deep peri-alpine lakes with similar trophic status and vertical mixing dynamics, characterized by phosphorus accumulation in the hypolimnion and variable mixing during late-winter turnovers.

3 Specifically, we assessed the effects of three different hypothetical pathways according to which: (I) winter air temperature controls MSDs by influencing mixing depth during turnovers and epilimnetic phosphorus replenishment; (II) vernal air temperature influences MSD by accelerating the timing of spring population peak; (III) summer temperature influences MSDs by increasing fish predation. We assessed the relative strength of these pathways using Structural

Equation Modelling on long-term datasets for the two lakes (29 years for Lake Lugano and 19 years for Lake Iseo).

4 Between the hypothesized pathways, the one driven by winter air temperature (through P replenishment) influenced *Daphnia* abundance in spring in both lakes, but the effects propagated to summer *Daphnia* abundance only in Lake Lugano. Additionally, summer *Daphnia* abundance was influenced by the summer air temperature through a positive (although weak) effect. By comparison, vernal air temperature had no detectable effects on summer *Daphnia* abundance.

5 The results revealed marked differences between the meromictic study lakes and the shallow hypertrophic water bodies that were the focus of previous research on *Daphnia* MSD, and also between the two study lakes. We suggested that the influence of epilimnetic P replenishment on the summer *Daphnia* abundance in Lake Lugano, which was recovering from past eutrophication, reflected the greater susceptibility of deep, stratified lakes to P depletion after spring compared to shallow hypertrophic lakes or reservoirs. This effect might not have been detected in Lake Iseo because P was more consistently depleted during the study period (i.e. variance in the predictor was too low to detect an effect).

6 Moreover, this study highlighted the complexity of the effects of climate variability on *Daphnia* MSD in deep lakes, showing that the responses can differ even between two neighboring lakes with similar vertical mixing dynamics and trophic status.

Keywords: temperature, clear water-phase, phytoplankton, epilimnetic phosphorus, food web.

Introduction

Herbivore zooplankton are of fundamental importance in lake food webs because they represent the major link in the energy flow between primary producers and secondary consumers (Persson *et al.*, 2007). Among the various species occurring in lakes, cladoceran *Daphnia* spp. are an important food resource for small fish and zooplankton predators and, at the same time, their grazing activity can regulate phytoplankton biomass (Lampert *et al.*, 1986; McQueen *et al.*, 1989).

In many lakes, populations of *Daphnia* follow regular seasonal patterns, where density starts to increase during late winter, reaches a maximum peak during spring, decreases during summer, and finally rises again at the end of summer (Benndorf *et al.*, 2001). In the scientific literature, lowered population densities of *Daphnia* in summer are commonly referred to as midsummer declines (MSD) (Hulsmann, 2003; Wojtal-Frankiewicz, 2012). Moreover, the *Daphnia* spring peak is followed by a phase characterized by low phytoplankton biomass and high water transparency, commonly named the clear-water phase (Talling, 2003). The clear-water phase might arise from intense grazing activity by zooplankton (Vadadi-Fülöp & Hufnagel, 2014) and nutrient (i.e., phosphorous) depletion, although physical settling of phytoplankton is also involved (Berger *et al.*, 2007). This phase acts as a food resource bottleneck to *Daphnia* and is a major cause of the population decline that occurs between spring and summer.

During the last decades, several studies have focused their attention on *Daphnia*'s MSD, especially in shallow and eutrophic lakes and reservoirs (Vijverberg *et al.*, 1990; Benndorf *et al.*, 2001; Hülsmann & Voigt, 2002; Hulsmann, 2003; Wagner & Benndorf, 2007). In these water bodies, the decreased density of *Daphnia* during summer has been attributed to several potential causes, which include fish predation (Vijverberg *et al.*, 1990), low food availability (Mehner *et al.*, 1998) and increasing seasonal temperature (Benndorf *et al.*, 2001; Wagner & Benndorf, 2007). This last hypothesis is supported by evidence which indicates that high temperature in early spring can result in an earlier maximum peak of *Daphnia*, an earlier and more extended clear-water phase and a stronger MSD due to prolonged food depletion during summer (Benndorf *et al.*, 2001). In addition, in these lakes, summer temperature can further contribute to *Daphnia's* MSD by stimulating the growth of young fishes and their predation on *Daphnia* (Wagner & Benndorf, 2007).

In deep temperate lakes (i.e. deeper than 100 m), the effects of temperature on *Daphnia's* phenology are probably more complex, because they include not only effects on growth and predation, but also indirect effects mediated by the influence of temperature on the vertical mixing depth during water turnovers. Deep lakes are often oligomictic or meromictic, that is, they are nearly-always or always stratified (Wetzel, 2001). Moreover, the turnover depth is variable and depends on meteorological conditions during the weeks or months preceding the turnover, with cold and windy weather usually leading to deeper mixing. Under these conditions, phosphorus accumulates in the monimolimnion (the deep layer that does not mix with the surface layers). During turnovers, part of this phosphorus is transferred to the epilimnion, replenishing the reserve depleted by settling during the stratification period. A key feature of these lakes is that the replenishment of phosphorus depends on the depth of mixing. Deeper vertical mixing tend to favor vertical transfer and the epilimnetic replenishment of phosphorus, whereas shallow mixing has the opposite effect. In turn, the degree of phosphorus replenishment during turnovers can have far reaching consequences for the succession of plankton during the following stratification period.

These complex interrelations between weather, maximum depth of mixing, phosphorus replenishment and plankton succession are well documented for oligomictic and meromictic lakes located at the edge of the Alps (Straile, 2000; Salmaso et al., 2013; Manca et al., 2015; Leoni et al., 2018). In short, in these lakes cold and windy winters lead to greater vertical mixing during late-winter turnovers and greater replenishment of phosphorus in the epilimnion (Straile, 2000; Salmaso *et al.*, 2013; Leoni, Garibaldi & Gulati, 2014; Lepori *et al.*, 2018a). The replenishment of epilimnetic phosphorus is thought to be important to the development of *Daphnia* population in spring (Leoni *et al.*, 2014; Manca, Rogora & Salmaso, 2015) because a greater replenishment is expected to increase food (phytoplankton) production (Manca *et al*., 2015) and/or improve phytoplankton nutritional quality (Anneville, Gammeter & Straile, 2005; Salmaso *et al.*, 2013).

Given the positive relationship between the epilimnetic phosphorus replenishment and the quantity and/or quality of food available to *Daphnia* in spring, we hypothesize that greater phosphorus replenishment due to deeper turnovers may help attenuate *Daphnia*'s MDS by alleviating food bottlenecks during the clear-water phase. This effect would determine a pathway between winter temperature (which controls phosphorus replenishment) and MSD, which should be added to the pathways between early-spring temperature and MSD already identified for shallow lakes (Benndorf *et al.*, 2001). Although the effect of winter temperature on summer biomass of large zooplankton grazer (including *Daphnia*) is consistent with recent empirical observations (Lepori, Roberts & Schmidt, 2018b), to our knowledge the influence of winter air temperature on *Daphnia's* MSD through effects on vertical mixing and phosphorus availability in deep meromictic lakes, has never been investigated. In addition, the causes of *Daphnia*'s MSD appear to be less studied in deep lakes than in shallow lakes and reservoirs.

The aim of this study was to try to disentangle the factors that lead to *Daphnia* midsummer decline in two deep peri-alpine lakes, Lake Lugano and Lake Iseo (located in Switzerland and Italy, respectively). In particular, we attempted to clarify which main factors affect *Daphnia* summer abundance in meromictic lakes, with phosphorus accumulation in hypolimnion, and similar current trophic status and vertical mixing dynamics. In this work, we assessed the effects of three different hypothetical pathways that may control *Daphnia* MSD. The first pathway represents the influence of winter air temperature on deep lake ecosystems. Specifically, we expected that warmer winters led to lower *Daphnia* abundance in summer by reducing vertical mixing, epilimnetic phosphorus replenishment and food availability (or quality) for *Daphnia* (P1). The second hypothesized pathway, drawn from previous studies on shallow lakes, represents the effect of higher spring air temperature, which causes a higher *Daphnia* peak in spring, an earlier and more severe exhaustion of food resource during spring and thus a greater midsummer decline (lower *Daphnia* density in summer) (P2). Finally, the third hypothesized pathway, also derived from literature on shallow lakes, represents the effect of summer temperature, which is expected to have a negative effect on *Daphnia* density in summer due to increased predation by young fish (P3).

Methods

Study sites

Lake Lugano (45°59’0’’N, 8°58’0’’E, 271 m a.s.l.) and Lake Iseo (45°43’11’’ N; 10°03’46’’E, 186 m a.s.l; Site LTER_EU_IT_102 – “Southern Alpine Lakes”; www.lter-europe.net) are deep natural lakes (maximum depth: 288 m and 251 m; surface area: 49 km2 and 61.8 km2 respectively) located south of the Alps, in southern Switzerland and northern Italy (Fig. 1). Lake Lugano is separated into two basins by a causeway built on a morainic front. Lake Lugano has been monitored since 1978, while Lake Iseo since 1993 through monthly analysis of chemical, physical and biological parameters (Table 1). For Lake Lugano, we used data collected from 1989 to 2017 at one station located in the

Northern basin, which is the deepest of the lake (Zmax=288 m) and has a theoretical renewal time of 12 years (Salmaso *et al.*, 2013). Data for Lake Iseo cover instead a 17-year period (from 1998 to 2016, no data were available for 2012) and they were collected at a sampling station located near the deepest point of the lake (Zmax=251 m).

Based on their geographical location, these two lakes would be expected to be warm monomictic, i.e. characterized by complete circulation once a year in late winter and stable stratification from spring onward (Wetzel, 2001). Nevertheless, due to their depth and eutrophic status they are holo-oligomictic, i.e., always nearly-always stratified, with occasional and irregular complete mixing events occurring only after exceptionally cold and windy winters. In both lakes, the last two complete mixing events occurred in 2005 and 2006 (Rogora *et al.*, 2018).

Between the early 1970s and 1980s all the south Alpine lakes were affected by a phase of severe eutrophication. The Northern basin of Lake Lugano and Lake Iseo are currently meso-eutrophic, but in the recent years they followed different trophic trajectories. In Lake Lugano, management schemes aiming to reduce phosphorus loads have been implemented since the late 1970s (Lepori *et al.*, 2018a). As a result, phosphorus pollution has considerably decreased; for example, the annual average concentration of TP across the epilimnion (0-20 m) decreased from 23 µgPL-1 to 15 µgPL-1 between the periods 1989-1995 and 2011-2017 (unpublished data). By contrast in Lake Iseo, during the last twenty years, TP epilimnetic concentration (0-20m) increased from 13 µgPL-1 to 17 µgPL-1 between the periods 1993-2000 and 2010-2015 (unpublished data).

Daphnia spp. represent the dominant grazer in both lakes, although other cladocerans (e.g. *Bosmina* spp.) and calanoid copepods are also present (Leoni, 2017; Lepori *et al.*, 2018b). In Lake Lugano, the pelagic fish fauna includes the planktivores whitefish (*Coregonus* sp.) and Italian shad (*Alosa fallax lacustris*) although annual yields and densities are low (EAWAG, 2014). The bleak (*Alburnus* sp.), which used to be abundant, became functionally extinct in the 1990s (Eawag, 2014). In Lake Iseo, the fish assemblage was dominated by the pelagic species Italian shad (*Alosa fallax lacustris*) and whitefish (*Coregonus lavaretus*) (Leoni *et al.*, 2014).

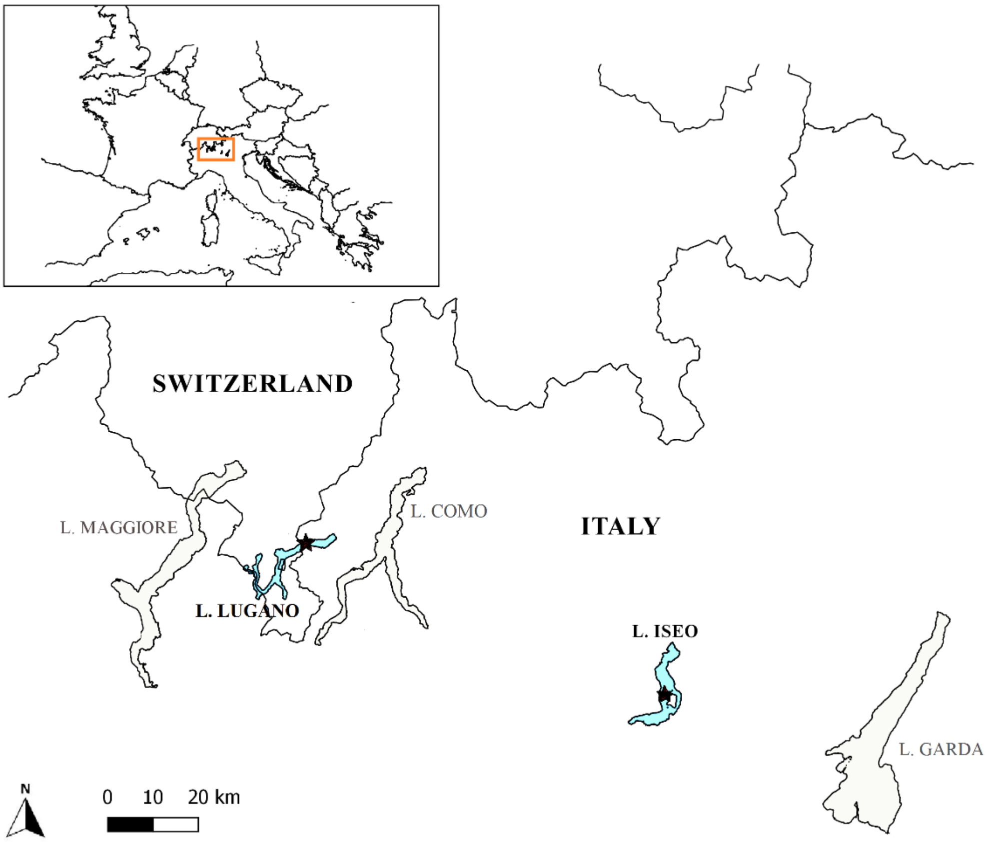


Figure 1- Geographical location of the study lakes. The stars indicate the location of the sampling stations. Bathymetric map of the lakes are reported in Lepori *et al.* (2018b) and by Valerio *et al.* (2015).

Structural equation model analysis

We used Structural Equation Model (SEM) analysis (Grace *et al.*, 2010; Grace, Scheiner & Schoolmaster, 2015) to assess the importance of the pathways causing *Daphnia* MDSs in our study lakes. We considered SEM particularly helpful because it allowed us to test effects of a complex set of hypotheses concerning factors that are interrelated and have a hierarchical structure.

