REVIEW

Long-term studies for evaluating the impacts of natural and anthropic stressors on limnological features and the ecosystem quality of Lake Iseo

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ABSTRACT

We review the state of the art of limnological studies in Lake Iseo and provide updated data concerning long-term investigations (from 1993 to 2018) carried out on chemical and physical parameters (*e.g.*, oxygen, phosphorus, silicon). Changes observed in Lake Iseo were compared with those reported in other Deep South alpine Lakes (DSLs) to highlight analogies and differences of long-term chemical, physical, and biological patterns. Until the 1960s, Lake Iseo and other DSLs were oligotrophic. The increase of anthropogenic pressure and global warming has led to a progressive and unrecovered process of eutrophication. Moreover, the decrease in frequency of full mixing episodes has induced a state of temporary meromixis. Other changes have been identified over the last two decades, especially concerning the phytoplankton and zooplankton communities, and new emerging chemical pollutants were detected. Given the important ecological and socioeconomic role of Lake Iseo, long-term investigations are of paramount importance to understand the response of the lake ecosystem to climatic and anthropogenic stressors. These two factors can also act coupled with new combined and synergic effects.

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INTRODUCTION

Lake Iseo is one of the deepest lakes south of the Alps, one of the largest Italian lacustrine basins, and an important resource for agriculture, industry (manufacturing and building centers are its peculiarities), fisheries, and drinking water, as well as for recreation and tourism.

Its inflow and outflow are the Oglio river, which is one of the major tributaries of the Po river, and it crosses the Po plain for about 156 km. The river is formed by the confluence of two alpine streams, one from the Presena glacier in the Adamello group, and the second from the Corno dei Tre Signori (in Stelvio National Park). The water level of the lake is controlled by a dam built in 1933 at the outflow of the Oglio river, which regulates water release in response to irrigation demand, while maintaining a sufficient flow in the river to sustain the health of downstream ecosystems (Rotiroti *et al.*, 2019). The lake is characterized by a high ratio between watershed area and lake surface (28.46); this feature, along with the mean annual precipitation value, which is about 900 mm y^{-1}, strongly influences the characteristics of Lake Iseo, particularly the theoretical renewal time that is about four years (Ambrosetti and Barbanti, 1999) (Fig. 1).

Considering its geographical position and morphological features, this lake is strongly influenced by increasing anthropogenic pressure and changes in meteoclimatic conditions. These aspects induce both direct and indirect impacts on the lake ecosystem. In effect, this lake should be a naturally oligotrophic water body; however, over the past 40 years, the increase of nutrient loadings brought it to a meso-eutrophic condition (Leoni *et al.*, 2014a; Salmaso *et al.*, 2014a). For an important system like the one of Lake Iseo, which provides many fundamental ecosystem services, continuous monitoring and reliable data to understand and predict physical, chemical, and biological evolution are crucial for social-welfare and management purposes. For this reason, since the 1970s, different research institutions have carried time series of limnological and ecological studies on Lake Iseo. They have worked independently or in synergy to improve knowledge on the ecosystems and to assess their dynamics. Since the 1990s, research group of Freshwater Ecology (Department of Earth and Environmental Sciences, University of Milano-Bicocca) has sampled the lake on a monthly basis, analyzing the physical-chemical parameters of the lacustrine water, as well as the biotic community and the pelagic food web. Monitoring programs have generated a wealth of data; details of parameters, sampling sites, depth, frequencies, sampling, and analysis methods are listed in Tab. 1. These data have justified the inclusion of Lake Iseo in the Italian and European Long-Term Ecological Research network (LTER) and in the Global Lake Ecological Observatory Network (GLEON).

The present study aims to synthesize the results of long-term investigations about the limnological state of Lake Iseo, providing updated data. Major achievements obtained during several decades of research help us to outline knowledge gaps and future perspectives. All that is discussed in the context of climatic changes that act coupled with pre-existing stressors like eutrophication; these factors can work in synergy and reveal their effects on the lake ecosystem. Changes observed in Lake Iseo were compared with those reported in the other deep south alpine lakes (DSL) to highlight analogies and differences of long-term chemical, physical, and biological patterns. In particular, we focus on the following aspects: i) the trophic evolution of the lake; ii) the thermal dynamic related to climatic changes that influences nutrient availability; iii) evolution in the phytoplankton community, with a special focus on cyanobacteria; iv) zooplankton and planktonic food webs; and v) a mention to emerging pollutants and microplastics.

TROPHIC EVOLUTION

Throughout the last century, the main environmental issue concerning Lake Iseo was eutrophication. Lake Iseo should naturally be an oligotrophic phosphorus limited water body; however, over the past 40 years, the increase of nutrient loadings brought it to a meso-eutrophic condition (Leoni *et al.*, 2014a; Salmaso *et al.*, 2014a).

After the 1960s, anthropogenic influences induced an acceleration of eutrophication that completely changed the ecological characteristics of this lake as well as of most of other DSLs (Barbieri and Mosello, 1992). This process had negative effects on physical-chemical and biological features, as well as on food-web functions, *e.g.*, decreasing the transparency of lake water, high oxygen concentration in the superficial layers, a marked decrease of oxygen in the hypolimnetic layers, and changes in the biovolume and structure of planktonic communities. Starting from the 1970s, the whole coast was engaged in a project of sewage treatment, with the building of two treatment plants located at the northern and southern ends of the lake (Garibaldi *et al.*, 1999).

