Long-term development of subalpine lakes: effects of nutrients, climate and hydrological variability as assessed by biological and geochemical sediment proxies

Manuela Milan

Department of Ecology and Environmental Science
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“Quando non sai dove stai andando, ricordati da dove vieni”

Proverbio africano

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List of papers

This doctoral thesis is a summary of the following papers, which are referred to in the text by their Roman numerals:

I. Milan M., Bigler C., Salmaso N., Guella G. and Tolotti M. (2015). Multiproxy reconstruction of a large and deep subalpine lake's ecological history since the Middle Ages. *Journal of Great Lakes Research 41: 982-994.*

II. Milan M., Bigler C., Tolotti M. and Szeroczyńska K. Effects of long term nutrient and climate variability on subfossil Cladocera in a deep, subalpine lake (Lake Garda, northern Italy). *Journal of Paleolimnology*, *submitted.*

III. Tolotti M., Lami A., Yang H. and Milan M. Different performances of independent sediment biological proxies in tracking ecological transitions and tipping points of a small sub-alpine lake since the Little Ice Age. *Manuscript.*

IV. Milan M., Bindler R., Tolotti M. and Bigler C. Tracing recent geochemical sediment properties in subalpine lakes using wavelength-dispersive X-ray fluorescence spectroscopy (XRF): understanding regional patterns of recent lake development. *Manuscript.*

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Author contributions

Authors are referred to by their initials.

Authors: **MM**: Manuela Milan, **MT**: Monica Tolotti, **GG**: Graziano Guella, **NS**: Nico Salmaso, **KS**: Krystyna Szeroczyńska, **AL**: Andrea Lami, **HY**: Handong Yang, **RB**: Richard Bindler, **CB**: Christian Bigler

Paper I
MT and MM conceived the study. Data collection was mainly carried out by MM (diatoms and geochemistry), GG (pigments) and external collaborators (dating). NS provided long-term limnological data. MM analyzed the data and wrote the majority of the manuscript, all authors contributed to final version of the manuscript.

Paper II
KS conceived the study. Data collection was carried out by MM (Cladocera) and external collaborators (dating). MM analyzed the data and wrote the majority of the manuscript. CB and MT contributed to final version of the manuscript.

Paper III
MT conceived the study. MM contributed to data collection and statistical analyses (Cladocera) and wrote the correspondent paper paragraphs. Sub-fossil pigments data were carried out by AL. HY performed ^{210}Pb-dating. MT wrote the majority of the manuscript, all authors contributed to the current version.

Paper IV
MM, RB, MT and CB conceived the study. Data collection was carried out by MM (XRF data) and external collaborators (dating). MM analyzed the data and wrote the majority of the manuscript, all authors contributed to the current version of the manuscript.

Abstract

Sediment records of two Italian subalpine lakes (Lake Garda and Lake Ledro) were analyzed in order to reconstruct their ecological evolution over the past several hundred years. A multi-proxy and multi-site approach was applied in order to disentangle the effects of local anthropogenic forcings, such as nutrients, and climate impacts on the two lakes and their catchments. Biological indicators (sub-fossil pigments, diatoms and Cladocera) were used to reconstruct changes in the aquatic food web and to define the lake reference conditions, while geochemical methods, i.e. wavelength-dispersive X-ray fluorescence spectroscopy (WD-XRF), were used to provide quantitative information on the different physical or chemical processes affecting both lake and catchment systems.

Sub-fossil pigments and diatoms, together with their respective inferred TP values, suggested very stable oligotrophic conditions in both lakes until the 1960s. The period following was affected by nutrient enrichment, which led to a drastic shift in the phytoplanktonic community. The response of sub-fossil pigments and diatoms to major climatic anomalies such as the Medieval Climatic Anomaly (MCA) and the Little Ice Age (LIA) were not pronounced, and the taxonomic composition remained relatively stable. On the contrary, these proxies showed an indirect response to climate variability since the beginning of the nutrient enrichment phase in the 1960s. In Lake Garda, the winter temperature regulates the water column mixing, which in its turn controls the degree of nutrient fertilization of the entire water column, and the related phytoplankton growth. In Lake Ledro a rapid reorganization of planktonic diatoms was observed only during the temperature recovery after the LIA, while recent temperature effects are masked by the prevailing nutrient effects. In Lake Garda, Cladocera remains responded in quantitative and qualitative terms to climatic changes, whereas in Lake Ledro they appeared to be mainly affected by variations in hydrological regimes, i.e. flood events. Cladocera remains corroborated the nutrient enrichment after the 1960s in both lakes as inferred by diatoms and pigments.