Modelling began by reviewing literature on lake ecosystems and *Daphnia* summer phenology to develop a preliminary model that summarizes the major causal pathways causing *Daphnia* MDS. We developed a preliminary model for both study lakes (Fig. 2). Second, we compiled the data available and parameterized the model variables. Third, we reduced the number of variables included in the preliminary model and developed a primary model. The simplification was necessary to keep a reasonable balance between the number of variables and the number of observations *(n*=28 for Lake Lugano, *n*= 19 for Lake Iseo; each year was treated as an observation in the model) and because data were not available for some of the variables included in the preliminary model. We applied SEM analysis to the primary model for the two study lakes in order to assess the strength of each causal path included in the model as well as the overall fit between the model and the measured data. Finally, based on the results of the primary model analysis, we developed two revised model, one for each lake. To develop the revised model, we removed weak pathways (based on path coefficient) and we added new ones, not considered in the primary model, that emerged as important during the analysis. What follows is a detailed explanation of these steps.

Preliminary model - The preliminary model is represented as a path diagram in Fig. 2. Essentially, this model represents the three hypothesized pathways outlined in the introduction. In this model we identified three exogenous variables, representing the air temperatures in winter ($T_{WI,}$), during the vernal period (T_{VE}) and in summer (T_{SU}). According to the first pathway in our model (P1), TWI influences the maximum depth of vertical mixing during late winter turnovers (MIXDEPTH), triggering cascading effects on the lake ecosystem. First, MIXDEPTH positively affects the concentration of phosphorus in surface layers after the winter turnover (TP_{TURN}), which reflects the TP availability to plankton. Second, TP_{TURN} positively affects *Daphnia* population abundance in spring (D.SPRING) by increasing food quantity and/or quality during the period of population growth preceding the spring peak. Third, higher TP_{TURN} can alleviate nutrient depletion during the clear-water phase, causing greater availability of phytoplankton (FOOD AVAILABILITY) and a less severe bottleneck for *Daphnia*. Fourth, in addition to nutrient limitation, grazing by *Daphnia* affects the abundance and composition of phytoplankton after the spring peak, indicating that FOOD AVAILABILITY results from the net effect of TPTURN AND *D*.SPRING. Finally, FOOD AVAILABILITY influences *Daphnia*'s population abundance in summer (*D.*SUMMER), with lower abundance and/or reduced edibility causing lower *D.*SUMMER.

According to the second pathway (P2), vernal air temperature has a positive effect on *D.*SPRING and as a consequence on its grazing rate on phytoplankton (FOOD AVAILABILITY). In turn, high grazing pressure in spring causes early food limitation and a decline in *D.S*UMMER.

According to the third pathway (P3), summer air temperature has a positive effect on the growth of young fish and their predation rate (FISH PREDATION), which results in reduced *D*.SUMMER.

Primary model - Because we lacked data on FISH PREDATION, in the primary model the third pathway was reduced to an effect of summer temperature on summer *Daphnia*'s abundance. In addition, we merged phytoplankton community composition (reflecting food quality and palatability) and phytoplankton biomass (representing food quantity) into a single variable (PHYTOPLANKTON COMPOSITION). This variable was obtained as the scores of the first axis of a non-metric multidimensional scaling applied on phytoplankton absolute biomass after *Daphnia* spring peak, when food resources are thought to be most limiting.

Model assessment - SEM analysis was performed using R (version 3.4.3) with the "Lavaan" package (version 0.5-23) and "SemPlot" (version 1.1). For the primary model, we estimated the global fit and the path coefficients (unstandardized and standardized) of every path included in the model. Model fit was assessed using the chi-squared test (χ2), which is a measure of the divergence between the fitted covariance matrix and the observed one (Hooper, Coughlan & Mullen, 2008). Consequently, high and non-significant p-values indicate good model fit.

Standardized path coefficients (β) indicate the strength of the causal effect of one variable on another and vary between -1 and +1 (Kline, 2015). To interpret their strength, we considered that a β= ±0.1 represents a weak effect, a β= ±0.3 represents a moderate effect and a β= ±0.5 or larger represents a strong effect (Cohen, 1977). We calculated the total effects as the sum of all direct and indirect effects between variables. Direct effects were measured using the path coefficients, whereas indirect effects were calculated as the product of the sequential path coefficients in a pathway (Cohen, 1988).

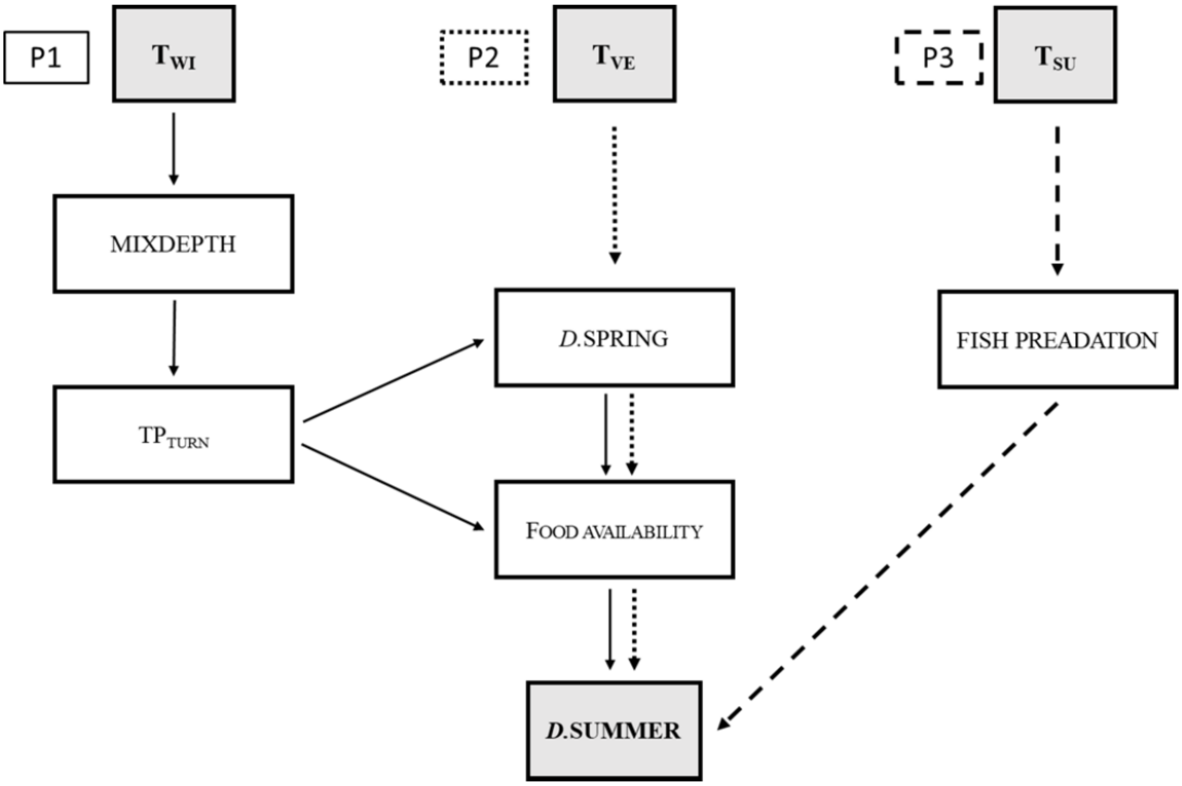


Figure 2- Preliminary model based on literature knowledge. We hypothesize that *Daphnia* abundance in summer (*D.*SUMMER) is regulated by three different pathways. The external variables of the model are winter air temperature (TWI), Spring air temperature (TVE) and summer air temperature (TSU). The first path (P1) is represented with continuous arrows, the second path (P2) is with dotted arrows and the third path (P3) with dashed arrows. MIXDEPTH represent the maximum turnover depth reached in late winter; TPTURN is the total phosphorus concentration at late-winter overturn; *D.*SPRING represented the *Daphnia* population abundance in spring.

Revised Model - The revision of the model started by eliminating the hypothesized links that were non-significant and had $\beta < 0.20$ (Hooper *et al.*, 2008). Next, we added links that were not included in the primary model but were signalled as potentially important when values of Modification Indexes (MI) were greater than 3 (Iacobucci, 2009). Modification indexes estimate the variation in $\chi 2$ that would occur if a fixed path coefficient would free to vary (Mitchell, 1992). A modification index is large if the model would fit better by adding a path that is not considered in the model (Iacobucci, 2009). We followed an iterative approach, where subsequent models were revised until all weak paths were removed and no additional ones were suggested (based on MI), all the while controlling that model fit remained satisfactory (based on the p-value of the $\chi 2$ test, see above).

Temporal trends- To understand the context of the changes in *Daphnia* MSD, we examined trends in several environmental characteristics of the lakes during the study period. In the analysis we included variables already considered in the SEM analysis (T_{WI}, T_{VE}, T_{SU}), TP_{TURN}, *D.*SPRING, *D.*SUMMER and an additional one, the maximum water transparency that occurred one month after *Daphnia* spring peak (i.e. in May if *Daphnia* peaked in April or in June if *Daphnia* peaked in May; Table 1). We considered that water transparency would add further information on potential food availability during the clear-water phase in addition to PHYTOPLANKTON COMPOSITION (Hülsmann & Voigt, 2002). However, we included water transparency only in temporal trend analysis and not as a predictor in SEM analysis to maintain an acceptable balance between the number of variables and the number of observations (e.g. ≥ 1:5; Kline 2015).

Data sources and model parameterization

The sources and the collection methods of the data used for the development of the SEM are summarised in Table 1.

Winter air temperature (T_{WI}) was calculated as the average temperature from December to February, vernal air temperature (T_{VE}) as the average temperature from February to May and summer temperature (T_{SU}) as the average temperature from June to August.

MIXDEPTH represents the maximum mixing depth during the late-winter turnover, before summer stratification. Following (Salmaso *et al.*, 2007) MIXDEPTH was estimated as the maximum depth at which an upper layer of uniform values (conductivity, pH, temperature and oxygens) met a lower layer of rapidly changing values.

TP_{TURN}, the average phosphorus concentration in the 0-20 m layer during the late-winter spring turnover, was calculated based on the concentration of total phosphorus within this layer during the date with the greatest vertical mixing depth.

We used the average value of *Daphnia* population abundance from April to May as an index of the maximum population density during the spring peak (*D.*SPRING) and the average value from July to August as an index of the minimum population density in summer (*D.*SUMMER). Population abundance (ind./m2) was calculated by multiplying the population density (ind./m3) by the thickness of the water layer from which zooplankton samples were collected in the two lakes (50 m in Lake Lugano and 20 m in Lake Iseo). We arbitrarily defined a MSD as an event when *Daphnia* population abundance in summer decreases below $50*10^3$ ind. /m2.

As mentioned above, the availability and quality of food available to *Daphnia* during late spring food bottleneck (clear-water phase) was parameterized as the first axis scores of a Non-metric

Multidimensional Scaling ordination (NMDS) based on phytoplankton biomass (PHYTOPLANKTON COMPOSITION). The NMDS scores reflect both the biomass and the composition of phytoplankton and were therefore considered to express food quantity and quality for *Daphnia*. To assist the interpretation of the NMDS axis-1 scores, these scores were correlated with the biomass of individual phytoplankton taxa using correlation analysis (Spearman rho). NMDS was performed on a Bray & Curtis' dissimilarity matrix (Legendre & Legendre, 1998), which was computed on the biomass of the phytoplankton genera. As indicator of fitness, a stress function that measures the fit between NMDS distance and actual dissimilarities was calculated. A stress value (STR) > 0.20 provides a representation not different from random, STR < 0.15 a good representation and

STR < 0.10 an ideal representation (Clarke, 1993). Rare genera, with biomass < 0.5% of the total biomass, were excluded from the analyses. The genera analysed represented 93.87% of the total biomass for Lake Lugano and 97.03% for Lake Iseo. Before NMDS computation, the data were square-root transformed. Finally, the NMDS solutions were rotated so that the variance of the scores was maximized along the first axes. In this study, NMDS scores on the first axis were considered as a proxy of phytoplankton community abundance and composition (PHYTOPLANKTON COMPOSITION), which in turn were thought to reflect food quantity and quality for *Daphnia*. To assist the interpretation of the NMDS axis-1 scores, these scores were correlated to the phytoplankton taxa biomass using a Spearman correlation matrix.

Table 1: Sources and collection methods of the data used to assess SEM analysis of Lake Lugano and Lake Iseo.

	DATA SOURCES		**METHODS**	
	Lake Lugano	**Lake Iseo**	**Lake Lugano**	**Lake Iseo**
MEASURED PARAMETERS				
Air tempearature	MeteoSwiss	Environmental protection agency (ARPA Lombardia).	Station of Lugano, daily mean value	Station of Costa Volpino, daily mean value
TP	University of Applied Sciences and Arts of Southern Switzerland (SUPSI, Lugano, Switzerland). www.cipais.org	Department of Earth and Environmental Sciences of the University Milano – Bicocca	Measured monthly across the water column	
Water Transparency			Measured monthly with a Secchi disk	
***Daphnia* density**			Sampled monthly or biweekly with a 100µm mesh. 0-50m	Sampled monthly with a 200µm mesh 0-20m
Phytoplankton density and biomass			Sampled monthly using Schröder bottle (0-20m). Samples analysed for density and biomass following (Simona, 2003)	
DERIVED PARAMETERS				
Mixing Depth	Lepori *et al.*, 2018	Leoni *et al.*, 2018	Depth at which conductivity (20°C) differed by >3µS/cm from surface value	Depth in which several physico-chemical parameters (pH, conductivity and oxygen) had homogeneous profiles (Salmaso et al., 2007).

Results

Long-term trends

Air temperatures in the two lakes followed similar trends in winter (T_{WI}) and early spring (T_{VE}), whereas T_{SU} in Lake Iseo was generally higher than in Lake Lugano (Fig. 3a, 3b and 3c). The minimum T_{WI} value was 2.70 and 3.41 °C for Lake Lugano and Iseo, respectively, while the maximum was 6.27 °C for Lake Lugano and 8.04 °C for Lake Iseo. T_{VE} ranged between 9.00 to 12.55 °C for Lake Lugano and from 9.90 to 15.76 °C for Lake Iseo. Finally, T_{SU} oscillated between 20.17 and 24.60 °C for Lake Lugano and between 21.52 and 27.09 °C for Lake Iseo.