The long-term dynamics of soluble reactive phosphorus (SRP) reflects complicated variations linked to the stratification patterns and to the release of P from the sediments under anoxic conditions. Mean annual SRP concentrations for the whole water column increased from around 10 µg P L^{-1} in 1967, to 20, 32, and 50 µg P L^{-1} in 1973, 1980 and 1990, respectively (Garibaldi *et al*., 1998). After 2001, concentrations progressively increased, reaching the maximum value of 77 µg P L^{-1} in 2018. The increase of P can also be explained by considering faulty sewage systems (Barone *et al.*, 2019) and the impact of external loads, of which the contribution should be taken into account for the evaluation of water quality (Nava *et al.*, 2019). Indeed, as reported by Garibaldi *et al.* (1999), a total load of 93 t y^{-1} of phosphorus was estimated in

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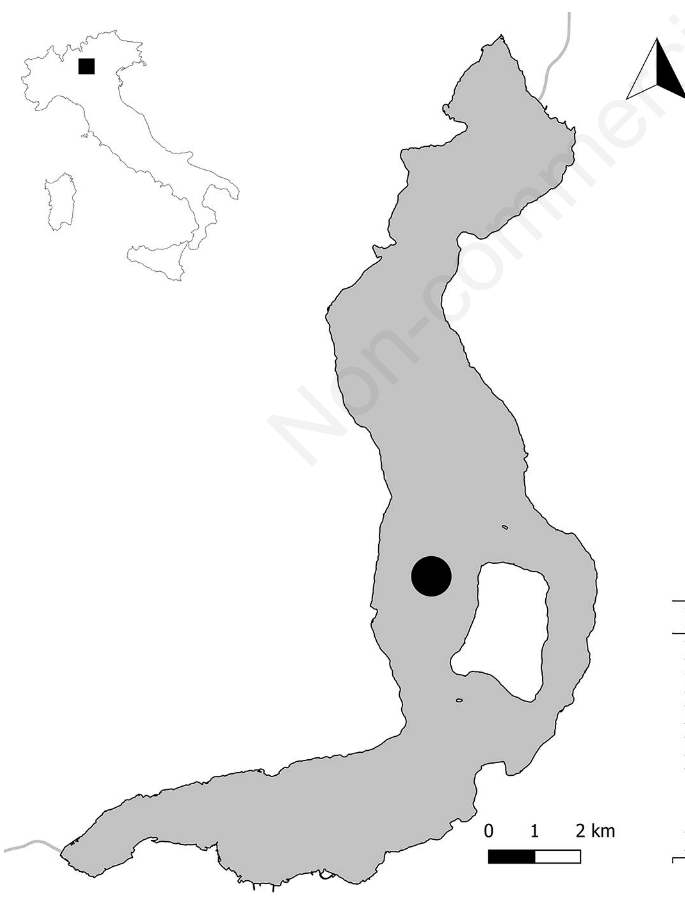

Parameter	Unit	Value
Lake altitude	m asl	186
Lake area	km^2	62
Maximum depth	m	251
Mean depth	m	123
Volume	km^3	7.57
Catchment area	km^2	1842
Mean outflow disch.	$m^3\ s^{-1}$	58.7

Fig. 1. Location, bathymetry and principal morphometric and hydrological characteristics of Lake Iseo. The dot indicates the position of the sampling point.

1995-1997 from the main tributaries, principally deriving from nonpoint sources.

The phosphorus trend observed in Lake Iseo is in contrast to those measured in other DSLs (lakes Garda, Lugano, Como, Maggiore), since the measures put in place in these lakes for the reduction of nutrient loads allowed a progressive decrease of external phosphorus load (Rogora *et al.*, 2018). However, due to a reduced tendency to the total mixing of the whole water column (see following paragraph), hypolimnion conditions of some of these lakes (*e.g.*, Maggiore) are experiencing a decrease in Dissolved Oxygen (DO) concentration that is likely to result in a release of phosphorus from sediment, consequently leading to an increase of this nutrient in the bottom layers (Rogora *et al.*, 2018). As long as mixing events do not occur, this phosphorus would be segregated at the lake bottom and not be available for primary producers.

THERMAL DYNAMICS, MIXING DEPTH, NUTRIENT AVAILABILITY, AND CLIMATE CHANGE

Lake Iseo should be classified as warm monomictic, as water temperature never drops below 4°C. However, it has to be considered a holo-oligomictic lake; indeed, owing to the great depth, complete circulation of the water column does not occur every year, but only after harsh and windy winters. This event merely concerns the superficial layers of the lacustrine water, and in the last few decades, the lake has sporadically experienced complete vertical overturn (Leoni *et al.*, 2014a).

Thanks to the growing availability of physical data, the significant warming and increased stability of the water column of Lake Iseo have been documented after 1990 (Ambrosetti and Barbanti, 1999). In Lake Iseo, the

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Tab. 1. List of the parameters collected in Lake Iseo since 1993 and description of instruments and methodologies adopted.

Parameter	Depth	Instrument	Period	References
Transparency	-	Secchi disk	1993-ongoing	-
Dissolved oxygen(mg L^{-1})	Discrete sampling*	WTW OXI 320 and Multi 3630 IDS	1993-ongoing	-
pH	Discrete sampling*	Radiometer PHM 83	1993-ongoing	-
Temperature(°C)	Discrete sampling *Continuous (0-250 m)	Mercury-filled Celsius reversing thermometer TD-Diver	1993-ongoing 2018-ongoing	-
Electrical conductivity(μS cm^{-1})	Discrete sampling *Continuous (0-250 m)	Radiometer CDM TD-Diver	1993-ongoing 2018-ongoing	-
Alkalinity(ppm $CaCO_3$)	Discrete sampling*	Acidimetric titration Gran's method	1993-ongoing	-
Total phosphorus(μg L^{-1})	Discrete sampling*	Spectrophotometer Molybdenum reaction	1993-ongoing	Valderrama (1981)
Total nitrogen(μg L^{-1})	Discrete sampling*	Spectrophotometer (272/220nm)	1993-ongoing	Valderrama (1981)
Soluble reactive phosphorus(μg L^{-1})	Discrete sampling*	Spectrophotometer Molybdenum reaction	1993-ongoing	APHA (1985)
Ammonia-nitrogen(μg L^{-1})	Discrete sampling*	Spectrophotometer Indophenol reaction	1993-ongoing	APHA (1985)
Dissolved silicon(μg L^{-1})	Discrete sampling*	Spectrophotometer Molybdenum reaction	1993-ongoing	APHA (1985)
Anions:NO_3^-, Cl^-, SO_4^{2-}(mg L^{-1})	Discrete sampling*	Ion-chromatography (Dionex ICS 1100)	1993-ongoing	Manual, Thermo Fisher Scientific In.c (2012)
Cations: Ca^{2+}, Mg^{2+}, Na^+, K^+ (mg L^{-1})	Discrete sampling*	Ion-chromatography (Dionex ICS 1100)	1993-ongoing	Manual, Thermo Fisher Scientific Inc. (2012)
Chlorophyll-a	Continuous (0-20 m)	Zullig's bottle for integrate samples	1993-ongoing	Lorenzen (1967)
Phytoplankton sampling	Continuous (0-20 m)	Zullig's bottle for integrate samples	1998-ongoing	-
Phytoplankton analysis	Subsamples	Zeiss Plankton IM inverted microscope using Utermöhl's technique	1998-ongoing	Lund *et al.* (1958) Smayda (1978)
Zooplankton sampling	Continuous (0-20 m)	Plankton nets (50-200 μm)	1998-ongoing	-
Zooplankton analysis	Subsamples	Zeiss Plankton microscope	1998-ongoing	de Bernardi and Canale (1995)