In Lake Garda, the geochemical data showed a pronounced shift in elemental composition since the mid-1900s, when major elements and lithogenic tracers started to decrease, while some elements related to redox conditions and other (contaminant) trace elements increased. The general trends since the mid-1900s agree with the biological records. However, some differences recorded in the two different basins of Lake Garda reflected the effects of local conditions, both related to hydrology and sedimentation patterns. Lake Ledro showed higher short-term variability for most elements, even though some features were comparable to Lake Garda. The geochemical record of Lake Ledro revealed a major influence of human-induced lake-level fluctuations and catchment properties.

This paleolimnological study allows us to place temporally restricted limnological surveys into a longer-term secular perspective, which is highly valuable for the definition of lake reference conditions. Because the restoration targets are usually based on the lake reference conditions, this study highlighted also the necessity to pay particular attention to the lake-specific sensitivity patterns. The multi-proxy and multi-site approach showed that the lake conditions of large and deep lakes in northern Italy, such as Lake Garda, are mainly driven by nutrient enrichment and/or climate change. In contrast, smaller lakes with larger catchment areas, such as Lake Ledro, are seemingly more impacted by conditions and processes occurring in the drainage basin.

Keywords: Paleolimnology, diatoms, Cladocera, sub-fossil pigments, geochemistry, wavelength-dispersive X-ray fluorescence spectroscopy, Lake Garda, Lake Ledro, reference conditions, nutrient enrichment, climate change, hydrological regime.

Introduction

The different geochemical and biological processes operating in the lake-water column, in the lake catchment and in the atmosphere produce material, which is deposited and accumulated at the lake bottom (Haworth and Lund 1984). Therefore, lacustrine sediments represent a powerful archive to track past human- and climate-driven impacts on the lake-catchment system. Paleolimnology is a multidisciplinary science, whose importance and relevance originates from the fact that lake sediments host a wide variety of environmentally valuable information (Last and Smol 2001). Moreover paleolimnology has developed "indirect" calibrations (i.e. transfer functions) based on different biological proxy indicators with the aim to reconstruct long-term changes in environmental conditions (Last and Smol 2006). The paleolimnological approach makes it possible to place limnological investigations within a secular temporal perspective, providing a longer timeframe with which environmental changes can be assessed as compared to monitoring data, which usually cover only a few decades or less (Smol 2008). Therefore, lake sediments are useful for both limnological research and environmental management because they provide retrospective analyses beyond the limited timescales available from monitoring programs, and allow a prediction of possible future lake developments within the context of human and climate impacts. Many studies have been carried out on lake sediments to quantify and better understand lake responses to different impacts, such as eutrophication (Anderson 1997), acidification (Renberg 1990; Renberg et al. 1993), lake-level fluctuations (Magny et al. 2001), pollution (Renberg and Wik 1985) and climate change (Bigler et al. 2002). Lakes located in remote and alpine regions are particularly interesting to assess climate impacts, because anthropogenically driven nutrient changes are often difficult to disentangle from climate effects in more-impacted low-altitude lakes (Battarbee et al. 2012). The multi-proxy approach tries to solve this problem, by combining the capability of each proxy in tracking different aspects of impacts (Bennion et al. 2015; Perga et al. 2015). Since the different techniques each exhibit both advantages and limitations (Rosén et al. 2010), the combined paleoecological and biogeochemical analyses can be used in support of each other, thereby strengthening paleoenvironmental interpretations (Michelutti and Smol 2013).