MIXDEPTH (Fig. 3d) showed similar values and trends in the two lakes, with a deep mixing event (>100 m) in 2000 and complete mixing events occurring in 2005 and 2006. After 2006, MIXDEPTH never exceeded 100 m. During the study period, TP_{TURN} (Fig. 3e) showed fluctuations from 19 to 110 µg P L-1 in Lake Lugano and from 15 to 72 µg P L-1 in Lake Iseo. Before 2012, TP_{TURN} values were higher in Lake Lugano than in Lake Iseo. However, after a maximum peak reached in 2006, TP_{TURN} concentrations showed a decreasing trend in Lake Lugano. The year-to-year variation

in summer oscillation of total phosphorus epilimnetic concentration (TP_{SUMMER}; Fig. 3f) differed between the study lakes. Lake Lugano showed greater variability, with values ranging from 11 to 70 µg P L-1 (measured in 2006). In comparison, Lake Iseo's TP_{SUMMER} ranged from 14 to a maximum of 32 µg P L-1 (measured in 2006).

During the study period, water transparency at the beginning of the summer (Fig. 3g) in Lake Lugano and Iseo ranged between 2 and 11 m. In general, Lake Lugano had greater water transparency than Lake Iseo.

*D.*SPRING (Fig. 3h) showed high year-to-year variability during the studied period, ranging from 24.3 to 679.0 · 103 ind./m2 in Lake Lugano and from 45.4 to 43.9 · 103 ind./m2 in Lake Iseo. In both lakes, *Daphnia* summer abundance was lower (Fig. 3i). During the study period *D.*SUMMER tended to be consistently lower and less variable in Lake Iseo than Lake Lugano. In Lake Iseo *D.*SUMMER ranged from 0.41 (in 2014) to 102.1· 103 ind./m2 (in 1999). For most of the study period in Lake Iseo *Daphnia*'s summer abundance was lower than 50 · 103 ind./m2, indicating MSD, except for the 1999, 2010 and 2013. In Lake Lugano *D.*SUMMER ranged from 1.00 (in 2016) to 295.0 · 103 ind./m2 (in 1989). The lowest densities, indicating MSD, were recorded in 1993, 1994, 1996 and at the end of the study period from 2013 to 2016, with the exception of 2015.

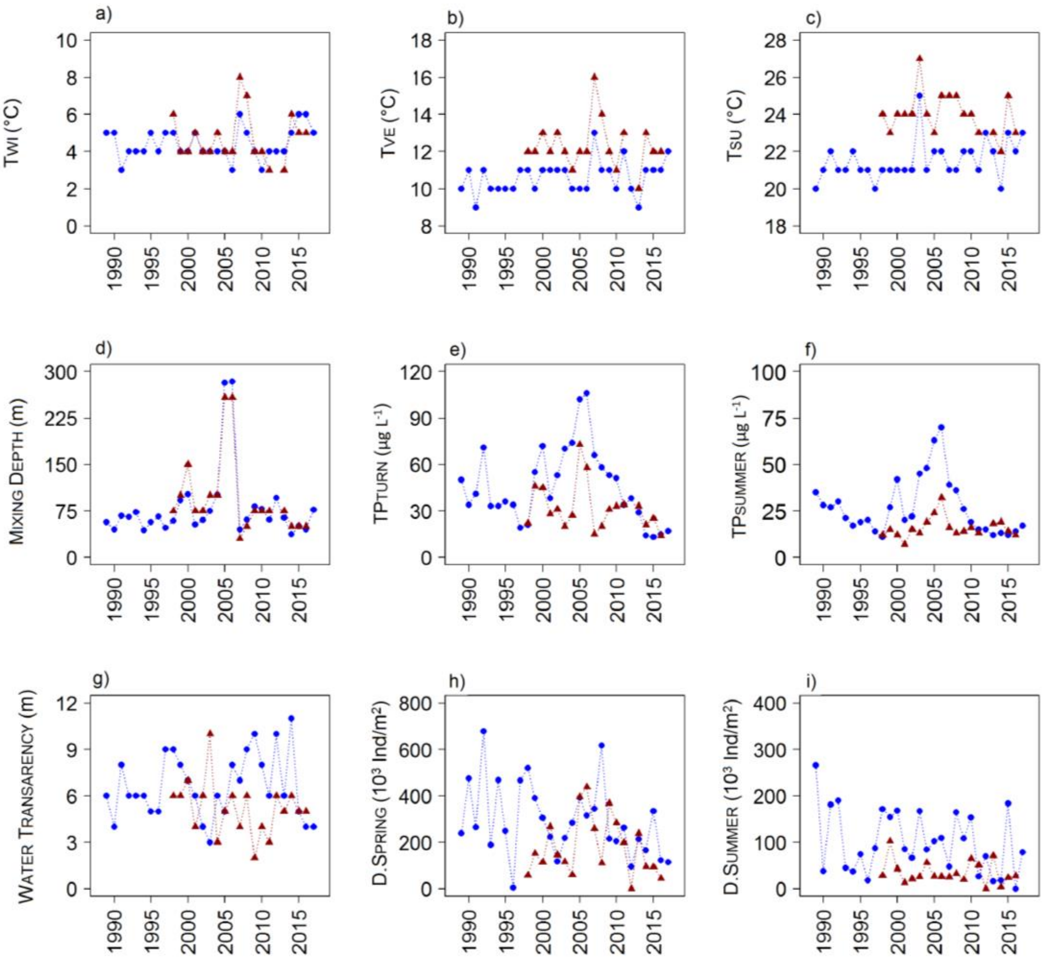


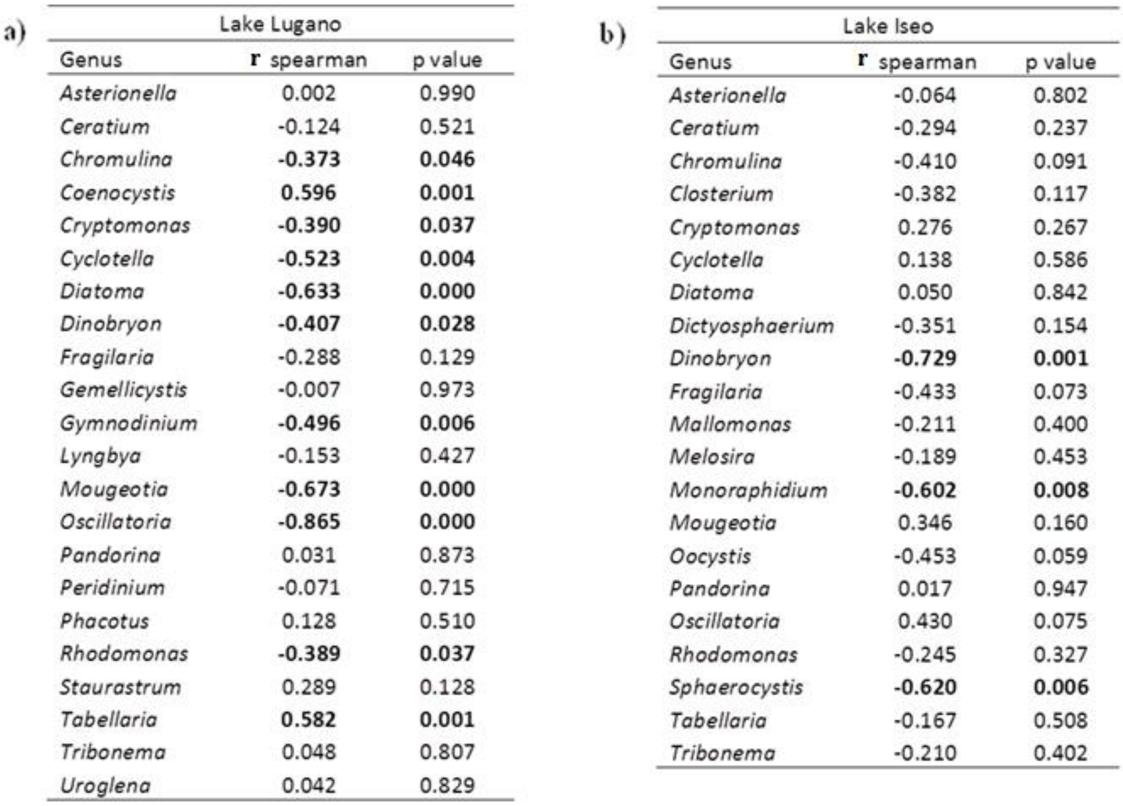
Figure 3 -Temporal trends of the principal chemical-physical and biological parameters estimated in Lake Lugano (blu circle) and Iseo (red triangle). a) Winter air temperature, T_{WI}, (from December to January); b) Vernal air temperature, T_{VE}, (from February to May); c) Summer air temperature, T_{SU}, (from June to August); d) Vertical Mixing depth (MIXDEPTH); e) total phosphorus at late winter overturn (TP_{TURN}); f) TP in summer (TP_{SUMMER}); g) Water transparency; h) *Daphnia* abundance between April and May (*D.*SPRING); i) *Daphnia* abundance between July and August (*D.*SUMMER).

Phytoplankton Non-metric Multidimensional Scaling (NMDS)

For Lake Lugano, the NMDS analysis produced a final 3-dimension solution with a stress of 0.107. Based on the correlation analysis (Table 2a), the NMDS for Lake Lugano had a negative and strongly significant association with the genera *Oscillatoria*, *Mougeotia* and *Diatoma* ($p<0.001$), and a positive and significant correlation with the genus *Coenocystis* ($0.001<p<0.01$).

For Lake Iseo, the NMDS analysis produced a final 3-dimension solution with a stress of 0.099. The NMDS for Lake Iseo had negative and significant correlations with the genera *Dynobrion, Monoraphidium* and *Sphaerocystis* ($p<0.01$) (Table 2b).

Table 2: Spearman correlation coefficients ("r spearman") and p-value of the correlation analysis between phytoplankton genera biovolume and the score of the first axis of non-metric multidimensional scaling in Lake Lugano (a) and in Lake Iseo (b). Bolded texted indicate significant correlation (p-value < 0.05).



a)

Lake Lugano		
Genus	r spearman	p value
Asterionella	0.002	0.990
Ceratium	-0.124	0.521
Chromulina	**-0.373**	**0.046**
Coenocystis	**0.596**	**0.001**
Cryptomonas	**-0.390**	**0.037**
Cyclotella	**-0.523**	**0.004**
Diatoma	**-0.633**	**0.000**
Dinobryon	**-0.407**	**0.028**
Fragilaria	-0.288	0.129
Gemellicystis	-0.007	0.973
Gymnodinium	**-0.496**	**0.006**
Lyngbya	-0.153	0.427
Mougeotia	**-0.673**	**0.000**
Oscillatoria	**-0.865**	**0.000**
Pandorina	0.031	0.873
Peridinium	-0.071	0.715
Phacotus	0.128	0.510
Rhodomonas	**-0.389**	**0.037**
Staurastrum	0.289	0.128
Tabellaria	**0.582**	**0.001**
Tribonema	0.048	0.807
Uroglena	0.042	0.829

b)

Lake Iseo		
Genus	r spearman	p value
Asterionella	-0.064	0.802
Ceratium	-0.294	0.237
Chromulina	-0.410	0.091
Closterium	-0.382	0.117
Cryptomonas	0.276	0.267
Cyclotella	0.138	0.586
Diatoma	0.050	0.842
Dictyosphaerium	-0.351	0.154
Dinobryon	**-0.729**	**0.001**
Fragilaria	-0.433	0.073
Mallomonas	-0.211	0.400
Melosira	-0.189	0.453
Monoraphidium	**-0.602**	**0.008**
Mougeotia	0.346	0.160
Oocystis	-0.453	0.059
Pandorina	0.017	0.947
Oscillatoria	0.430	0.075
Rhodomonas	-0.245	0.327
Sphaerocystis	**-0.620**	**0.006**
Tabellaria	-0.167	0.508
Tribonema	-0.210	0.402

Structural equation model analysis (SEM)

Lake Lugano primary model (Fig. 4a) – The SEM analysis for Lake Lugano converged after 68 iteration. The $\chi2$ test, with a p-value of 0.203, indicated a good model fit. In Lake Lugano there was a strong negative effect of T_{WI} on MIXDEPTH (β= -0.60), while MIXDEPTH had a strong positive effect on TP_{TURN} (β= 0.77). TP_{TURN} had a positive and moderate effect on *D.*SPRING (β= 0.30) and PHYTOPLANKTON COMPOSITION (β=0.26). Moreover, *D.*SPRING had a positive and moderate effect on PHYTOPLANKTON COMPOSITION (β= 0.29). In turn, PHYTOPLANKTON COMPOSITION positively influenced *D.*SUMMER (β=0.72). These effects, indicated that lower TP_{TURN} and *D.*SPRING were associated with low scores of the first axes of NMDS and, due to the correlation presented above, with higher biovolumes of *Oscillatoria* and *Mougeotia* and *Diatoma*, and that in turn higher biovolumes of these phytoplankton taxa were associated with lower densities of *Daphnia* in summer.

Additionally, T_{VE} had a positive and weak-to-moderate effect on *D.*SPRING (β=0.20). The hypothesized negative effect of T_{SU} on *D.*SUMMER was not supported by the analysis. On the contrary, T_{SU} had a positive and moderate impact on *D.*SUMMER (β=0.29).

Lake Lugano revised model (Fig. 5a)- The model was revised by removing the weak (β=0.20) path TV_{E}→*D.*SPRING and adding a path not hypothesized *a priori*, the direct influence of *D.*SPRING on *D.*SUMMER, whose inclusion in the model was suggested by a MI value of 4.45. The SEM analysis of this revised model converged after 56 iteration and the χ2 test indicated a good model fit (p-value=0.258). The revision supported the existence of causal links between winter air temperature, mixing depth, total phosphorus, phytoplankton composition, *Daphnia* spring abundance and *Daphnia* summer abundance (Fig. 5a). In addition, it suggested the existence of a direct and positive relation between *D.*SPRING and *D.*SUMMER (β=0.26).

Lake Iseo primary model (Fig. 4b)- The SEM analysis for Lake Iseo converged after 77 iteration and the χ2 test indicated a moderately good model fit, with a p-value of 0.099. In Lake Iseo there was a strong negative effect of T_{WI} on MIXDEPTH (β= -0.68), and, in turn, MIXDEPTH had a strong positive effect on TP_{TURN} (β= 0.88). TP_{TURN} had a direct and positive influence on *D.*SPRING (β= 0.73), whereas the effect of TP_{TURN} on PHYTOPLANKTON COMPOSITION were weak (β=0.13).

However, PHYTOPLANKTON COMPOSITION had a moderate and positive effect on *D.*SUMMER ($\beta=0.27$).
TVE had a positive and weak-to-moderate effect on *D.*SPRING ($\beta=0.17$), whereas *D.*SPRING had no effect on PHYTOPLANKTON COMPOSITION ($\beta= -0.04$). TSU had a positive, but weak to moderate, impact on *D.*SUMMER ($\beta=0.21$).