*Discrete sampling depths are the following: 0, 10, 20, 30, 50, 75, 100, 150, 200, 245 m.

rate of temperature increase in the water column and/or hypolimnetic waters in the period between the 1970s and 2005/2009 ranged between 0.01 and 0.03°C y^{-1} (Salmaso and Mosello, 2010). The increasing trend of water temperature is common in DSLs, as synchronous temporal patterns of water temperature were evident across the lakes, particularly since 2006, after the complete turnovers of 2005-2006, which caused a sudden cooling of the whole water column (Rogora *et al*., 2018). During the period between 1986 and 2015, the warming trend in the surface waters of the Lake Iseo and other DSLs was further evidenced through satellite data (Pareeth *et al.*, 2017).

During the last four decades, mixing episodes involving the deepest waters of Lake Iseo occurred at the beginning of the 1980s, in 1999, 2000, 2005, and 2006. In other years, mixing depths ranged from 30 to 200 m with no regular pattern. In the most recent years (2014-2018), mixing depths reached less than 30% of the water column and less than 50% of lake volume (Fig. 2; Rogora *et al.*, 2018). Thermal stratification that began in spring was usually well-established between June and August, with thermocline depth varying from 10 to 20 m. Owing to climatic factors (mild winters) and the accumulation of salts in the hypolimnion, Lake Iseo may currently be defined as temporary meromictic. The accumulation of salts mainly depends on the precipitation of calcium carbonate, which is a direct effect of high productivity in water rich in calcium and bicarbonate, as is the case with Lake Iseo (Garibaldi *et al*., 1999).

Numerical simulations were applied to Lake Iseo to predict the effects of climate-change scenarios on lake thermal evolution (2012-2050). Results showed an overall average increase in lake-water temperature (0.012°C y^{-1}) and reinforced Schmidt thermal stability of the water column in the winter, which may further hinder the deep circulation process (Pilotti *et al.*, 2013;Valerio *et al.*, 2015). A comparable result was obtained in the study of Fenocchi *et al.* (2018), in which the application of the General Lake Model (GLM) to Lake Maggiore suggested that persistent lack of complete mixing, severe water warming, and extensive effects on water quality are to be expected for centuries to come for all DSLs.

During the second half of the 1980s, there was a marked decrease in hypolimnetic oxygen concentrations, and anoxia conditions were established during the 1990s (Garibaldi *et al*., 1999, 1995). The full mixing of 2005 and 2006 partially recharged dissolved oxygen in the water column, reaching around 60% saturation (Leoni *et al*., 2014a). However, the effects of these events did not persist, and deep-water DO again started to decrease soon afterwards. A hypoxic/anoxic condition was again established from 2010 onwards, with DO concentrations in the deepest layers (>150 m depth) steadily below 1.2 mg O_2 L^{-1}. On the basis of the available data, the period from 2010 to 2018 represents the longest recorded phase of anoxia that Lake Iseo experienced (Rogora *et al*., 2018).

The volume-weighted average DO concentration on the whole water column measured during the spring turnover showed stable values between 4 and 6 mg O_2 L^{-1} in Lake Iseo; a slight tendency towards decreasing DO levels was

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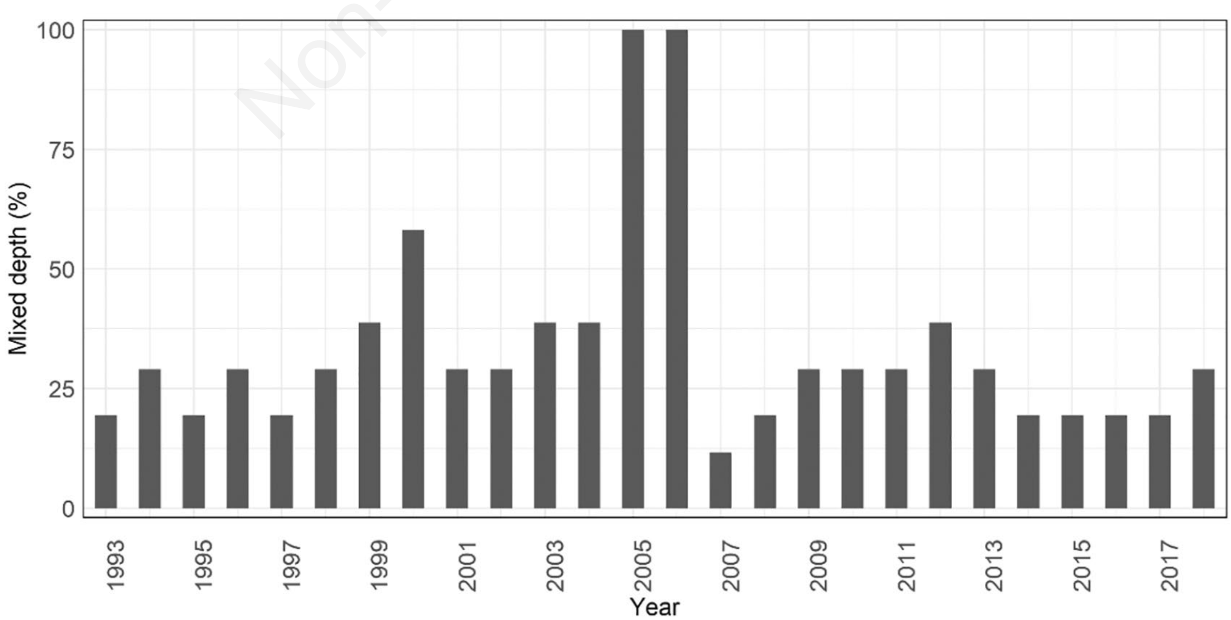