Remains of different groups of organisms are preserved in the sediments, such as diatoms (Battarbee et al. 2001), plant macrophytes (Sayer et al. 2010; Spierenburg et al. 2010), Cladocera (Guilizzoni et al. 2006; Jeppesen et al. 2001; Szeroczyńska 1998), Mollusca (Ayres et al. 2008; Walker et al. 1993), chironomids (Brodersen et al. 2001; Langdon et al. 2010) and fish (Davidson et al. 2003). Diatoms are sensitive to changes in water quality in terms of pH, nutrients, salinity and temperature regimes (Bennion et al. 2004). In fact, each taxon requires specific habitat and particular water-chemistry conditions (Hall and Smol 2010). Together with their ability to react rapidly to environmental changes, diatoms are therefore considered as a valuable indicators of environmental changes (Bigler et al. 2007). Moreover they are widely used to define a lake's reference conditions, which are intended as the pre-human impact lake status (Bennion et al. 2011a). For many European lakes, the reference conditions are identified as prior to the mid-19th century, before the intensification of human activities following along industrialization. Industrialization and population growth degraded the ecological quality of many temperate lakes through eutrophication and acidification since then (Bennion et al. 2011a). Nevertheless, the major nutrient impact started in the majority of European lakes in the 1950s, just after the end of World War II (Bennion et al. 2011b; Lotter et al. 1998; Thies et al. 2012). Establishing the reference conditions of a lake is now required by the Water Framework Directive (WFD) to prevent further deterioration, to maintain ecological functions and services, and to improve the status of water resources (Bennion and Simpson 2011). In order to define reference conditions, different diatom-based transfer functions have been developed for the hindcasting of past lake trophic levels. In particular, the optima and tolerances of diatom taxa in respect to important environmental variables have been estimated by assembling numerous regional training-sets, including lakes of different typology (Lotter et al. 1997; Weckström et al. 1997). However, the majority of training-sets include small and shallow lakes, while large and deep lakes are often underrepresented. This is potentially a limitation of this methodological approach, as large lakes differ from small lakes in many important physical, chemical and biological aspects (Bennion et al. 2011b).

Cladocera occupy a key level in the lake food web between top-down regulators, i.e. fish and invertebrate predators, and bottom-up factors, such as nutrients and phytoplankton (Jeppesen et al. 2001). Living in both the pelagic and the littoral zone of lakes makes them good indicators of changes in water level (Korhola et al. 2000; 2005), temperature (Nevalainen 2012; Szeroczyńska 2006), nutrients (Jeppesen et al. 2001), pH (Jeziorski et al. 2008; Paterson 1994), submerged macrophyte distributions (Davidson et al. 2007) and food webs (Finney et al. 2000; Jeppesen et al. 2001). Due to their chitinous body, Cladocera are well preserved in the sediments, which facilitates long-term environmental reconstructions (Hofmann 1987). Moreover different indices based on Cladocera were developed in order to infer past lake evolution. The ratio of planktonic to littoral Cladocera is calculated to identify episodes of reduced water level in relation to climate change, but also to evaluate nutrient variations (Sarmaja-Korjonen 2001). Fish pressure on Cladocera and fish abundances are inferred, respectively, from the Planktivory Index (Leavitt et al. 1994) and Catch-Per-Unit-Effort index (CPUE; Jeppesen et al. 1996). Recently Korosi et al. (2010) and Manca et al. (2007) investigated the ability of pelagic Cladocera, such as *Bythotrephes* spp., *Bosmina* spp. and *Daphnia* spp., in adapting their body size and body appendages over longer periods in response to fish and invertebrate predation, as well as to temperature and nutrient shifts. On the other hand, Cladocera as paleolimnological indicators exhibit also some limitations, such as the complex patterns of distribution, the varying preservation of species and type of remains (Eggermont and Martens 2011), and the formation of interspecific hybrids, possibly in relation to environmental changes (Lampert and Sommer 2007).

Züllig (1981) demonstrated that past phytoplankton development could be detected from the specific carotenoids preserved in lake sediments. Sub-fossil pigments have been used not only to reconstruct past algal composition, but also to infer past lake-water pH (Guilizzoni et al. 1992; Züllig 1981) and phosphorus concentrations (Guilizzoni et al. 2011). Nevertheless, the use of sub-fossil carotenoids for environmental reconstruction is possibly limited by their chemical degradation under oxygenated conditions at the lake bottom and the pronounced degradation in very deep lakes (Leavitt 1993), which can alter the pigment concentrations.

Geochemical analyses of lake sediments make it possible to assess the sediment conditions and diagenetic processes related to the lake-catchment system in response to human activities and climate (Boës et al. 2011; Brisset et al. 2013; Last and Smol 2001). Land-use changes strongly influence the soil stabilization and erosion (García-Ruiz et al. 2010), while climatic variability plays an important role in both chemical and physical weathering of the catchment area (Vannière et al. 2013). In addition, trace metals are deposited at the lake bottom, providing information of important atmospheric contaminants (Koinig et al. 2003). Wavelength-dispersive X-ray fluorescence spectroscopy (WD-XRF) is a rapid, non-destructive and accurate method for the determination of the geochemical composition of lake sediments (Rydberg 2014). WD-XRF analysis of bulk sediment samples provides information on past productivity (e.g., Si/Al ratios to infer biogenic silica), redox conditions (Fe/Mn ratios), atmospheric pollution (e.g., Pb), human impact (lithogenic elements), climate change and weathering rates (weathering indices such as K/Al ratios) in the catchment area (Martín-Puertas et al. 2011). As outlined for biological proxies, the geochemical analyses have also some limitations. In fact, diagenetic alterations and ion migration within the sediment have to be considered when such data are interpreted in sediment cores (Naeher et al. 2013). Moreover, a significant proportion of dissolved elements is held in the water column of deep lakes (Boyle 2001).