Lake Iseo revised model (Fig. 5b)– The model revision started by removing the weak paths ($\beta<0.20$) TP_{TURN}→PHYTOPLANKTON COMPOSITION, T_{VE} → *D.*SPRING, and *D.*SPRING→PHYTOPLANKTON COMPOSITION. The SEM analysis of the revised model converged after 71 iteration and the $\chi2$ test indicated a better fit with respect to the primary model, with a p-value of 0.088. The relation between winter air temperature, vertical mixing depth, phosphorus concentration after the turnover and *Daphnia* abundance in spring were also confirmed. The total indirect effect of T_{WI} on *D.*SPRING was moderately strong and negative ($\beta=-0.37$). The positive relation between T_{SU} and D.SUMMER was confirmed in Lake Iseo revised model ($\beta=0.21$).

However, in the revised model for Lake Iseo, the hypothesized causal effects of TP_{TURN} on PHYTOPLANKTON COMPOSITION and of PHYTOPLANKTON COMPOSITION on *D.*SUMMER were weak and non-significant ($\beta<0.2$), indicating that the pathway driven by winter air temperature had no substantial influence on *Daphnia* midsummer decline.

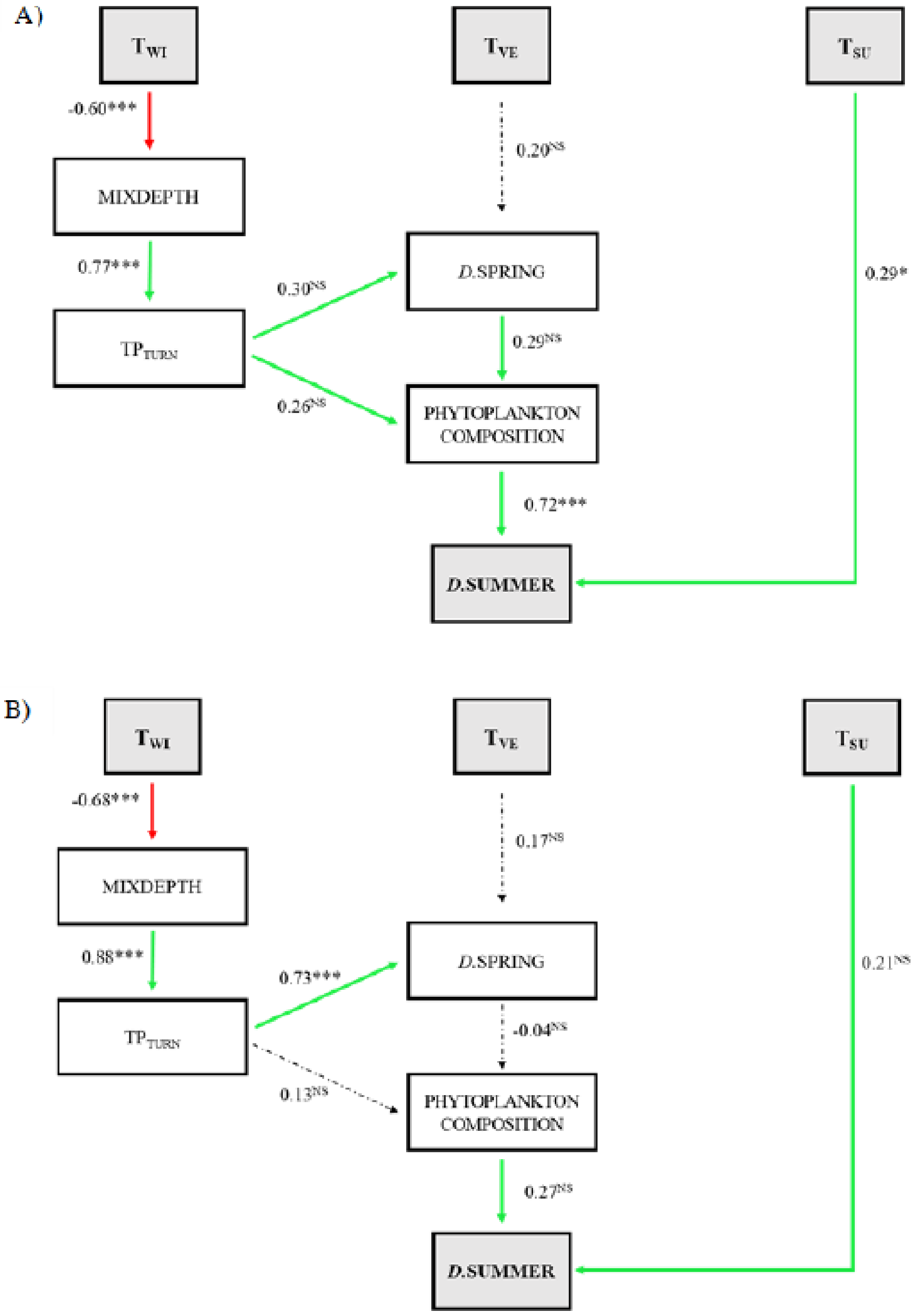


Figure 4 -SEM analysis diagrams of the primary models For Lake Lugano (A) and Lake Iseo (B). The numbers next to the arrows represent the associated path coefficients (β). *, ** and *** indicate, respectively, p-values <0.5, < 0.01 and <0.001; NS indicates a non-significant effect. Conventions as in figures 2. The positive relations are represented by green arrows, while negative are represented by red arrows.

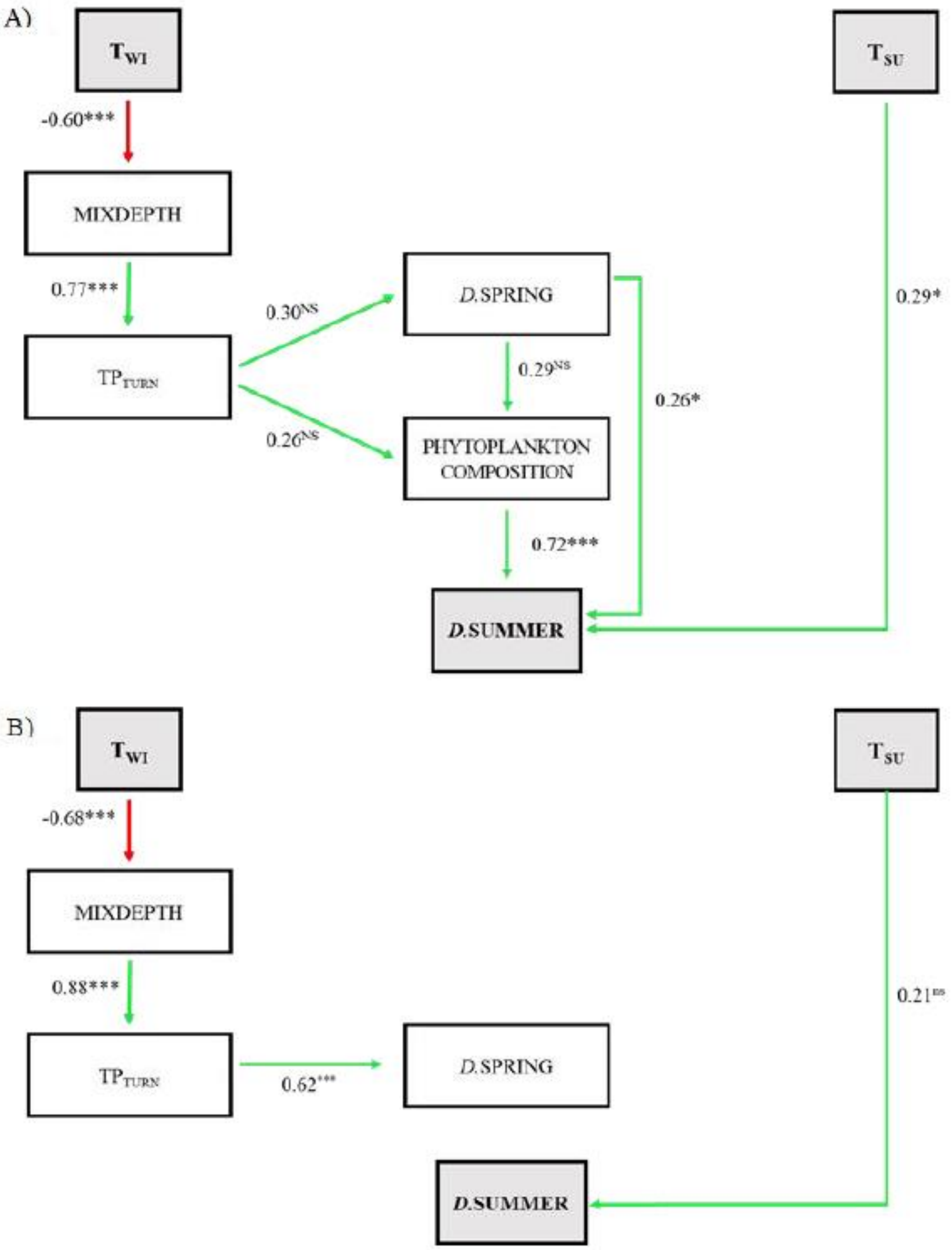


Figure 5: SEM analysis diagrams of the final models of Lake Lugano (A) and Lake Iseo (B). Conventions as in figures 2 and 4.

Discussion

In meromictic lakes, such as Lake Lugano and Lake Iseo, the effects of temperature on *Daphnia*'s phenology are complex, because they potentially include several indirect effects mediated by the influence of temperature on mixing depth during turnovers and, as a consequence, on the availability of nutrients in the epilimnion. The results of the SEM analysis indicated that, between the considered pathways, the one driven by winter air temperature had the greatest influence in controlling *Daphnia* abundance in spring and summer in the study lakes, whereas vernal air temperature had weak effect (Fig. 5). In addition, counter to one of our hypotheses, the summer air temperature had a positive (although moderate-to-weak) effect on the summer *Daphnia* abundance. These results mark key differences between the deep meromictic subalpine lakes, that were the object of this study, and the shallow hypertrophic reservoir (Bautzen reservoir, Germany) that were the focus of most of the previous research on *Daphnia* MSD (Mehner *et al.*, 1998; Benndorf *et al.*, 2001; Hülsmann & Voigt, 2002; Wagner & Benndorf, 2007). In this shallow reservoir, early spring (vernal) and summer temperature were identified as main factors controlling *Daphnia* MSDs. In particular, vernal temperature appeared to play a major role influencing *Daphnia* spring development, with higher temperature leading to a greater peak population density in spring and, as a consequence, to earlier and more severe food depletion during the clear-water phase (Benndorf *et al.*, 2001). Moreover, the summer air temperature was negatively associated with the *Daphnia* abundance in summer, potentially due to effects on the development of young fish and their predation rate on *Daphnia* (Wojtal-Frankiewicz, 2012).

In comparison, in Lake Lugano and Lake Iseo, two deep subalpine lakes, the effect of vernal temperature on *Daphnia* was not detected. The effect of summer temperature was instead positive, although only weak-to-moderate, meaning that higher summer temperature positively influenced the abundance of *Daphnia* in summer. Moreover, in Lake Lugano, winter air temperature, which controls vertical mixing dynamics during later winter turnovers (Lepori *et al.*, 2018b), emerged as a main driver of *Daphnia* MSD, through cascading effects on several pathways.

We suggest that these differences reflect dissimilarities in the way phosphorus is supplied to plankton between deep meromictic lakes and shallow and less-stratified reservoirs. In eutrophic shallow lakes with weaker summer stratification, as Bautzen reservoir, phosphorus is rarely in short supply because it tends to be more homogeneously distributed across the water column throughout the year (Kerimoglu & Rinke, 2013). In contrast, in stratified lakes phosphorus can become strongly limiting to phytoplankton in the epilimnion soon after the beginning of summer stratification. Therefore, phosphorus may have stronger influence on food availability and quality to *Daphnia* from the clear-water phase through summer (Brett, Muller- Navarra & Park, 2000; Van Donk *et al.*, 2008). As a result, the effects of phosphorus limitation on phytoplankton and *Daphnia* (through food quantity and/or quality) may be expected to play a greater role in stratified lakes than in eutrophic shallow lakes and reservoirs.

Contrary to one of the hypothesized pathways (P3), summer temperature had a positive (although weak) effect on *Daphnia* summer abundance. Summer temperature may have effects at two different time scales on *Daphnia* (mediated by fish predation). The first effect is seasonal, due to the positive effect of temperature on predation by young fish in summer (see P3). The second effect may be long-term, due to a compositional, community-level change towards smaller and more zooplanktivorous fish (Jeppesen et al., 2010). The positive effect of summer temperature on *Daphnia* summer abundance indicates that the hypothesized seasonal effect by young fish is not an important driver of *Daphnia* summer abundance in the study lakes (this effect should cause a negative, not positive effect of summer temperature). The pathway TS_{U}→FISH PREDATION→*D*.SUMMER was hypothesised based on results from shallow lakes, where predation by fish can be important. The lack of support for this pathway in this study is consistent with the idea that predation by fish has weaker effects in deep lakes, where zooplankton can find refuge from fish predation in deep waters (Jeppesen *et al.*, 2003)., In contrast, the long-term effect cannot be easily rejected, because it may become detectable only over longer time scales (e. g., several decades). However, for the study lakes there is no evidence of increasing fish predation on zooplankton. In Lake Iseo the structure of the fish assemblage has remained almost constant during the last decade (Leoni *et al.*, 2014). In Lake Lugano, the structure of zooplankton (dominated by large-bodied species) during the study period and fishing yield data (see Methods) suggest that predation pressure by fish has remained low (Lepori *et al.*, 2018b; Lepori, 2019).

According to our results, in both Lake Lugano and Lake Iseo, *Daphnia* abundance in spring (*D.*SPRING) was influenced by the epilimnetic concentration of total phosphorus after the turnover (TPTURN). This effect was expected because, in oligomictic lakes, cold winters tend to increase the vertical extension of mixing depth and, as a consequence, lead to greater replenishment of phosphorous in surface waters from deeper waters. Conversely, warm winters tend to reduce vertical mixing depth and phosphorus concentration after the turnover (Lepori *et al.*, 2018b). The effect of TPTURN on *Daphnia* confirms what observed in other deep subalpine lakes, where phosphorus availability positively influences *Daphnia* population growth in spring, e.g. leading to higher population densities of *Daphnia* after complete vertical mixing events (Manca *et al.*, 2015; Leoni, Nava & Patelli, 2018). These peaks have been attributed to greater phosphorus replenishment in epilimnion, which can improve food quality for grazers and in particular for *Daphnia* (Gulati & Demott, 1997; Leoni *et al.*, 2014).