Fig. 2. Mixed depth as % of the maximum lake depth at winter-spring turnover in the Lake Iseo in the period 1993-2018.

observed during the last five years, declining below 4 mg O_2 L^{-1}. In particular, anoxic conditions affect a growing hypolimnion volume due to a reduction of the renewal time of the deepest waters. These conditions promote phosphorus release from the sediment and the accumulation of reduced chemical species, such as sulphide, and methane, with severe consequences for the entire lake ecosystem (Posch *et al*., 2012; Rogora *et al*., 2015). These changes could have a huge impact on water usability, particularly for fishing, drinking, and recreational purposes.

In Lake Iseo, TP concentrations gradually increased in the hypolimnion (150-251 m) after 2005, from about 60 to 90-100 μg P L^{-1}, and even more in the deep-water layer (below 100 m: from 100 μg P L^{-1} during the period 1993-2005 to 140-160 μg P L^{-1} during 2014-2018 (Rogora *et al*., 2018).

A similar pattern to phosphorus emerged for hypolimnetic (150-251 m) dissolved silica (DSi) in Lake Iseo (Fig. 3). Indeed, DSi concentration was quite constant from 1993 to 2004, with average values (±standard deviation) of around 1509±92 μg Si L^{-1}. These values are higher than the ones observed for the same period in the superficial layers (0-20 m), where average values were 652±346 µg Si L^{-1}. In 2005, DSi concentration abruptly decreased, reaching a hypolimnetic value of 1300 μg Si L^{-1} as a result of the complete circulation event that mixed bottom and superficial waters. However, after this abrupt decrease, DSi concentration gradually increased, reaching a value of about 2300 μg Si L^{-1} in the most recent years. This increase can be linked to the change of the thermal behavior of Lake Iseo from oligomictic to meromictic, which is leading to the segregation of dissolved silica in the deepest layers. That may have consequences on diatom populations, dominating and supporting the pelagic community (see following paragraph), and, in a broader context, on the trophic web and productivity of Lake Iseo. In fact, several studies documented that the Si internal loading from sediments can contribute over 40% of total annual DSi to lakes, and may thus be an important flux in the overall Si budget and for sustaining the growth of siliceous phytoplankton (Wang *et al.*, 2016).

The resistance to mixing, which causes detrimental effects to the deeper layers, could have apparent beneficial effects on surface waters and induce the reoligotrophication of the epilimnetic layers of the lake (Fig. 4). However, considering Lake Iseo, even if the internal load is segregated at the bottom of the lake, external loads are enough to maintain high levels of primary productivity (Barone *et al*., 2019). On the other hand, future complete mixing with the subsequent replenishment of the superficial layers with nutrients and reduced substances, may have detrimental effects on the entire lake ecosystem, as what happened in the past in Lake Lugano (Holzner *et al*., 2009).

Many studies and synoptic analyses performed on long-term data (20-27 years) demonstrated that both the local climate (*e.g*., air temperature) and some large-scale climate patterns (*e.g*., teleconnection indices) impacted spring-water temperatures and the mixing pattern of large and deep lakes south of the Alps (Manca *et al*., 2015; Salmaso *et al*., 2014). Climate variability in winter, thermal structure, mix-

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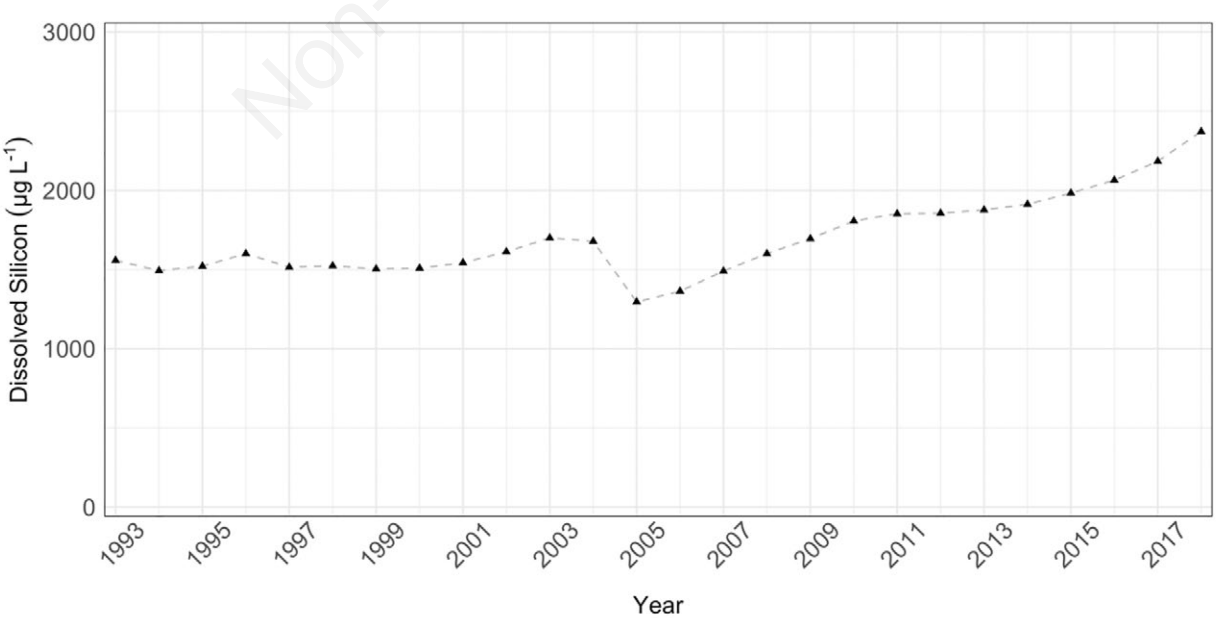