Numerous small lakes were chosen for paleolimnological investigations aimed at understanding the different drivers of long-term lake evolution (Bigler et al. 2007; Guilizzoni et al. 2006; Lotter et al. 1997; Thies et al. 2012). In comparison, sediment records from large and deep lakes were analyzed more rarely. In the northern Alpine region, some large lakes such as Lake Constance, Mondsee, Ammersee and Geneva were investigated to reconstruct past trophic conditions (Alefs and Müller 1999; Berthon et al. 2013; Wessels et al. 1999). In the southern Alpine region, only sediments from Lake Maggiore have been intensively studied (Marchetto et al. 2004). As a consequence, information on both the secular ecological evolution and on effects of recent climate variability on the ecological dynamics of large subalpine lakes in the southern Alpine region is very limited. The multi-site approach, intended as the combined study of several sites within an homogeneous region, has been recognized as valuable to understand the individualistic response patterns of different lakes to common external forcings (Battarbee 2000) and to recognize the impact of local factors in driving the lake's evolution (Johnson et al. 2010). Usually the deepest point of a lake basin is considered to be the best coring point, because it exhibits regular sedimentation patterns and is less impacted by currents and resuspension (Dåbakk 1999). On the other hand, Haworth and Lund (1984) recommended the application of a multi-site approach within a lake, intended as the study of several parallel cores collected from different parts of the same lake, in order to gain a better picture of the distribution of chemical components within a lake.

Objectives

The overarching aim of this thesis was to understand the impacts of human pressure and climate change on two different subalpine lake ecosystems during the past few centuries. Lake Garda and Lake Ledro are lakes of glacial origin located in the southern Alpine region. The choice to analyze these two lakes, which have similar location and origin, but different morphology and catchment area properties, was derived from the intention to understand the responses of different lake-catchment systems to common external forcings. In fact, as highlighted by Battarbee (2000), even lakes located close to each other may show individualistic responses. A multi-proxy and multi-site approach appears to be the best tool to disentangle the effects of human and climate impacts on the lake itself and its catchment area (Battarbee et al. 2012). In particular, biological proxies are mainly used to reconstruct aquatic food web changes and to define the reference conditions within each lake, while geochemical proxies provide information on the different processes, which affect lakes and catchments.

Although Lake Garda has been surveyed monthly since AD 1996 (as part of the Italian Long Term Ecological Research Network, www.lternet.edu) and some sporadic information on the lake status is available since AD 1974, the time span covered by limnological investigations is too short to assess the lake response to different longer-term external impacts (Bennion et al. 2011a). A paleolimnological study was carried out on sediment cores collected from the two different lake basins, which were analyzed for sub-fossil diatom assemblages and pigments. Paper **I** aimed at reconstructing the basins' long-term trophic evolution from non-impacted reference conditions to the present. A further objective of this study was to evaluate differences in the basins' long-term diatom assemblage composition changes, and in lake phosphorus concentrations, which may be possibly related to differences in their physiography and/or the performance of different inferential approaches. As the role of the climate variability on lake dynamics became more important in recent years, the trophic evolution of Lake Garda was compared with local air temperature patterns and teleconnection indices, in order to understand the role of climate changes on deep subalpine lakes.

In large temperate lakes the climate-driven ecological perturbations are typically smoothed (Michelutti et al. 2015). The inertia to environmental changes was particularly evident in the deeper main basin (Brenzone) of Lake Garda, while the shallower basin in the southeast (Bardolino) showed a slight change in diatom assemblage composition already before the recent eutrophication period, which is probably attributable to changes in water temperature. As this hypothesis could not be verified, the use of a specific proxy was necessary to understand the climatic impact on the biological community. Cladocera remains are widely used to reconstruct temperature and trophic changes (Jeppesen et al. 2001). Paper **II** aimed at reconstructing species distributions and abundances of Cladocera in both lake basins during the late Holocene, with special focus on their relation to direct human impacts and climate change. Due to the preference of the majority of Cladocera species to live in the littoral zone, a new coring point from the littoral zone was added for this study. The long-term differences in Cladocera assemblage composition and abundances in different profundal and littoral lake compartments were analyzed and related to historical limnological and climatic variability. Cladocera results were compared to other biological proxies, such as diatoms, pigments and *Pediastrum* remains, in order to discriminate lake's response to nutrient enrichment and climate change.