In comparison, the mechanisms underlying *Daphnia* MSD differed between the study lakes. In Lake Lugano, *Daphnia* abundance in summer depended on the composition of phytoplankton during the clear-water phase. In this lake, the replenishment of phosphorus availability in the epilimnion, following turnover, and *Daphnia* abundance in spring controlled the composition of phytoplankton community during the clear-water phase. Specifically, reduced replenishment of epilimnetic phosphorus (caused by weaker mixing following warmer winters) and reduced *Daphnia* abundance in spring were associated with prevalence of filamentous genera of cyanobacteria and green algae (*Oscillatoria* and *Mougeotia*) and/or large colonial diatoms (*Diatoma*). This effect is consistent with previous observations that these taxa are favoured by conditions characterised by decreasing phosphorus concentration in epilimnetic layers and sharp transitions between nutrient-poor surface waters and nutrient-rich into deep waters (Reynolds, 1980; Anneville et al., 2005). Filamentous phytoplankton, such as

Oscillatoria and *Mougeotia*, are generally considered inedible to *Daphnia*, either because they are too large, or because filaments can clog the meshes of the filtration apparatus and decrease the food ingestion rate (Wejnerowski, Cerbin & Dziuba, 2017). Therefore, we suggest that in Lake Lugano early TP depletion in epilimnetic layers heightened *Daphnia* MSD (i.e., decreased summer density) by reducing the edibility of the food resource (phytoplankton) and exacerbating the resource bottleneck during the clear-water phase.

This proposed mechanism explain why summer abundances of *Daphnia* in Lake Lugano appeared to decline during the study period. In this lake, epilimnetic phosphorus decreased during the last decades due to phosphorus management and weaker mixing (Lepori *et al.*, 2018a), causing phosphorus segregation in deeper waters. As a result, TPMIX and *D.*SPRING have probably decreased during the study period, whereas densities of filamentous and colonial phytoplankton have presumably increased, causing increasingly severe MSDs.

Interestingly, in Lake Lugano high densities of *Daphnia* in spring appeared to be associated with lower biovolumes of *Oscillatoria* and *Mougeotia* during the clear-water phase. This result may seem counterintuitive, because, based on the keystone predation hypothesis (Leibold, 1996), grazing pressure may be expected to promote inedible phytoplankton. However, high grazing rates by *Daphnia* may promote the regeneration of nutrient in epilimnetic layers (Carney & Elser, 1990), a factor that can positively influence the development of edible genera of phytoplankton. This positive effect may overshadow any negative effect mediated by keystone predation, which, at present, remain uncertain (e.g. Sarnelle [2005] could not detect on effect of *Daphnia* grazing on phytoplankton vulnerability based on an experimental study). Therefore, this result suggests that high grazing pressure by *Daphnia* may benefit the overall edibility of phytoplankton in this lake, perhaps through efficient regeneration of nutrients Conversely, lower grazing in spring may accelerate the loss of phosphorus from the epilimnion and, as a result, shift conditions in favor of inedible phytoplankton able to grow in nutrient-depleted conditions.

Moreover, in Lake Lugano we detected a positive relation between the abundance of *Daphnia* in spring and in summer. We think that a large population abundance in April and May can reverberate to the summer months, due to *Daphnia* life cycle duration and fecundity (Wojtal-Frankiewicz, 2012). Indeed, the *Daphnia* life cycle is generally longer than a month and it can range from 33 days to more than 60 days (Meise, Munns & Hairston, 1985). Accordingly, we hypothesize that a large population abundance in April and May can contribute to maintain a higher population abundance in the summer month, due to the survival of the individuals born in late spring.

In comparison with Lake Lugano, in Lake Iseo, the winter air temperature affected *Daphnia* in spring, but the abundances of *Daphnia* in spring and in summer were unrelated. Moreover, for this lake, our analysis did not identify any factor explaining variation in summer densities of *Daphnia*. The inter-annual variability of *Daphnia* abundance was consistently lower in Lake Iseo than in Lake Lugano (Fig. 3 h, i), especially in the years that followed the last complete vertical mixing events (2005 and 2006). We suggest that the difference between the lakes reflects differences in the recent history of the lakes. Lake Lugano was eutrophic at the beginning of the study and moved toward mesotrophic conditions in recent years. Therefore, severe MDSs, which are associated with low P availability, are a relatively recent phenomenon, which emerged especially toward the end of the study period. Instead, Lake Iseo has been characterized by stable mesotrophic conditions since the beginning of the 1990s and, as a result, the decline of *Daphnia* during summer, probably due to a physiological decline of the population after the spring peak, has been a constant condition during the last decade. In this lake, the driving effect of winter air temperature and phosphorus replenishment on summer densities of *Daphnia* could not be detected probably because the temporal variation in P concentration was too low (i.e. variation in the predictor was too low to detect a statistical effect).

Our study highlights the complexity of the effects of climate variability on *Daphnia* phenology in deep lakes, showing that the responses differed between two temperate deep lakes with similar vertical mixing dynamics and current trophic but different past trophic evolution. These results are important in the context of growing evidence that large lakes worldwide are facing warmer water temperature due to climate change, and that warming can affect zooplanktonic organism and freshwater food webs in different ways (Wojtal-Frankiewicz, 2012; Vadadi-Fülöp & Hufnagel, 2014). For example, it has been predicted that, in the future, lakes that are currently monomictic (i.e., that experience full turnovers), are facing an increase in water stratification stability and may eventually become meromictic (Fenocchi *et al.*, 2018). Based on our results, in these lakes the *Daphnia* midsummer declines may become more severe. In particular. the effects of climate change are similar to that of eutrophication and in some cases they can interfere with management measures aimed to restore lakes from past nutrient pollution (Lepori *et al.*, 2018b). *Daphnia* are known for the ability to control algal densities with their grazing activity, thanks to the ability to feed on a wide array of algal species, excluding large colonial ones (Scheffer & Rinaldi, 2000). For these reasons, robust populations of *Daphnia* are desirable from ecological and a water-quality management point of view. Our results suggest that a progressive increase in winter air temperature can contribute to cause critically low densities of *Daphnia* during spring and summer, and these declines may compromise the ability of zooplankton to control phytoplankton biomass (Lepori *et al.*, 2018b).

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Data Availability Statement

Limnological data for Lake Lugano are published in yearly reports in aggregate form (www.cipais.org). The Institute of Earth Sciences of SUPSI maintains the database. Limnological data for Lake Iseo are available from the author Barbara Leoni (UNIMIB), upon request.

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4 A PALEOLIMNOLOGICAL STUDY OF A DEEP PERIALPINE LAKE (LAKE ISEO) IN RELATION TO CLIMATE CHANGE AND TROPHIC EVOLUTION

In the previous chapters, it has been pointed out how lake ecological states and pelagic food webs can be compromised by human activities and by the complex effects of climate change. However, as pointed out in previous studies, the biological responses to climate change resulted more complex to predict than the responses of limnological characteristics, as water temperature, chemical parameters, vertical mixing regime (Hering *et al.*, 2010; Perga *et al.*, 2015). Indeed, for biological parameters, climate change triggers complex cause-effects chains (Leoni, Nava & Patelli, 2018; Lepori, Roberts & Schmidt, 2018), including several interconnected variables that already responded individually to the combined effect of climatic and anthropic pressures.

In order to target good and efficient management policies aimed to conserve and improve lake ecological status and water quality it is crucial discern the respective contributions of the environmental pressures to ecological alterations and allow to predict the possible effects of future climate change on the freshwater quality and availability.

Paleolimnology consist in the reconstruction of past lake environmental conditions and ecological status based on the study of lake sediments proxies (Tolotti *et al.*, 2018). It is considered a powerful tool, in particular when combined with present limnological surveys, because it allows filling the knowledge gaps between the present and the past ecological status of fresh water ecosystems. Indeed, lake sediments analysis allows to prolong limnological perspective before the period impacted by human activities and this can help in discriminate between natural and anthropogenic variability effects (Twiss, 2008). In particular, one of the most valuable biological proxies that can be studied for reconstruction purposes are subfossil Cladocera. Indeed, they are widespread in both the pelagic and littoral zones of lakes and they often represent the dominant component of zooplankton in terms of biomass(Tolotti, Milan & Szeroczyńska, 2016). The chitinous parts of their body, as head shells, post abdomen and carapace are well preserved in lake sediments (Pociecha *et al.*, 2019). In addition, the taphonomic taxonomy is well established, and is possible to classify the Cladocera remains at the species level(Szeroczynska *et al.*, 2007). Due to their fundamental role in the pelagic food web, their subfossil remains have the capability to track long term changes in both bottom-up drivers (such as nutrients, physical and chemical stressors) and top- down regulation (as predation by zooplankton secondary consumers or fish)(Jeppesen *et al.*, 2001; Xu *et al.*, 2017).

This third part of my PhD thesis focused on a paleolimnological study of the deep oligomicitic Lake Iseo. The main aim is to explore possible synergic combination of effects of nutrients and climate on Lake Iseo ecosystems through the study of species composition and abundance of pelagic and littoral subfossil Cladocera remains. Understanding how zooplankton community changed in the past period will help in understand the possible future change in pelagic food webs structure and composition. Indeed, paleolimnology allow investigating lake past evolution, under the effects of different levels of natural and anthropogenic pressure, passing from period characterized by low level of anthropic pressure to period characterized by the synergic impact of climate warming and human activity.

Lake Iseo appeared to be an ideal site to conduct this kind of paleolimnological investigation thanks to the high sedimentation rate due to the high ratio between catchment area and lake surface area (Leoni *et al.*, 2019) . Moreover, on Lake Iseo a monitoring campaign is being carried out since the beginning of 1990s and this allows the interpretation and comparison of paleolimnological results in the light of those provided by the decadal freshwater investigations.

In particular, I have extracted subfossil Cladocera remain from lake sediment core, I have counted and identified the several taxa from subfossil remains analysis at the microscope and I have developed the data analysis and the results elaboration and interpretation. The results collected, presented and discussed in following section represent a first version of a manuscript that will be submitted to a scientific journal.

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The impact of anthropogenic and climatic stressors on a deep perialpine lake ecosystem (Lake Iseo, Italy): a paleolimnological perspective.

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Abstract

In large lakes with strong anthropogenic pressure, it is usually difficult to disentangle the impacts of climate variability from those driven by eutrophication. Sediment records can be useful to disentangle the effects of climate change and nutrient enrichment in deep lakes, because they span over long time periods, before and after human disturbance. The chitinous bodies of Cladocera organisms are well preserved in lake sediments and their typical morphological features are useful for species identification. The present work aims at the reconstruction of change in the species distribution and abundance of subfossil Cladocera in Lake Iseo in relation to climate and anthropogenic pressure. We relate subfossil Cladocera species composition and abundance in a 80 cm sediment core collected in the pelagic zone of Lake Iseo, to long term temperature trends and phosphorus concentration inferred by diatoms frustules. The sediment records of Lake Iseo suggest that Cladocera rapidly respond to environmental change and that climate change combined with eutrophication can induce changes in community composition and species abundance. Moreover, catchment-related processes may decisively affect both species composition and abundances of the lake planktonic communities due to the decrease of lake water transparency induced by input of inorganic material from the catchment area to the lake. The effects of hydrological variability on the ecology of Lake Iseo appear to be amplified by the large ratio between the catchment and the lake area.

Introduction

Multiple anthropogenic and natural stressors can compromise lake ecological quality and ecosystems services. Although it is widely accepted that eutrophication is one of the most impacting problem for lake water quality, in the last years it has become evident that also climate change can strongly impact lake ecosystem and lake water quality (Adrian *et al.*, 2009; Perga *et al.*, 2015). Climate change involves changes in air and water temperature, in hydrological cycles, in precipitation and storm frequency and intensity (Deng *et al.*, 2016).

In large lakes affected by multiple and strong anthropogenic pressure, it is usually difficult to disentangle the impacts driven by climate from that driven by eutrophication. Indeed, the increase in water temperature can produce effects that mimic those induced by an increase in nutrient concentration, and thus lead to increases in algal productivity, modifications in the vertical mixing regime (with consequent hypolimnetic hypoxia and phosphorus segregation, (Rogora *et al.*, 2018), to enhanced nutrient loading due to change in stream hydrology (Battarbee *et al.*, 2012), etc. Cladocera (Crustacea) are key organisms in the pelagic food web of deep lakes, as they represent the link between bottom-up factors (nutrient and phytoplankton) and top-down regulators (fish and other invertebrate predators) (Leoni, 2017). Moreover, zooplanktonic organisms are particularly sensitive to environmental stressors, such as climate change and nutrient variations (Vadadi-Fülöp & Hufnagel, 2014).

Sediment records can be useful to disentangle the effects of climate change and nutrient enrichment in deep lakes, because they span over secular time periods, that reach the time before the beginning of major human disturbance (Twiss, 2008). The chitinous bodies of Cladocera organisms are well preserved in lake sediments, and they present some typical morphological characteristics that are useful for the identification at species level. However, some recent studies highlighted that the best way to track different aspects of past ecological responses to overlapping stressor is a multi-proxy approach, combining the analysis of paleolimnological biotic (i.e. subfossil cladoceran and diatoms frustules) and abiotic proxies (e.g. inorganic and organic content) and measured long-term data (e.g. air temperature, teleconnection indices) (Milan *et al.*, 2015; Perga *et al.*, 2015).

The present work aims at reconstructing changes in the species composition and abundance of subfossil Cladocera in Lake Iseo in relation to combined eutrophication and climate change. In order to disentangle the effects of these stressors on lake biological communities, we relate subfossil Cladocera recorded in a 80 cm sediment core, collected in the pelagic zone of Lake Iseo, to measured air temperature trends, teleconnection indices and phosphorus concentration inferred by diatoms frustules.

Methods

Study Area

Lake Iseo (Site LTER_EU_IT_008_"Southern Alpine Lakes"; http://www.lter-europe.net) is the fourth largest Italian perialpine lake. It is located in northern Italy, in the Alpine foothills (190 m a.s.l.) at the lower end of a large populated prealpine valley (Val Camonica). The inflow and outflow of water in the lake are from the River Oglio. Lake Iseo has a surface area of 61.8 km^2, water volume of 7.6 km^3, a maximum depth of 258 m, and an average depth of 124 m (Nava *et al.*, 2017). The surface area of the watershed, including the lake, is 1842 km^2, with a mean altitude of 1429 m a.s.l and a maximum of 3554 m a.s.l (Garibaldi *et al.*, 2003). Water temperatures of deep perialpine lakes typically do not drop below 4 °C, so they are commonly classified as "warm monomictic", as they are characterized by complete water circulation once a year in late winter and stable stratification from spring period (Leoni, 2017). Nevertheless, due to the great depth of Lake Iseo, late winter vertical mixing occurs only during harsh and windy winters. During the last twenty years, complete winter mixing occurred only in 2005 and 2006, so the lake is to be regarded as holo-oligomictic. Lake Iseo experienced a relatively rapid eutrophication process since the 1970s that was mainly attributed to nutrient loading from the inflows. The increase in nutrient loadings brought the lake to a meso-eutrophic condition, as total phosphorus concentrations in the water column increased from approximately 60 μgP L^{-1} in the 1990s to 80 μgP L^{-1} between 2006 and 2016 (Rogora *et al.*, 2018).