Fig. 3. Trend of annual concentrations of dissolved silicon (DSi) in the hypolimnetic layers (150-251 m) of Lake Iseo from 1993 to 2018.

ing regime, and the vertical redistribution of oxygen and nutrients in DSLs appeared to be controlled by two inversely correlated modes of atmospheric circulation relevant for the Mediterranean area: the East Atlantic (EA) pattern and the Eastern Mediterranean Pattern (EMP; Salmaso *et al*., 2014). In particular, the negative value of winter EA (or positive of winter EMP) led to cold winter-air temperature in the Lake Iseo area that caused deeper mixing during vertical spring turnovers and an increase of epilimnetic-nutrient (*e.g*., phosphorus) replenishment. However, a positive value of winter EA (or negative of winter EMP) leads to warm winter-air temperature, a shallow mixing depth during vertical spring turnovers, and a segregation of hypolimnetic layers (Leoni *et al*., 2018).

PHYTOPLANKTON COMMUNITY AND CYANOBACTERIA

Plankton communities are at the foundation of aquatic-ecosystem functioning, and an in-depth comprehension of their dynamics is fundamental to manage aquatic resources, and to predict and face future environmental changes. Long-term series of plankton analyses provide essential datasets for characterizing reliable patterns of seasonal and multiannual trends, and shifts of populations and communities in response to global or local impact (Morabito *et al.*, 2018).

Standardized phytoplankton studies in the euphotic layers (0-20 m depth) of Lake Iseo have existed since the 1990s. In the last thirty years, about 60 phytoplankton taxa belonging to seven taxonomic groups were identified. At least three main algal groups dominated in terms of Iseo's biovolume community throughout the last three decades: Bacillariophyceae, Conjugatophyceae, and Cyanobacteria (Garibaldi *et al.*, 2003; Leoni *et al.*, 2014b). Studies of vertical phytoplankton distribution showed that spatial heterogeneity in the vertical dimension is much more significant than that in the horizontal dimension. Indeed, results on phytoplankton biovolume and Chl-*a* indicated that there is no significant variability in spatial distribution among sampling sites located some kilometers from each other (Leoni *et al.*, 2014a). Phytoplankton can optimize their depth in the water column to maximize their growth rate using temperature, light, and nutrient gradients (Reynolds, 2006). In summer, water temperature and light decrease with depth, while nutrients usually increase. In Lake Iseo, chemical parameters and inorganic nutrient concentrations showed a typical pattern for monomictic lakes, with low values of conductivity, phosphorus, nitrogen, and silica compounds in the surface layer in summer (Diehl, 2002; Minella *et al.*, 2016; Salmaso *et al.*, 2012). A combination of field observations and 3D hydrodynamic simulations were used to identify phytoplankton species, and estimate the various time scales of the dominant physical and biological processes in Lake Iseo during a stratified period, deriving phytoplankton patch categorization and growth interpretation that provides a general framework for the spatial distri-

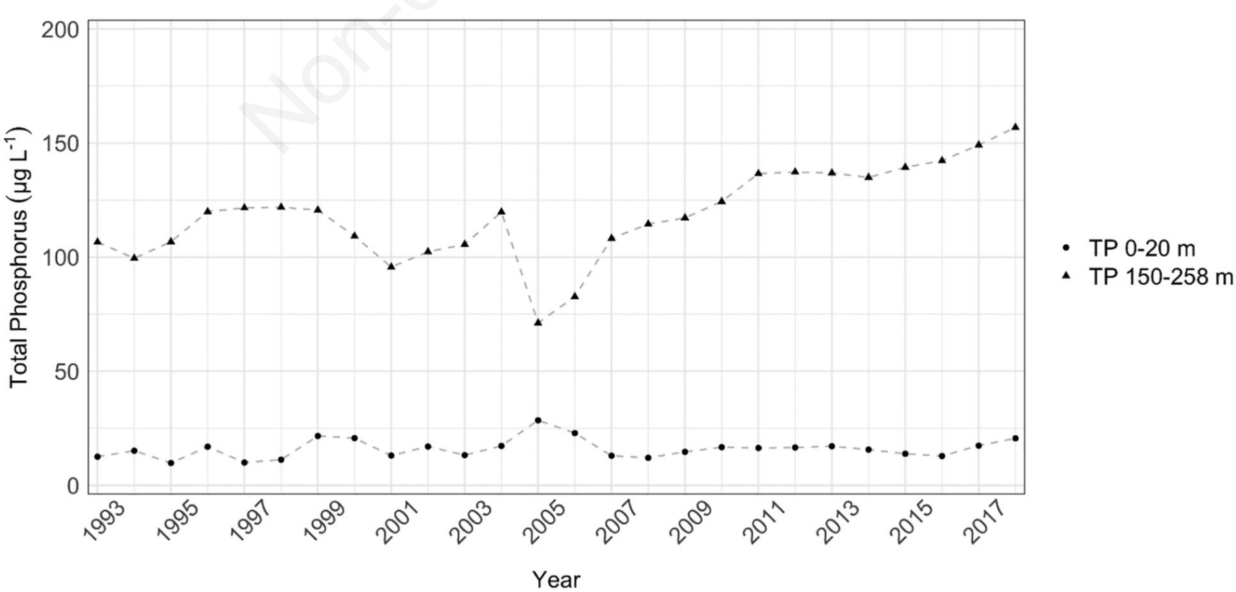

Fig. 4. Trend of annual concentrations of total phosphorus (TP) in surface (0-20 m) and hypolimnetic layers (150-251 m) of Lake Iseo from 1993 to 2018.