Different biological proxies are often studied simultaneously to strengthen the paleoenvironmental interpretations (Michelutti and Smol 2013). In Paper **III** the multi-proxy approach was applied to a short sediment core collected from a small subalpine lake close to Lake Garda (Lake Ledro), in order to assess whether lakes of different size located in the same region respond similarly to common external forcings. In this study, photosynthetic pigments, diatoms and Cladocera were analyzed and compared in order to track the ecological transitions and the tipping points related to major environmental perturbations, such as nutrients and climate in a small subalpine lake. The multi-proxy approach was also applied in order to refine the reconstruction of past ecological evolution of Lake Ledro considering the scarcity of limnological and climatic information available.

The multi-proxy studies in Lake Garda and Lake Ledro were mainly based on biological proxies. Therefore geochemical analyses were carried out (Paper **IV**) to expand the knowledge of human and climate impacts not only on the lake system, but also on their catchment area. Both lakes were analyzed for concentrations of different elements and elemental ratios in order to assess the human and climate pressures on the two lake ecosystems and their catchment areas, as well as in relation to the results from the paleoecological analyses. Because both lakes are directly or indirectly exploited for hydropower production, the study focused on recent human-induced lacustrine dynamics.

Study sites

Lake Garda is the largest Italian lake (Area = 368 km^2; Vol= 49 km^3; Z_{max} = 350 m), and contains 34% of the Italian freshwater resources. A submerged ridge divides the lake into two sub-basins: the main western basin (Brenzone, 350 m deep) and the smaller eastern basin (Bardolino, 81 m deep), which accounts only for 7% of the total lake volume. The main lake inlet, the River Sarca, flows through the siliceous Adamello-Presanella mountain range and enters at the northern end of the lake, while the outflow River Mincio is located at the southeastern end of the lake (Salmaso 2010). The catchment area covers about 2350 km^2, from an altitude of 3556 m a.s.l. (Monte Presanella) to 65 m a.s.l. with a mean altitude of about 1000 m a.s.l.. The ratio of catchment area to lake area is relatively small (6:1) (Bonomi 1974). The lithological composition of the catchment area is dominated by sedimentary rocks (such as limestones, marls, sandstones), but also partly consists of glacial and fluvial deposits, together with igneous and metamorphic rocks (Sauro 1974). Lake Garda represents a key regional resource for irrigation, drinking water, recreational activities and tourism (Salmaso et al. 1997).

Lake Ledro is a small, mid-altitude lake (Area = 3.7 km^2; Vol = 0.08 km^3 ; Z_{max} = 49 m) located only 5 km NW of Lake Garda. It has two temporary tributaries, Massangla and Pur rivers, and one outlet, Ponale River, which is responsible for downcutting in a morainic dam. The catchment area extends over 111 km^2 from 2250 m a.s.l. (Monte Cadria) to 652 m a.s.l., and the ratio of catchment area to lake area is 30:1. The drainage basin is composed of Mesozoic rocks with Triassic dolomites and Jurassic and Cretaceous limestones. Due to the torrential regime of the tributaries and the high catchment/lake area ratio, Lake Ledro is exposed to floods events (Simonneau et al. 2013). Tourism and recreational activities around Lake Ledro represent an important fraction of the local economy.

The two lakes are connected by underwater pipes, which force the water from Lake Ledro down to Lake Garda and pump it back to Lake Ledro. Both catchment-lake systems have been the target for hydroelectrical exploitation during the first half of the 20th century, which modified the transport of organic and minerogenic material to the lakes. In particular, since the AD 1929 the water level of Lake Ledro has been regulated according to the necessity of hydroelectricity production. Moreover, both catchment areas are strongly impacted by human activities, such as tourism, agriculture and forestry.

Methods

Coring and sediment sampling

All sediment cores were collected with a gravity Kajak corer (UWITEC, Austria). Two cores were collected from the deepest point of the main Lake Garda basin (Brenzone; core length 56 cm) and two additional cores from the deepest point of the second basin (Bardolino; core length 65 cm). Bren 1-09 and Bar1-11 were analyzed for radionuclides and biological proxies, while the parallel cores Bren2-09 (core length 63 cm) and Bar2-11 (core length 54 cm) were used for geochemical analyses. In addition, one short core was collected from the littoral zone of the Bardolino basin (water depth 39 m; core length 44 cm) to study Cladocera remains. The different cores collected from each basin were correlated through comparison of dry weight percentage values.