Sediment coring, chronology and lithological parameters

A 80 cm long sediment core was collected in the deepest point of Lake Iseo (45°43′11″N; 10°03′46″E) using a Kajak gravity corer (UWITC, Austria) in late June 2014. The core was vertically extruded and sliced in the laboratory: the first 30 cm of the core were sliced at 0.5 cm contiguous intervals, while from 31cm to the bottom at 1cm intervals. Sediment visual aspects, i.e. color and texture presence of macroscopic remains, were annotated during slicing. The core chronology, from the surface to 74 cm, was established through radiometric analysis of ^{10}Pb and ^{137}Cs, ^{226}Ra and ^{241}Am (Appleby, 2005) by Ensis Ltd. (University College London, UK).

For all the subsample were determined the wet density, water content (measured by drying the sediment at 105°C for at least 12 hours) and the organic matter content (measured as Loss On Ignition (LOI) after heating the sample at 550°C in a furnace for three hours (Heiri, Lotter & Lemcke, 2001).

Diatoms inferred phosphorus

In order to analyze diatom frustules in the sediment, around 0.75 g of wet sediment from each subsample was treated with H_2O_2 (30%) and HCl (10%) according to standard procedures (Battarbee *et al.*, 2001). The cleaned diatom suspensions were permanently mounted using Naphrax® resin (refraction index=1.7). For each slide, at least 500 valves were counted under a light microscope at 1000x magnification and using interference contrast. Lake total phosphorus concentrations were inferred based on subfossil diatoms (Diatom Inferred Total Phosphorus—DiTP) using the Swiss-TP calibration set (including a set of low to medium altitude lakes mainly located in the Swiss Plateau) and a weighted-average regression with inverse deshrinking (TP CH WA-INV).

Subfossil Cladocera

Cladocera remains were analyzed in 72 of the 110 subsample sliced from the core. The sample have been treated in order to clean and concentrate the subfossil remain following the method described by Szeroczynska et *al*. (2007).

About 2.5 cm^3 of wet sediment were heated in KOH (10%) and then treated with HCL (10%), after the treatment the subsample were washed through a mesh of 35μm and concentrated in falcon tubes. One-two drops of a safranin-glicerol mixture was added to the cleaned subsamples in order to facilitate the identification of the remains under an optical microscope (100× or 400× magnification). Taxonomical identification of Cladocera remains was based on Szeroczynska and Sarmaja-Korjonen (2007). Cladocera remains (headshield, shell, postabdomen, postabdominal claws, mandible) were counted, and converted to number of individuals following Frey (1986). Three to six slides for each sample were counted in order to obtain a minimum of 100 individuals (Milan, Bindler & Tolotti, 2016), however this number had not been reached in a few samples characterized by very scarce abundance of remains.

Data elaboration and analysis

In order to detect general changes in Cladocera community, the total pelagic species abundance (Total Pelagic), the total littoral species abundance (Total Littoral) and the total Cladocera abundance (Total) were calculated. Additionally, the percentage of pelagic species above the total (Pelagic %) was computed in order to detect the proportion between pelagic and littoral taxa.

Starting from sedimentation rates (provided by the radiometric dating) and the percent organic content of each sediment layer (LOI%) the inorganic sedimentation rate (Inorganic Sed Rate) has been calculated. This parameter has been used as proxy of the inorganic materials carried from the catchment area.

Homogenized monthly mean air temperatures provided by the HISTALP data set (Auer et al., 2007) for Torbole-Riva station were used as proxy for climate variability at Lake Iseo. In particular, the average value of Temperature from March to October (representing the vegetative period) and in winter period (December-January) have been calculated. Similarly, average value of East Atlantic pattern (EA) from March to October and from December to January have been calculated. The values of the teleconnection index East Atlantic pattern (EA), computed by the National Oceanic and Atmospheric Administration-Climate Prediction Centre (NOAA-CPC, www.cpc.ncep.noaa.gov), was considered to outline possible relations between ecological dynamics and global atmospheric circulation patterns. We choose to use East Atlantic pattern because several studies verified the effectiveness of this atmospheric mode of variability in representing meteoclimatic variation and the influence on limnological characteristic and zooplankton phenology in the deep subalpine lakes (Leoni, Nava & Patelli, 2018a; Rogora *et al.*, 2018)

Mean annual and winter values of EA were calculated for the period 1950-2014. A Pettitt test (Mallakpour & Villarini, 2016) was performed on the total pelagic Cladocera abundances in order to detect changing point in Cladocera community development.

A Spearman correlation analysis was performed in order to explore relations between the principal Cladocera taxa abundances, and to detect intra-species relation, and between the principal Cladocera taxa abundances and LOI% and Inorganic Sed Rate aiming at clarifying the relations between the abundances and the load of organic and inorganic materials to the Lake Iseo ecosystems.

Performing several tests on the same set of data may result in an increased risk of type I statistical error, i.e., the rejection of the null hypothesis of no difference between the zooplankton taxa densities and/or core lithological variables due to mere chance. A high conservative approach to reduce the risk of type I errors is to adopt Bonferroni correction of significance of statistical tests (Rice, 1989). However, there is no general consensus on the procedure of application of Bonferroni correction (Nakagawa, 2004), partially because the application of this high conservative approach may lead to considerably reduced power of the tests and consequently to increased risk of type II statistical errors, whereby the null hypothesis being tested is unduly accepted (Nakagawa, 2004). In addition, several studies on relationships between actual zooplankton predator and prey, as well as among actual limnological characteristics and zooplankton population development, supported the existence of many direct correlations between the tested parameters in this study. Considering the above mentioned well known relationships and the absence of a clear theoretical framework for how to apply correction of significance to data of this nature, we decided to consider significant the uncorrected p-values lower than 0.05. However, we have also applied a

Bonferroni correction and we highlighted relationships that were significant according to this procedure.

The data analysis and environmental variable graphical elaboration has been performed with R (version 3.6.1). The graphical elaboration of the stratigraphic data has been performed using the software C2 (version 1.7.7).

Results

Sediment core analysis

The ^{210}Pb and ^{137}Cs based core chronology spanned from 1922 ±13 at depth 73.5 cm and 2014 on the surface with an average error of ±6 year. Sediment accumulation rate (Fig. 1a) presented an average value of 0.39 g cm^{-2} year^{-1} from the sediment top down to the beginning of the 1980s, and then it decreased under 0.2 g cm^{-2} year^{-1} with the exception of two peaks over 0.5 g cm^{-2} year^{-1} in 1995 ±4 and in 1998 ± 3.

Organic matter content presented as percentage of Loss On Ignition (LOI) showed minimum values in the deeper layer of the core and gradually increased in the upper layer (Fig. 1b). The minimum and the maximum recorded values are, respectively, 4.2% at 76.5 cm depth and 18.5 % at 16.75 cm. In the upper layer, from the beginning of 1980s until 2014, is possible to observe a particularly accentuated variability, with values spanning from 8.8% to 18.5%.

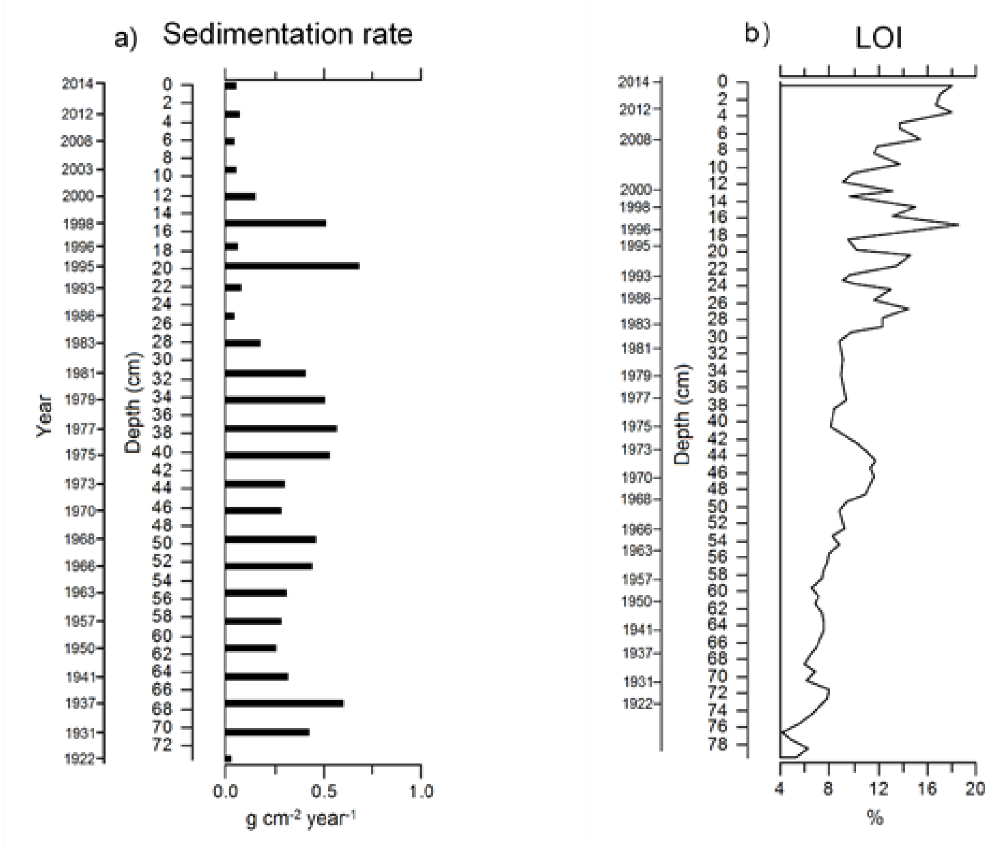

Figure 1- Sedimentation rate (a) and percentage of Loss On Ignition (LOI) (b) in the core layers.

Subfossil Cladocera remains

The subfossil Cladocera assemblage of Lake Iseo sediment core featured 29 taxa, including 8 pelagic taxa and 21 littoral taxa. The identified taxa belonged to the families Leptodoridae, Daphniidae, Bosminidae, Cercopagidae and Chydoridae. The Cladocera belonging to the first four families consist of open water zooplankton (pelagic), while taxa belonging to Fam. Chydoridae are characteristic of the littoral zone. The total Cladocera densities are presented in Figures 2 and 3. The pelagic species prevailed over littoral ones and represented for most of the layers the total abundance, while only in the layers deeper than 61.5 cm (year 1950±10 AD) pelagic species were less than 50% of the total remains. The Cladocera abundances were particularly low in the deeper layers of the core, from 79.5 to 64.5 cm depth, that were deposited from the early 20th century until 1941 ±12, while are quite swinging in the upper layer but considerably higher. A Pettit test applied on the total of the pelagic species pointed out three significant changing points in the pelagic abundances, in 1941 ± 12 years (p-value <0.001), in 1969 ±8 years (p-value <0.001) and in 1993±4 years(p-value< 0.05).

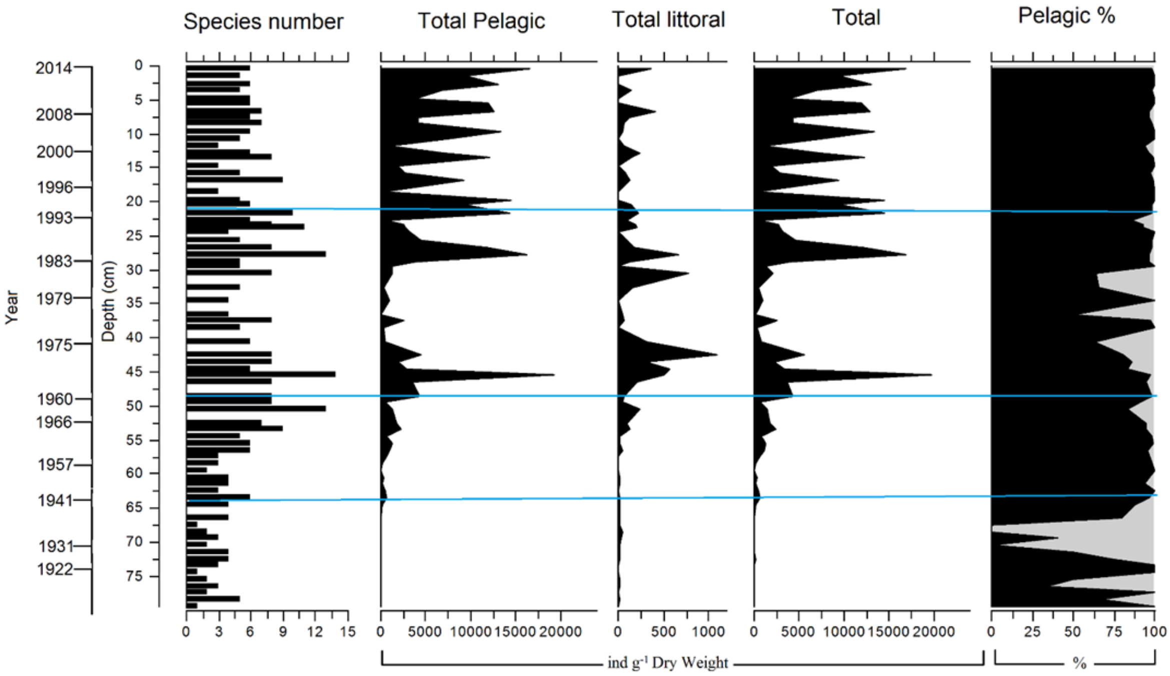

Figure 2-Depth profiles of subfossil Cladocera abundance: Number of Species: number of species identified in every layer; Total Pelagic: total pelagic taxa abundance (ind g^{-1} Dry Weight); Total Littoral: total littoral taxa abundance (ind g^{-1} Dry Weight; Total Cladocera abundance (ind g^{-1} Dry Weight); Pelagic %: percentage of pelagic Cladocera over the Total. The horizontal lines identify the significant changing points pointed by the Pettitt test performed on the total pelagic abundance.