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bution of phytoplankton concentration in Lake Iseo (Marti *et al.*, 2015). During high water stability, vertical-transport time scales were much shorter than horizontal transport and vertical dispersion, leading to a growth niche for the observed phytoplankton.

The large Bacillariophyceae were dominant mainly during late winter and early spring (*Aulacoseira* spp., *Melosira varians, Asterionella formosa*), Conjugatophyceae (mostly *Mougeotia* spp.) developed mainly in spring and summer, and Cyanobacteria (mostly *P. rubescens*) developed in summer and autumn, following a fairly regular temporal trend during the years. Furthermore, over the last few years, there have been several episodes of blooms of cyanobacterium *Dolichospermum lemmermannii*, in particular, in conditions of high nutrient concentrations and high water temperature (Salmaso *et al.*, 2015). However, the described sequence can be integrated by the development of other algal taxa such as Dinophyceae and Chlorophyceae (Garibaldi *et al.*, 2003; Hernández-Avilés *et al.*, 2018; Leoni *et al.*, 2014b).

The greater importance assumed by the different algal groups in each year may be due to changes in meteoclimatic conditions during the growing season and to the different nutrient amounts recycled from the deep to the surface layers. In particular, in Lake Iseo, spring nutrient enrichment of epilimnetic layers, promoted by great vertical mixing depths due to harsh and windy winters, did not affect the increase of total phytoplankton biomass and Chl-*a* concentrations, probably because an additional input of phosphorus in the already enriched epilimnion had minor effects (Leoni *et al.*, 2018). However, some effects on phytoplankton composition have been observed as, after deep mixing events, a great development of algal taxa with a high level of P content occurred (Leoni *et al.*, 2014a).

During the last two decades, the predominant species in the cyanobacterial taxa was *Planktothrix rubescens*. However, since 2014, the presence of "cryptogenic" species of Cyanobacteria, *Tychonema bourrellyi*, able to produce consistent biomasses and harmful toxins (anatoxin-a and homoanatoxin-a), was detected. Stratified conditions in the aquatic system seem to be a key factor for the settling of *T. bourrellyi*, which cannot proliferate in lakes with deep mixing events. In Lake Iseo, enhanced stability triggered by higher air temperature promoted the development of these allochthonous Cyanobacteria, which has created their niche as a result of the more stable water column and reduced mixing depths during the winter (Nava *et al.*, 2017). This important element of change is a good example of the taxonomic and ecological lack of knowledge on lake microbiota, and of the need for a more detailed list of potentially toxic species and the distribution of cyanotoxins in freshwater bodies (Salmaso *et al.*, 2018b). Interesting insights for the monitoring of cyanobacteria and phytoplankton blooms in Lake Iseo, among other DSLs, have been provided by the use of satellite data (Bresciani *et al.*, 2018, 2011). The first account on cyanotoxin distribution was carried out by Cerasino and Salmaso (2012) and updated in 2016 (Salmaso *et al.*, 2018a). In Lake Iseo, the conspicuous seasonal development of *P. rubescens* during spring and early summer corresponds to the increase of microcystin concentrations. Instead, the coexistence of *Planktothrix* and *Tychonema* could explain the seasonal increase in anatoxin-a (Salmaso *et al.*, 2018b).

ZOOPLANKTON AND FOOD WEBS

In Lake Iseo, the zooplanktonic community has been regularly sampled since the 1990s until today with monthly or bimonthly frequency. The huge quantity of collected data allowed to conduct long-term studies concerning the response of zooplankton-population dynamics to climatic variability and environmental changes, helping to clarify the effect that a change in zooplankton phenology can have on the entire lake pelagic food web. Furthermore, this component is an early warning bioindicator of hydrophobic pollutants, as the zooplankton response to pollution changes resulted particularly prompt.

The majority of research about zooplankton-population dynamics in Lake Iseo focused on the effects of vertical mixing dynamics on epilimnetic phosphorus replenishment and on plankton phenology. In particular, Leoni *et al.* (2014a) investigated the effects of mixing-depth changes, during the late-winter and early-spring period, on the clutch size and population development of the *Daphnia longispina* group, the fundamental primary consumers in the pelagic food web. The study highlighted the effects that the great replenishment of phosphorus in epilimnetic water levels, due to complete vertical mixing events, had on the growth rate of *Daphnia* spp. The phosphorus increase in the surface-water layers seemed to predict the reproduction and population density of *Daphnia* species, probably due to an increase in food quality (Chlorophyta and large diatoms).

Winter climatic variability is a key factor in controlling vertical mixing depth and consequent phosphorus replenishment, triggering a cascading effect on lake limnological characteristics and on zooplankton phenology. Leoni *et al.* (2018) verified that winter large-scale circulation patterns (EA and EMP) control a chain of linked causal factors affecting winter-air temperature, spring-water temperature, resulting water vertical-mixing depth, and the epilimnetic concentration of total phosphorus and plankton phenology, including both primary and secondary consumers. This study indicated that, in Lake Iseo, characterized by high phosphorus concentration, a harsh winter corresponded to less favorable conditions for plankton development. In correspondence of a particu-

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larly harsh winter, primary and secondary consumers postponed the time in which the population starts to increase after winter diapause, and the time in which primary consumers reach maximum density. This study demonstrated a different answer to climatic variability in eutrophic lakes such as Lake Iseo, compared to oligotrophic ones. Indeed, in oligotrophic lakes, the increase of *Daphnia* is triggered by an increase of phytoplankton growth deriving from spring enrichment in nutrients (Manca *et al.*, 2015). In Lake Iseo, spring enrichment in nutrients did not result in a considerable increase of phytoplankton growth, as phosphorus is always available in sufficient concentrations. Thus, we hypothesized that the increase of zooplankton density can be linked to different phytoplankton quality instead of quantity (Leoni *et al.*, 2018; Patelli *et al.*, in press).