Two short cores were retrieved from the deepest point of Lake Ledro (water depth 49 m; core length 83 cm and 77 cm, respectively). Ledro1-11 was analyzed for radionuclides, biological proxies and geochemical elements, while Ledro2-11 was used for analyzing the sediment macroscopic aspects and texture. Each core, except for Ledro2-11, was vertically extruded and sliced in the laboratory (E. Mach Foundation, San Michele all'Adige, Italy) at 0.5 cm intervals from 0 to 30 cm and at 1 cm intervals from 31 down to the core bottom. Ledro2-11 was opened longitudinally in the laboratory of the Institute of Ecosystem Study - National Research Council (Verbania-Pallanza, Italy).

Radiometric dating

^{210}Pb is a naturally produced radionuclide, derived from atmospheric fallout, while ^{137}Cs and ^{241}Am are artificially produced and introduced by atmospheric fallout from nuclear weapons testing and nuclear reactor accidents. ^{210}Pb is removed from the atmosphere by precipitation or dry deposition and stored on land, lakes and oceans (Last and Smol 2001). Radiometric dating with ^{210}Pb makes it possible to date the last 100-150 years of a sediment core (Rose et al. 1998). Physical and biological processes could mix the superficial sediment, thus impacting the ^{210}Pb dating. To overcome this problem, the analysis of artificial radionuclides, such as ^{137}Cs and ^{241}Am, is widely used for the validation of the ^{210}Pb ages for the last ~50 years. In central Europe, atmospheric fallout is recorded in the sediment as a ^{137}Cs peak around AD 1963 (extensive atmospheric testing of nuclear weapons) and in AD 1986 (nuclear reactor accident in Chernobyl). ^{241}Am is used to confirm the 1963 peak, as Am represent a derivate of Cs (Last and Smol 2001). Sediment samples (c. 1 g DW) were analyzed for ^{210}Pb, ^{137}Cs and ^{241}Am by direct gamma assay at ENSIS Ltd (University College London, UK), using an ORTEC HPGe GWL series well-type coaxial low background intrinsic germanium detector. Due to the non-monotonic variations in unsupported ^{210}Pb activity, core chronologies were calculated using the CRS (constant rate of ^{210}Pb supply) dating model (Appleby 2001).

The few vegetal remains found in the Brenzone and in the Ledro cores, were dated based on ^{14}C at the Poznan Radiocarbon Laboratory, Poland (http://radiocarbon.pl/index.php?lang=en), using the age calibration curve r:5, atmospheric data from Reimer et al. (2009) and the software OxCal v.4.1.5 (Bronk Ramsey 2010).

Spheroidal carbonaceous particles (SCPs)

Spheroidal carbonaceous particles (SCPs) are used as marker of atmospheric deposition of particulate pollutants originating from fossil fuel combustion (Renberg and Wik 1985). In fact, the majority of the European regions show three common steps in the SCPs temporal evolution. SCPs appeared since the middle 1800s (i.e. starting of the Industrial Revolution) and increased rapidly after around 1950 in concomitance with the major expansion in the construction of coal-fed power stations and the availability of more fuel oil. A peak between 1950 and 1980 (depending on the area) reflected the development from a larger number of small local power stations to a smaller number of larger ones. The following decrease in concentration derived from the increase in combustion efficiency and the introduction of more rigorous control legislation (Rose 2001). SCPs analysis were carried out by ENSIS Ltd (University College London, UK), following the method outlined by Rose (1994).

Diatoms

According to standard procedures (Battarbee et al. 2001), about 1 g of wet sediment samples was treated with H_2O_2 (30%) and HCl (10%). The rinsed samples were permanently mounted on microscope slides using Naphrax® resin (refraction index = 1.7). At least 500 valves per sample were counted at 1000× magnification under a light microscope (LEICA DM2500, Germany). Diatoms were identified at the lowest possible taxonomic level, on the basis of standard literature (Krammer 2002; Krammer and Lange-Bertalot 1986-1991; Lange-Bertalot 2001) and recent literature on singular taxa (Houk et al. 2010, 2014; Lange-Bertalot and Ulrich 2014).