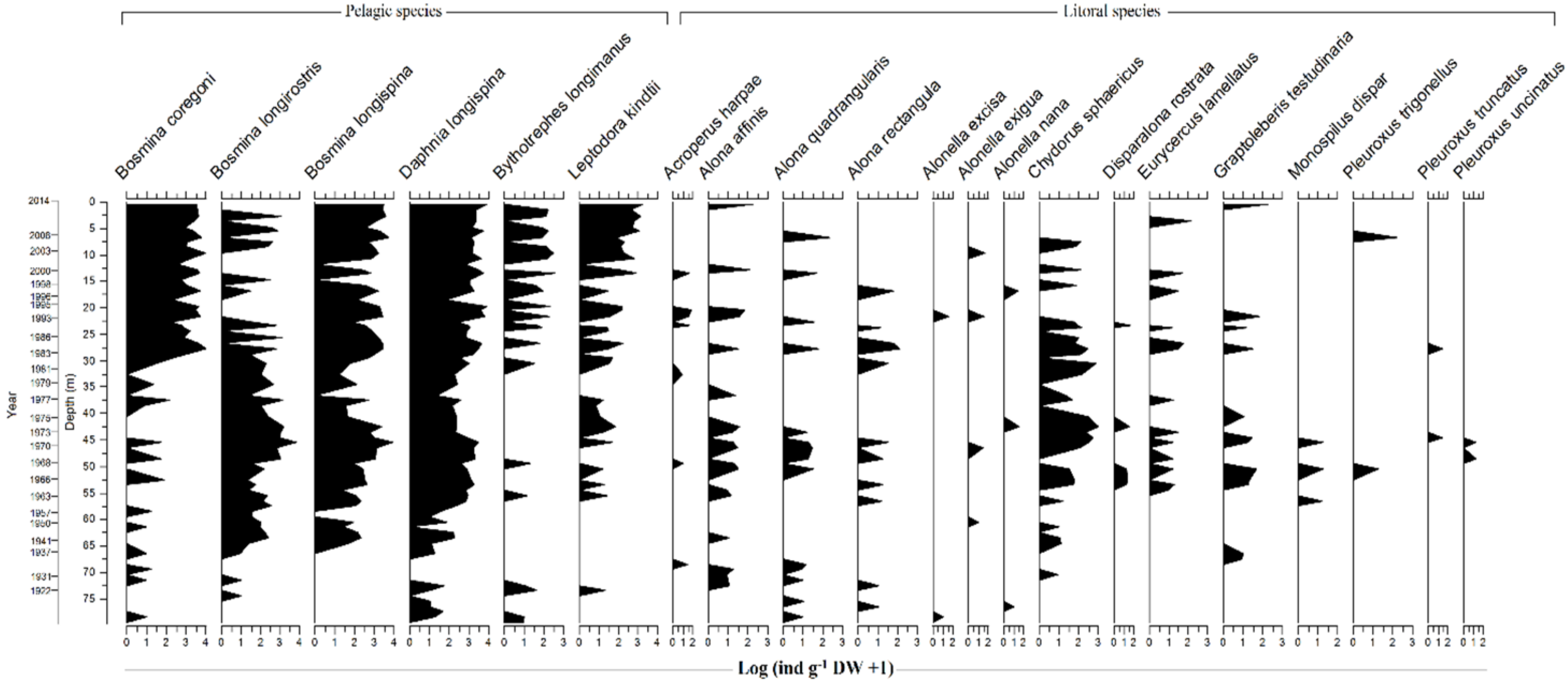

Figure 3- Diagram of the abundances of the principal Cladocera taxa in Lake Iseo sediments. Abundances are expressed as logarithm of the number of individuals per g of Dry sediment (DW).

The first section, from 79.5 to 64.5 cm, was characterized by scarce Cladocera abundance and a number of taxa spanning from 1 to 5 per layer. The most relevant between the pelagic are *Bosmina longispina* (Leydig, 1860), *Bosmina longirostris* ((Leydig, 1860)), *Daphnia longispina* (O.F. Muller 1785) and the zooplanktivourus *Bythotrephes longimanus* (Leydig, 1860) (Fig. 3). The second section, from 64.5 to 48.5 cm, showed higher abundance of both pelagic and littoral taxa and higher species variability. In particular, *Bosmina longispina*, and the littoral species *Chydorus sphaericus* (O.F. Milller, 1785) and *Graptoleberis testudinaria (*(Fischer, 1848)) appeared here for the first time. The third section, from 48.5 to 21.75 cm, was characterized by swinging abundances, as the pelagic species prevailed in some layers while in others prevailed the littoral ones. It was possible to observe two peaks, i.e. 19000 ind g^{-1} DW and 16000 ind g^{-1} DW respectively, in pelagic species abundances at 45.5 and at 27.75 cm, corresponding to years 1971 ±7 and 1984±4, respectively (Fig. 2). Conversely, at 36.5 cm depth it was possible to observe a general decline in the identified subfossil Cladocera remains, with a total abundance of 91 ind g^{-1} DW. The high abundances of the pelagic species were particularly due to *Bosmina* spp., *Daphnia longispina* and the two zooplanktivourus *Bythotrephes longimanus* and *Leptodora kindtii* ((O.F. Muller, 1776)) (Fig. 3). On the contrary, *Chydorus sphaericus* resulted the most abundant littoral taxa. The most recent core section, from 21.75 cm to the core surface, is characterized by the prevalence of pelagic species that represented more than 95% of the total abundance (Fig. 2). However, periods of high Cladocera abundance alternated with period characterized from lower levels, with a minimum of 393 ind g^{-1} DW at 18.5 cm (1995 ±4) and a maximum of 16900 ind g^{-1} DW at 0.5 cm (2014). In this core section, it was possible to observe the increase in *Bosmina coregoni* ((Baird, 1857)) abundance and a decreased abundance of *Bosmina longirostris*, the latter being identified only in 6 layers (Fig. 3). Included in the most abundant pelagic species there were also *Bythotrephes longimanus*, *Leptodora kinditii* and *Daphnia longispina.* The Spearman correlation between Cladocera taxa abundances outlined some positive and significant relations between the pelagic species (Tab. 1a). In particular, it is possible to notice a positive and significant correlation between *B. coregoni* and *B. longispina* and between *B. coregoni* and *D. longispina*. Both the latter species resulted to be positively correlated with the main predators *B. longimanus* and *L. kindtii*. In addition, a positive and significant correlation was found between the littoral species *C. sphaericus* and the pelagic *B. coregoni* and *B. longispina*.

The Spearman correlation between Cladocera abundance and Inorganic Sed Rate pointed out positive and significant relationship between Inorganic Sed Rate and *B. longirostris*, *C. sphaericus* and the total littoral abundance, while negative relation existed between Inorganic Sed Rate and *B. coregoni* and *L. kindtii*. Conversely, the organic matter content (LOI %) had positive and significant relation with *B. coregoni, D. longispina, L. kindtii* and the total pelagic abundance (Tab. 1b).

Table 1- Results of the Spearman correlation between (a) Cladocera taxa abundances and (b) between the Cladocera and the organic content (LOI %) and Inorganic sedimentation rate (Inorganic Sed Rate) of each layer. Only significant and marginally not significant relations are presented in the table: * p-value < 0.05; ** p-value < 0.01; *** p-value < 0.001; $^{+}$ p-value < 0.06. The values that resulted significant after Bonferroni correction are presented in bold.

a)

	r Spearman
B. coregoni - B. longispina	**0.65*****
B. coregoni - D. longispina	**0.74*****
B. coregoni - B. longimanus	**0.51*****
B. coregoni - L. kindtii	**0.66*****
B. longirostris - B. longispina	0.27*
B. longirostris - C. sphaericus	**0.49*****
B. longispina - D. longispina	**0.84*****
B. longispina - B. longimanus	**0.36****
B. longispina - L. kindtii	**0.70*****
B. longispina - C. sphaericus	0.25*
D. longispina - B. longimanus	**0.45*****
D. longispina - L. kindtii	**0.73*****
B. longimanus - L. kindtii	**0.52*****

b)

	r Spearman
Inorganic Sed Rate - *B. coregoni*	-0.28*
Inorganic Sed Rate - *B. longirostris*	0.29*
Inorganic Sed Rate - *L. kindtii*	**-0.39****
Inorganic Sed - *B. longimanus*	-0.24^{+}
Inorganic Sed Rate - *C. sphaericus*	0.36**
Inorganic Sed Rate- Total littoral	0.36**
LOI % - *B. coregoni*	**0.46*****
LOI % - *D. longispina*	0.29*
LOI% - *L. kindtii*	0.29*
LOI % - Total pelagic	**0.36****
LOI% - Total abundance	0.35*

Diatoms inferred total phosphorus

Total phosphorus inferred by diatoms presented an increasing trend from the deeper to the surface layers (Fig. 4). In particular, a constant total phosphorus concentration of 28 μg L^{-1} was estimated from the core bottom until 48.5 cm (year 1968 ± 7). From 48.5 to 20.5 cm the total phosphorus concentration increased, reaching a maximum of 67 μg L^{-1} at 25.75 cm (year 1993 ± 4). The surface layers, i.e. from 25.75 until the core surface, total phosphorus variability was quite high spanning from a minimum of 28 μg L^{-1} at 14.75 cm (year 1998 ± 3) to a maximum concentration of 62 μg L^{-1} at 24.5 cm (year 1989 ±6).

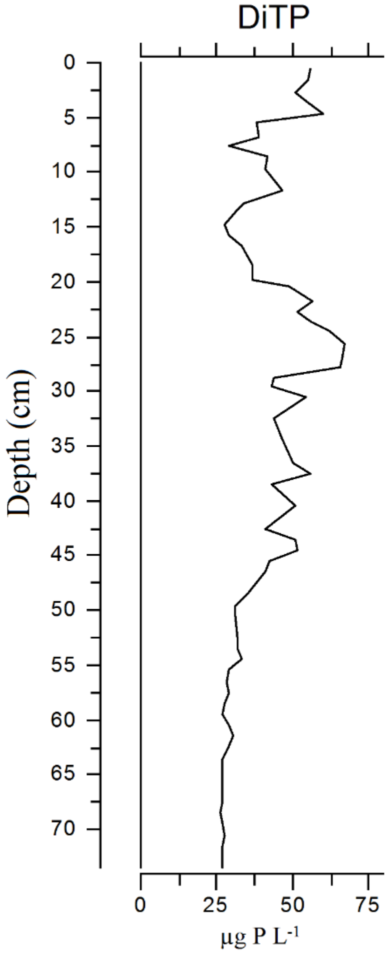

Figure 4- Phosphorus inferred by diatoms in Lake Iseo sediment core (DiTP).

Environmental variables

The mean air temperature from March to October have increased stepwise by almost 2.0°C since the beginning of the 20th century (Fig.5a). In the first 20 years of the century (1900-1920) the average air temperature was 16 ± 0.67 °C, while was 18 ±0.53°C from 1990 to 2010. was. Winter air temperature follow a similar pattern with an increasing trend in particular from the 90s (Fig. 5b).

In addition, the average value of East Atlantic pattern in winter and from March to October (Fig 5c and 5d) showed an increasing trend, with more positive values in the last decades. Since the second half of the 1990s EA showed high inter-annual variability in particular in winter, spanning from -1.64 in 2005 to 1.60 in 2014.

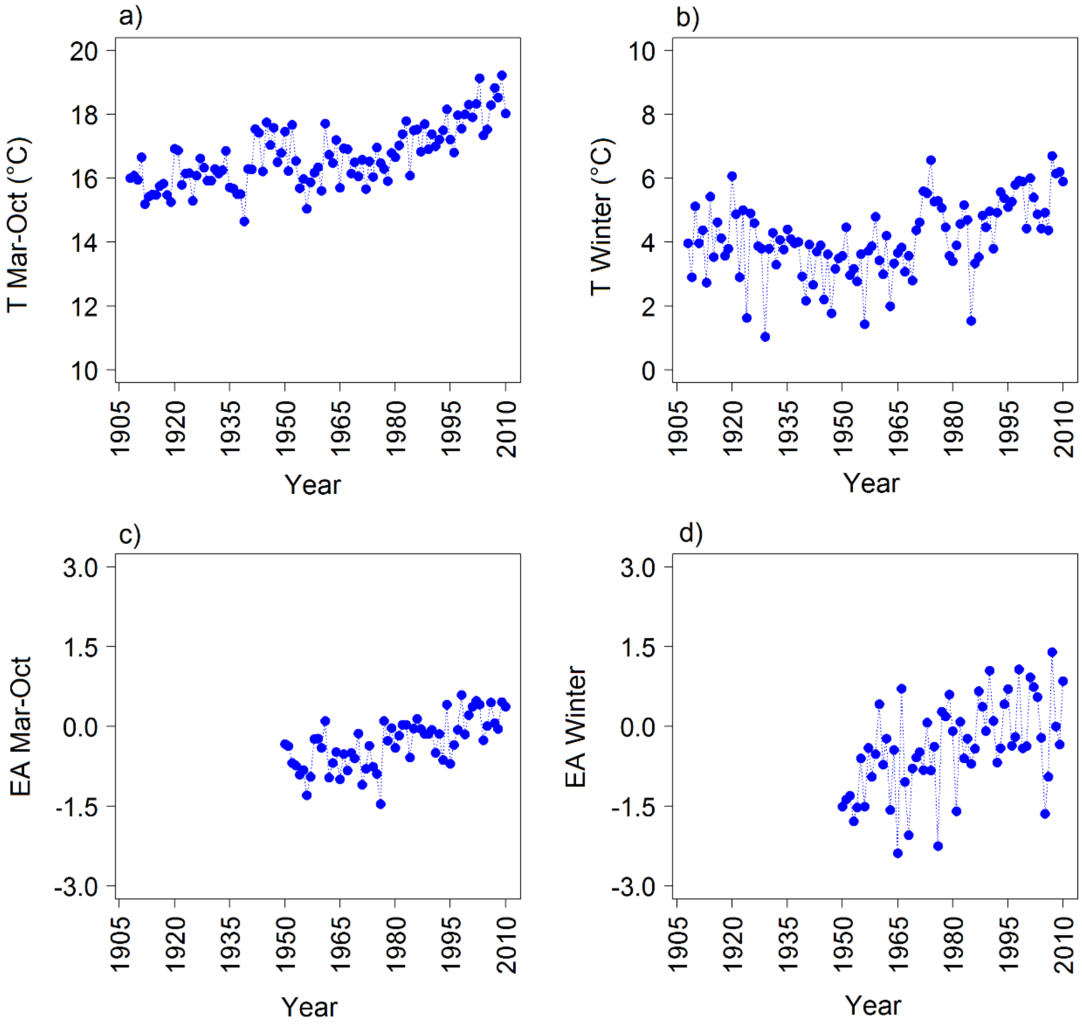

Figure 5- Temporal trend of climatic proxies: a) Air temperature from March to October ($T_{MAR-OCT}$). b) Winter air temperature, from December to February (T_{WINTER}). C) East Atlantic pattern from March to October ($EA_{MAR-OCT}$). d) Winter East Atlantic pattern, from December to February (EA_{WINTER})

Discussion

The analysis of Lake Iseo sediment core confirmed that paleolimnology is a useful tool to reconstruct the lake trophic evolution and the zooplankton species composition and abundance, and to provide important information about the long-term development of Cladocera community. The Cladocera remains detected in Lake Iseo sediment reflected the species composition and abundance of modern pelagic Cladocera assemblages (Leoni, 2017; Leoni *et al.*, 2018a). In particular, a relation between the principal pelagic species has been pointed out, highlighting a strong relation between the abundance of predators, as *B.*

longimanus and *L. kindtii*, and their principal prey, *Daphnia longispina* and *Bosmina* spp. Indeed, Leoni (2017) reported that in summer period big zooplanktonic secondary consumers could feed of smaller zooplankton, as *Bosmina* spp. instead of *Daphnia.* The high and positive relation between the two main predators *L. kindtii* and *B. longimanus* pointed out from our results suggested that their development is favoured from the same condition of food availability and temperature. This can seems counterintuitive because this two species are potentially competitor, however in deep lakes has been verified that the coexistence of *B. longimanus* and *L. kindtii* is possible thank to the temporal and spatial separation of their ecological niches, with shift in population seasonal cycles and vertical distribution on the water column (Manca, Portogallo & Brown, 2007; Manca, 2011).