The cascading effect triggered by winter-air temperature on spring- and summer-zooplankton phenology in Lake Iseo was also confirmed by Patelli *et al.* (in press). The aim of this research was to disentangle the factors causing a *Daphnia* population decline in summer *(Daphnia* midsummer decline) in the lakes of Lugano and Iseo. Winter-air temperature (through phosphorus replenishment) influenced *Daphnia* abundance in spring in both lakes, but the effects were only propagated to summer *Daphnia* abundance in Lake Lugano. Additionally, summer *Daphnia* abundance was influenced by summer-air temperature through a positive effect. Results revealed some differences between the two study lakes, suggesting that, in Lake Iseo, which was characterized by stable mesotrophic conditions since the beginning of the 1990s, the decline of *Daphnia* during summer is probably due to a physiological population decline after the spring peak. Indeed, the intensity of the *Daphnia* midsummer decline in this lake has very low interannual variability.

The impact of the long-term chain of effects triggered by winter EA on *Daphnia* were also documented for the lakes of Garda and Maggiore (Manca *et al.*, 2015). The effects of epilimnetic nutrient replenishment triggered by winter temperatures and plankton-biomass mixing were detectable in the meromictic Lake Lugano. Despite being associated with lower P concentrations, warmer-than-usual winters and summers led to increases in summer chlorophyll-a concentrations through complex direct and indirect (food-web) effects (Lepori *et al.*, 2018).

In Lake Iseo, a study about the zooplanktonic food-web structure was conducted in order to detect the predator-prey relation considering organism-body size (Leoni, 2017). In particular, the relation between the main predator, *Leptodora kindtii*, and the potential prey (*Daphnia* spp. and *Eubosmina longicornis*) was reported by analyzing the stable isotope of C and N. Size-specificity was crucial for addressing space and time changes in trophic links between organisms composing the two hierarchical levels within the open-water zooplankton community. Indeed, among prey encountered by a free-swimming *Leptodora*, only those able to fit into the basket opening could be captured. As basket diameter increases with animal-body length, prey size selection depends on *L. kindtii* body length. In particular, in spring, small-sized *E. longicornis* can be more suitable prey for *L. kindtii* than *Daphnia*. Cyclopoid adults were at the top of the zooplankton food chain in Lake Iseo, and they could potentially be feeding on *Daphnia*. They, however, likely fed in a different habitat (>20 m deep water).

EMERGING POLLUTANTS AND MICROPLASTICS

Industrial and agricultural production has contributed to the input of several pollutants in Lake Iseo. Studies have focused on legacy (*e.g.*, DDT, PCB) and current-use POPs (*e.g.*, polycyclic aromatic hydrocarbons, PAHs) (Binelli and Provini, 2004, 2003; Mazzoni *et al.*, 2019; Pascariello *et al.*, 2019). Salmaso and Mosello (2010), and recently Guzzella *et al.* (2018) in detailed studies reported the presence and concentrations of several organic and inorganic pollutants in Lake Iseo, as well in many other southern alpine lakes.

Several recent studies demonstrated the presence of micropollutants in different aquatic species (Beone *et al.*, 2011; Bettinetti *et al.*, 2012a); some of these originated from the melting of glaciers that released pollutants accumulated in the past (Bettinetti *et al.*, 2012a). Even in the case of Lake Iseo, an input of DDTs from secondary sources was detected in the 2000s; sediment analyses confirmed that this load caused a drastic increase of DDTs from 1991 to 2008, with higher values than those measured during the 1970s, when DDT was largely used in agriculture (Bettinetti *et al.*, 2011). A similar situation was observed in another DSL (Maggiore; Bettinetti *et al.*, 2012b).

An issue of emerging concern is microplastics, of which the presence in freshwater ecosystems is increasingly reported. In 2018, during the 10th edition of *Goletta dei Laghi*, a first monitoring of microplastics presence in the lakes of Iseo, Garda, and Maggiore was carried out. Results showed that microplastics (<5 mm) were found in all samples from the surveyed lake surfaces. The highest concentration was reported in Lake Iseo, with a value of 57,000±36,000 particles km^{-2} in the sampling transect performed between Lovere and Pisogne (Sighicelli *et al.*, 2018).

KNOWLEDGE GAPS AND FUTURE PERSPECTIVES

In these last few decades, Lake Iseo has experienced important and continuous changes attributable to both

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well-known stressors and emerging or never-before-considered types of impact. Many studies were already published, monitoring activities are always in progress, and several other research topics have to be addressed. Its responses are, in some cases, comparable to those globally reported in other lakes, but different local effects can also be highlighted. Indeed, the complex interactions of different drivers (both climatic and anthropogenic) can affect aquatic systems on both a global and a local scale, on which the intrinsic characteristics of individual lakes also play a role. The complexity of these interactions has only been studied to a limited extent, and much is yet to be known about their single and/or synergic effects, and about lacustrine ecosystem responses.

Long-term investigation is an important component of our activities, giving us the power to detect and attribute the causes of environmental change, and to provide insights into how lakes function and react. However, further studies are still required to understand the legacy of processes that occurred in the past that are acting in combination with new and emerging stressors.

In other DSLs, long-term lake-monitoring data are now supplemented by high-frequency data collections that provide almost real-time information on lake condition and function, which is particularly valuable for lake modelling. To implement this technology on Lake Iseo, it could be beneficial to increase the ability to model and forecast long-term trends in lake thermal structure and P dynamics, crucial for developing reliable recovery plans. It is compelling to assess the role of climate change since climatic variations are somehow intensifying the symptoms of pre-existing negative types impact in freshwaters (Moss *et al.*, 2011).