In order to define the reference conditions and to track the nutrient enrichment history of Lake Garda and Lake Ledro, diatoms were used to reconstruct total phosphorus concentrations (DI-TP). Even though the Northwest European training set (NWEu-TP) includes data from 152 lakes located in the United Kingdom, Northern Ireland, Denmark and Sweden (Bennion et al. 1996), it appeared to be the best model for Lake Garda (Paper **I**). On the other hand, in Paper **III,** two training-sets were considered to provide accurate models for the DI-TP reconstruction in Lake Ledro: the NWEu-TP and the European Combined training-set (Eu-Comb), which includes 477 European lakes (Battarbee et al. 2001). In both papers the training-sets were selected based on a good agreement between DI-TP values and TP concentrations measured in the lake water, the better statistical performance with high regression coefficients and the higher cumulative relative abundance of fossil diatoms represented in the modern training-set samples. Diatoms were analyzed at E. Mach Foundation (San Michele all'Adige, Italy).

Sub-fossil pigments

Concentrations of total carotenoids (TCar) relative to the content of organic matter were determined spectrophotometrically in accordance with the method described by Züllig (1982) and Guilizzoni et al. (1983; 2011). Fresh subsamples (3 g) were extracted in 4 mL acetone/water (90/10) at 4 °C and the 430:410 spectrophotometric absorbance ratios of the acetone extracts were calculated to assess the extent of chlorophyll degradation to phaeopigments (Guilizzoni et al. 1992). Past total phosphorus concentrations (Car-TP) was inferred by TCar concentrations for both lakes, while past lake pH (Car-pH) was inferred only for Lake Ledro (Guilizzoni et al. 1992). Specific sub-fossil pigments were analyzed by reversed phase High-Performance Liquid Chromatography (HPLC) at University of Trento (Trento, Italy) for Lake Garda and at Institute of Ecosystem Study - National Research Council (Verbania-Pallanza, Italy) for Lake Ledro. Detailed information on the methods is reported in Paper **I** and **III**.

Cladocera

In Paper **II** and **III** Cladocera remains were analyzed using the methods described by Frey (1986) and Szeroczyńska and Sarmaja-Korjonen (2007). About 2 cm^3 of wet sediment were heated in KOH (10%), filtered through a 40 µm mesh, and finally treated with HCl (10%). The residue was stained with a glycerol-safranine mixture. Each slide was examined using a light microscope (LEICA DM2500, Germany) at 100-400x magnification and all of the visible Cladocera remains were counted. The taxonomic identification of Cladocera individuals was based on Flössner (2000), Margaritora (1983), and Szeroczyńska and Sarmaja-Korjonen (2007). The ecological preferences of the species were defined based on Flössner (2000), Frey (1986), Kamenik et al. (2007), Korhola (1990) and Margaritora (1983).

In Lake Garda (Paper **II**), ephippia and cell-walls of the coccal green algae *Pediastrum* spp. were identified in the same samples as Cladocera remains. The identification at the species level of *Pediastrum* spp. is based on Komárek and Jankovská (2001). Cladocera analyses were conducted partly at the Polish Academy of Science (Warsaw, Poland) and partly at E. Mach Foundation (San Michele all'Adige, Italy).

Geochemical analyses

Loss-on-ignition (LOI) is a widely used method to estimate the amount of organic matter and carbonate mineral content in sediments. Sediments are combusted at different temperatures, in order to study the sediment components. Water content was determined after drying about 2 g of wet sediment at 105 °C for 24 h, while organic matter, estimated as loss-on-ignition, was determined after heating at 550 °C for 2-3 h. These analyses were carried out at E. Mach Foundation (San Michele all'Adige, Italy).

In Paper **IV** sediment samples from Lake Garda and Lake Ledro were analyzed by wavelength-dispersive X-ray fluorescence spectroscopy (WD-XRF). Major and trace elements were measured using Bruker S8 Tiger WD-XRF analyzer equipped with an Rh anticathode X-ray tube at the Department of Ecology and Environmental Science (Umeå University, Sweden). The calibration was developed for WD-XRF for the matrices of the powdered samples, as presented in Rydberg (2014). Different ratios were considered for this work, such as Mn/Fe for the reconstruction of the redox conditions, Si/Al to infer the biogenic silica concentration, and Zr/Ti for the effects on hydropower establishment. Moreover Pb enrichment factor (PbEF) was analyzed in order to outline the atmospheric pollution in this region.