From the combined study of subfossil Cladocera remains, diatoms frustules and some climate proxies as air temperature and East Atlantic pattern, we could identify several stages in the ecological evolution of Lake Iseo during the 20th century. From the beginning of the IXth century to the early of 1960s Lake Iseo resulted characterized by low total phosphorus concentration, low organic matter content and scarce abundance of Cladocera, with the prevalence of littoral species on the pelagic one. In particular, the lowest value of phosphorus concentration inferred by diatoms frustules (28 μg L^{-1}) in the beginning of 20th century highlighted a mesotrophic condition for Lake Iseo, higher than the oligotrophic original condition theorized for deep subalpine lakes (Ambrosetti *et al.*, 1992; Tolotti *et al.*, 2018). However, it will need further attention and validation through the algorithm chosen for phosphorus concentration estimation. In addition, this period was characterized by lower air temperature and EA value. East Atlantic pattern has been shown to drive climate pattern over the Mediterranean basin impacting on the limnological characteristics of deep subalpine lakes and on zooplanktonic communities (Manca, Rogora & Salmaso, 2015; Leoni *et al.*, 2018a). In particular, since negative and lower value of EA are associated with colder temperature in the Mediterranean region (Leoni *et al.*, 2018a; Salmaso *et al.*, 2018), the lower abundance of Cladocera in the deepest core layers can be due to overall unfavourable conditions for the development of zooplankton community. Indeed, scarce food availability and lower winter air temperature are known to negatively affect lake primary productivity and zooplankton fecundity and growth rate (Zawisza & Szeroczyńska, 2007; Manca *et al.*, 2015). This result is consistent with that found in other studies on deep subalpine lakes, where the deepest layer are characterized by low species diversity and abundance (Milan *et al.*, 2015).

From the beginning of 1960s diatom-inferred phosphorus concentrations began to increase. The increasing trend is confirmed by several studies that observed a period of strong eutrophication affecting the principal deep subalpine lakes, including Lake Iseo (Salmaso *et al.*, 2008; Rogora *et al.*, 2018). Cladocera community seems to react to the higher nutrient availability, indeed a progressive increase of planktonic species density and richness, indicators of warm and nutrient-rich water were observed. In this period, became dominant species as *Bosmina longirostris*, *Bosmina longispina* and *Daphnia longispina.* In particular *Bosmina longirostris* is considered a typical indicator of eutrophication (Zawiska *et al.*, 2014; Milan *et al.*, 2017). The increase of pelagic taxa in period of high nutrient availability was an attended result following the bottom up mechanisms reported in literature and verified in other lakes (McQueen *et al.*, 1989). In deep lakes, phosphorus availability controlled the abundance and the composition of phytoplankton community, influencing food availability and quality for primary consumers. A higher biomass of zooplankton, due to high phosphorus concentration, is usually associated with high biomass of primary producers (Leoni *et al.*, 2014; Manca *et al.*, 2015). The increase in the abundance of primary consumers is known to positively affect their predators, and this trigger the entire pelagic food web through a bottom up mechanism (primary producers).

The massive presence of *Bosmina longirostris* reversed at the end of the 1980s, when the most abundant species of *Bosmina* became *Bosmina coregoni* that required less eutrophic but warmer waters (Stenson, 1976). In this period inferred total phosphorus decreases but it is possible to observe an increment in air temperature and in EA values, corresponding to warmer climate. We hypothesised that the warmer climate combined with the reduced phosphorus concentration favoured the development of *B. coregoni* instead of *B. longirostris*. It is known that most of the subalpine lakes, after the strong phases of eutrophication that peaked between 1960s and 1980s, faced a phase of re-oligotrophication, but in Lake Iseo the process wasn’t so marked and the lake is still in an eutrophic condition (Leoni *et al.*, 2018a; Rogora *et al.*, 2018). However, in Lake Iseo a partial reduction of epilimnetic phosphorus concentration is plausible in this period. Indeed, from the second half of 1980s the first measures to reduce nutrient loading to freshwater were taken, as the development of the first waste water collectors and treatment plants (Garibaldi *et al.*, 1999). Another factor that contribute to the partial reduction of the phosphorus concentration after the 1980s may be the decrease of the late winter vertical mixing depth. Indeed, a reduction in the depth reached by water turnover in late winter has been observed in all the subalpine lakes, connected with the increase in winter air and water temperature. In deep oligomictic lake, as Lake Iseo, water turnover in late winter represents a key factor in determining spring phosphorus concentration in the epilimentic layers, because it favours the vertical transfer from the hypolimnion and the replenishment of phosphorus in the epilimnion. Shallow mixing has the opposite effect (Salmaso *et al.*, 2007; Leoni *et al.*, 2018b; Rogora *et al.*, 2018).

Concomitantly with the increased abundance of *Bosmina coregoni* it was possible to observe a generalized increase in all the pelagic species in the upper layers of the cores, including the predators *Leptodora kindtii* and *Bythotrephes longimanus*. This can be due to the increased water temperatures related to the warmer climate, as suggested by the air temperature trend and East Atlantic pattern. Moreover, several recent studies reported increases in water temperature and in particular, Pareeth *et al.* (2017) reported an increase in surface water temperature of 0.017 °C per year in the last thirty years (1986-2015). This period corresponded to the highest abundances and the major species variability recorded in Lake Iseo sediment core, and the concomitance between not limited phosphorus concentration and warmer water favour the development of lake primary and secondary production (Schalau *et al.*, 2008; Salmaso, 2010). As verified in other deep lakes, higher temperature positively influenced the development of zooplankton organism and of phytoplankton, their primary food resource, in some cases miming the effects of eutrophication (Lepori, Roberts & Schmidt, 2018).

Interestingly, in Lake Iseo the general taxa abundance along the core showed pronounced oscillations, with alternating phases of high Cladocera abundance to phases of very low abundance, in particular of the pelagic species. Lake Iseo is characterized by a high sedimentation rate, in particular if compared to other deep subalpine lakes as Lake Garda. Due to very different average sedimentation rate, the 80 cm long sediment core for lake Iseo covers around 100 years, while in Lake Garda a shorter sediment core could cover several centuries (Milan *et al.*, 2017). The high sedimentation rate points out the strong impact that catchment area dynamics can have on lake ecological characteristics. A pattern similar to that of Lake Iseo, with period of high abundance alternated to strong decreases in pelagic taxa abundance, was detect by Milan *et al.* (2016) in Lake Ledro. In this lake, characterized by a big ratio between catchment area and surface area as Lake Iseo, this shift were imputed to the effect of hydrological variability and in particular to flood events. In Lake Iseo a similar hypothesis can be plausible. High amounts of inorganic material coming from the catchment area through a flood event can negatively affect lake production increasing water turbidity and nutrient availability and leading to unfavourable condition for pelagic community development (Adrian *et al.*, 2009). Indeed, in Lake Iseo it was possible to observe a negative correlation between the inorganic sedimentation rate and some vulnerable pelagic species as *B. coregoni* and *L. kindtii*. Moreover, the littoral species *C. sphaericus* and the high adaptable *B. longirostris* (Milan *et al.*, 2017) increased in period characterized by high sedimentation rate. We hypothesize that this relation can be due to a major material transportation from the littoral zone to the centre of the lake, caused by high hydrological variability in the catchment area. Indeed the increase in sediment resuspension, due to the increase of inorganic material input, may increase water turbidity and negatively affect lake primary production (Graham & Vinebrooke, 2009; Morabito *et al.*, 2018).

The present paleolimnological investigation of Lake Iseo allowed identifying the role of the principal drivers of change in the structure and composition of pelagic zooplankton abundance, suggesting that climatic factors should be considered together with nutrient availability as a key factor in controlling the temporal development of plankton communities and pelagic food web structure. In the period characterized by stable trophic condition and not-limiting nutrient level, climatic variation and in particular increased temperature became the most important driver of lake ecological characteristic. Moreover, this study provides the basis for understanding the combined effect of climate and eutrophication over deep lake ecosystems and to interpreter possible future changes.

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5 CONCLUSION

Zooplankton are fundamental components in lake pelagic food webs, acting as the connector of primary producers and big secondary consumers they are the centre of top-down and bottom-up effects that regulate the structure and functioning of pelagic food webs. They are also good indicators of the changes occurring in lake ecosystems because they showed a fast answer to environmental variability, they are widespread in lakes all over the world and their morphological characteristics are well studied. Nevertheless, in deep subalpine lakes there is still an only partial understanding of how natural and anthropogenic pressure can alter plankton phenology in freshwater. For this reason, in the present PhD thesis, long-term limnological investigations and a paleolimnological study on deep subalpine lakes, and in particular on Lake Iseo, were combined in order to expand the knowledge on the zooplankton communities and on their changes in relation to the synergic effect of climate change and eutrophication in deep and oligomictic lakes.

Long-term series of chemical physical and plankton observations have been analysed and the results were compared with that reported for Lake Lugano and other subalpine lakes, characterised by different trophic levels and ecological evolution. In that way, some important trends of populations and communities' responses to global and local impacts were detected. Paleolimnological study on Lake Iseo confirmed and reinforced long-term investigation results allowing to investigate period of time for which measured limnological data are not available. Moreover, it makes possible reconstruct past lakes ecological evolution and examine biological community changes in the passage from phases dominated by local stressors as nutrients loads, to phase in which local and global stressor synergically impacted the lake ecosystems.

Long-term investigation of deep lakes limnological dataset highlighted a strong relation between climate variability, well represented by teleconnection indices, and zooplankton phenology. Warmer water temperature in spring anticipated the timing of population recovery after diapause of both primary and secondary consumers. Additionally, in deep oligomictic lakes the effects of climate change on zooplankton community and pelagic food web resulted particularly complex. The winter climatic conditions trigger a cascading effect on the lake ecosystem and on pelagic food webs, influencing nutrient availability. It has been verified that in deep lakes, the increase of water temperatures and the consequently enhanced stratification

period caused a reduction in the vertical extension of the late winter water turnover. This reduction has a strong impact on the phosphorus replenishment of the epilimnetic layers and leads to phosphorus segregation in hypolimnion.
An important finding of my PhD thesis is that in lake characterized by different trophic levels food webs components answer differently to climatic variations. More precisely, in oligotrophic lakes, the cascading effects triggered by climate influenced the algal carrying capacity and primary consumers population density. Conversely, in a lake characterised by high phosphorus concentration, the spring enrichment in nutrients did not result in either a considerable increase of phytoplankton growth, nor, consequently, in a zooplankton density peak.

It has been proved that regular lake seasonal events, which patterns have been well studied in several kind of lakes, as the clear water phase and *Daphnia* midsummer decline, resulted affected and altered by climate change leading to severe consequences to the entire food web structure. Indeed, as demonstrated in some oligomictic lakes, as Lake Lugano, the cascading effect triggered by winter air temperature reverberated until primary consumers summer phenology, altering *Daphnia* midsummer decline intensity. Warmer temperature negatively affected primary consumers (in particular, *Daphnia*) abundance through the control of food availability during the clear water phase. On the bases of these findings, a progressive increase in winter air temperature could contribute to causing critically low densities of primary consumers, and these declines may compromise the ability of zooplankton to control phytoplankton biomass.

The paleolimnological investigation, through the combined study of biotic and abiotic factor, allowed clarifying the synergic effects of the most important driver of change in lake ecosystems, suggesting that climatic factors should be considered with nutrient availability as determinant element in controlling the temporal development of plankton communities and pelagic food web structure. At the beginning of 20th century, when global warming was not yet so accentuated, the nutrient increase in water resulted as the principal driver in determining the long-term development of plankton communities and pelagic food web structure. Moreover, in Lake Iseo a potential effect of catchment area hydrological variation on the biological community has been detected. Even if there are still some difficulties in relating punctual data as a floods event with paleolimnological data that integrate information about a longer period of time, paleolimnology can be a powerful tool to track the role of hydrological variability in affecting environmental and ecological lake conditions.

The results presented in the previous chapters highlight the high vulnerability of deep lakes to climate change and the difficulties in disentangling the effects of global stressor on biological community, due to the interaction with other anthropic and local pressures as eutrophication. In a scenario of generalized and worldwide increasing temperature and in the light of the obtained results is possible to predict that other temperate lakes that are actually monomictic may eventually become meromictic and face new emerging problems as primary consumers decline and increased summer algal blooms. Moreover, the enhanced water stability and the consequent phosphorus segregation in hypolimnetic layers leads to a re-oligotrophication process in the surface water with a supposed reduction of the eutrophication issue. However, in temperature-induced meromictic conditions an occasionally complete water turnover, due to a particularly harsh winter, could redistribute the segregated phosphorus among the water column and interfere with lake management strategies.

The deep subalpine lakes are all located in the same climatic region and they have very similar mixing vertical mixing dynamics. However, they show different biological responses to climatic condition on the bases of their trophic levels and evolution. This remarked the difficulty in predicting the possible effects of future natural and anthropogenic stressors on lake ecosystems because the lake response is strongly dependent on its ecological characteristics. Comparing the underling mechanism in more than one study site resulted crucial in order to define patterns of variability and to hypothesize causes and consequences. The availability of long-term limnological data sets, collected with standardized methods, resulted crucial in conducting comparative studies on lakes. Indeed, long-term data sets provide a precious opportunity to recognize common or contrasting patterns of variability and to clarify how those patterns change at different scales and which are the possible causes and consequences.

In conclusion, my PhD work allowed reconstructing the complex effects of climatic variability and in particular winter air temperature on deep lakes pelagic food webs. Combining long-term limnological investigation on different lakes with paleolimnological studies allowed disentangling the main driver of change in pelagic food web structure and functioning. The presented results can be the bases to calibrate efficient lake management strategies aimed to improve and maintain lake water quality, ecosystem services and biodiversity.

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