One of the most important open issues that strictly involve lake management is eutrophication. Major efforts should be made to study catchment processes, especially focusing on the watershed load of nutrients on which lake communities rely, i.e., nitrogen and phosphorus. Indeed, even if investigations were done to estimate the load linked to the watershed (Barone *et al.*, 2019; Garibaldi *et al.*, 1999), information is still scarce, and more and more detailed studies should be performed to obtain more reliable and accurate estimations. Given hypolimnion segregation that is no longer mixed with lake surface water, catchment changes and processes can play a more important role on lake features.

P and N concentrations primarily regulate total algal growth, but the relative availability of Si to N and P can influence the composition of the phytoplankton community. Despite this, the study of this important element is often dismissed. Global projections on future climate predict an overall decrease in river discharge in southern Europe (Van Vliet *et al.*, 2013); therefore, since silica is introduced to the lake from inflowing surface waters, there could be a possible reduction in DSi concentrations in the surface water of Lake Iseo, as already verified in other systems, *e.g.*, in Lake Baikal (Sorokovikova *et al.*, 2019). Moreover, we already observed silica segregation in the deepest layers of Lake Iseo (see paragraph "Thermal dynamics, mixing depth, nutrient availability, and climate change"). If the meromixis condition in this system becomes permanent, silica replenishment would not occur. This, coupled with decreased input from the watershed, results in a depletion of this important compound in superficial layers. A new investigation is being developed to model and understand the biogeochemical cycle of this compound that has an important effect on the phytoplankton community and, therefore, on the whole pelagic community.

Changes that occurred in the phytoplankton communities (such as the increased importance in Lake Iseo, as well as in the lakes of Garda, Maggiore, Como, of *Tychonema bourrellyi*, a cryptogenic species that had never before been observed, potentially toxic Cyanobacterium that is radically changing phytoplankton features and the toxicological fingerprint of the largest DSLs), underline the lack in knowledge concerning the taxonomy and ecology of lake microbiota, and the potential consequences for the ecological functioning of ecosystems, and for human health. Furthermore, that highlights the need for developing next-generation monitoring approaches to use technological tools, such as the high-throughput sequencing (HTS) of environmental DNA. These innovative culture-independent approaches on the study of the biodiversity of prokaryotic and eukaryotic microorganisms in freshwaters, could radically modify our knowledge about the diversity, function, and biogeography of aquatic microbiota (Salmaso *et al.*, 2018a).

Remote sensing could help in monitoring the high spatial and temporal variability of some water-quality variables, such as those used in the evaluation of the ecological status of lakes according to the WFD. It provides frequent data for large-scale studies of water-quality parameters such as chlorophyll-a, which could significantly vary over short time periods and in the different areas of a lake (Bresciani *et al.*, 2018).

Zooplanktonic organisms, because of their relatively short life cycle, rapidly respond to local and global stressors. Studies on the long-term data on Lake Iseo were crucial to understand changes taking place in the lake ecosystem; in particular, it was demonstrated that changes in phenology, such as growth rate, abundance, and species composition, can reflect climate shifts well. Such changes can create a mismatch between consumers and food resources affecting the entire pelagic food webs. From this perspective, recent studies of food-web structure using stable isotopes highlighted the trophic relationship between taxa and the fate of hydrophobic pollutants in biota.

The presence of micropollutants represents hidden and underestimated problems for aquatic biota and human health, and it is less perceived by citizens. In particular, the effects of emerging micropollutants (*i.e.,* PFAS) and mixtures of micropollutants are unknown, and they have an unpredictable impact level considering the thousands of toxic species released in hydrographic basins, lakes, and groundwater (Cleuvers, 2003; Ebele *et al.*, 2017; Valsecchi *et al.*, 2015). The presence of microplastics in the surface water of Lake Iseo was verified (Sighicelli *et al.*, 2018). However, many studies highlighted that deep sediments can represent a long-term sink for these pollutants, so future investigations in Lake Iseo are needed to understand its role as a possible microplastics sink, through accumulation and segregation in deep sediments, or as a source, due to possible preferential distribution in the water column. A study is in place to disentangle the fate of microplastics in lakes, and to design a standardized method for the separation and analysis of microplastics from sediments, comparing the effectiveness of different methods and proposing a new one (Nava *et al.*, 2019).

An important catchment-related aspect that it is often dismissed is the relationship between groundwater (GW) and surface waters (SW), since these systems create a hydrological continuum that could explain processes that would otherwise be difficult to understand. Evaluating pathways and quantifying the fluxes between GW and SW systems are important for evaluating water-resource allocations. Furthermore, a comprehensive understanding of GW-SW interactions supports the identification of pollutant migration pathways and, therefore, of their potential impact within the aquatic environment. As of now, this aspect has been marginally addressed, with a few studies performed at the basin scale (Rotiroti *et al.*, 2019). However, many aspects still remain to be considered, and studies could be performed to deeply understand the studied system due to its important implications on lake management. Future studies on the Lake Iseo watershed could combine hydrodynamic, hydrochemical, and isotopic analyses of surface water and groundwater as an appropriate method for investigating the complex relationship between these compartments, and for evaluating anthropogenic hydrosystem modifications.

In this contribution, we highlighted some important and continuous changes experienced by Lake Iseo, attributable to both well-known stressors and new emerging types of impact. We performed synoptic analysis providing updated data about the state of the art of long-term investigations and the most advanced studies carried out on this lake. We documented the effects of anthropogenic climate change on the lake ecosystem at different levels and scales, focusing on changes in physical and chemical features and on the distribution, abundance, and physiology of organisms, species interactions, and food webs. We highlighted ongoing studies and future perspectives to fill some knowledge gaps regarding lake ecological processes. To better understand key lacustrine dynamics and responses is essential for efficient management strategies to counteract climate change and mitigate its effects, and to conserve lake ecosystems, biodiversity, and natural capital.

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