Sampling of monitoring data

Regular monitoring analysis of water chemistry and biology has been carried out every month since 1996 in the deepest part of the Brenzone basin over the entire water column (0-350 m) and the trophogenic layer (0-20 m). Limited data for specific physical variables are available since 1974 (Salmaso and Mosello 2010). Chemical and biological data for the Bardolino basin are available only for the period 1996-2008. Measurements of temperature, oxygen, pH and conductivity have been carried out using underwater multi-parameter probes, while chemical analyses were carried out at E. Mach Foundation (San Michele all'Adige, Italy). Only sparse and irregular chemical and biological data are available for Lake Ledro (Casellato 1990, Autonomous Province of Trento, unpublished data). Since 2009, four seasonal surveys are being carried out yearly by the Provincial Environmental Agency according to the EU WFD. Detailed data were collected from 2011-2012 within a local research project, funded by the Autonomous Province of Trento, Italy (Boscaini et al. 2012; Salmaso et al. 2013).

Climatic data

Long-term monthly mean air temperature and precipitation data were obtained from the HISTALP data set (Auer et al. 2007) and used as a proxy for climate variability (Paper **I**, **II** and **III**). For Brenzone and Lake Ledro the homogenized data recorded during the period AD 1760-2008 at the weather station of Torbole-Riva del Garda were considered, while for Bardolino the data from the Villafranca weather station were used, available for the period AD 1788-2006. For Lake Ledro, weather data are registered only since 2002 at a weather station in Bezzecca, located close to the NE shore and run by GIS Unit of the E. Mach Foundation (San Michele all'Adige, Italy). These data were integrated in the HISTALP dataset from 2008 onwards.

The North Atlantic Oscillation (NAO) and East Atlantic pattern (EA) indices were considered in order to relate the ecological dynamics of Lake Garda to global atmospheric circulation patterns (Paper **I**). Indices were computed by the National Oceanic and Atmospheric Administration - Climate Prediction Centre (NOAA-CPC, www.cpc.ncep.noaa.gov).

Numerical analysis

A non-metric multidimensional scaling (NMDS) was used for the multivariate analyses of biological sediment proxies. Being based on the graphical representation of a Bray & Curtis dissimilarity matrix (Legendre and Legendre 1998), such ordination is particularly suitable for sparse biological matrices, which typically include numerous zero values (Kruskal and Wish 1978). The NMDS was applied in order to identify patterns in the temporal evolution of the different biological proxies in Paper **I**, **II** and **III**. A locally weighted scatterplot smoothing (LOWESS) interpolation of radiometrically determined dates for non-contiguous sediment layers was performed to assign an age to each sediment layer, which made it possible to couple and correlate sediment, limnological and climatic information. After a Kolmogorow-Smirnow Normality test, non-parametric Spearman rank order correlation analysis without data transformation of relative abundances (%) was applied to assess the significance of the relationships between different variables (Paper **I** and **III**). Vector (Paper **II** and **III**) and surface (Paper **II**) fitting analyses were applied to the NMDS ordinations aiming to identify the major drivers for long-term changes in sediment biological proxies in Lake Garda and Lake Ledro.

Major results and discussion

The paleolimnological studies on Lake Garda and Lake Ledro provided an overview of the long-term changes that occurred in the water column as well as in the catchment area during the last few centuries. Both lakes showed coherent and strong responses to the anthropogenic nutrient enrichment since the 1960s. The responses of both lake ecosystems to climate variability emerged to be indirect and modulated by lake size and depth, ratio of catchment area to lake area, and the complex interactions between climate variability and physical, chemical and biological lake dynamics (Leavitt et al. 2009).

The biological proxies studied in Paper **I** showed that a major ecological change occurred in both sub-basins of Lake Garda (Brenzone and Bardolino) during the 1960s. A drastic shift in diatom assemblage composition was observed, consisting of a pronounced increase in the abundance of mesotraphentic colony-forming taxa, such as *Asterionella formosa*, *Fragilaria crotonensis*, *Tabellaria flocculosa*, and *Aulacoseira* spp., which replaced the small unicellular oligotraphentic *Cyclotella* species, which had been steadily dominant in the previous centuries. The ecological evolution of Lake Garda since the Middle Ages appeared to include two major stages, with the 1960s representing the threshold. The pigment- and diatom-based reconstructions of TP concentrations indicated very stable, ultra-oligotrophic conditions before the 1960s. Diatom assemblage composition and abundances remained very stable during this long period in both cores. Even major climate events characterizing these centuries, such as the Medieval Climatic Anomaly (MCA) and the Little Ice Age (LIA), did not affect either the diatom assemblages or the sub-fossil pigments. This suggests a considerable inertia of Lake Garda towards climatic changes, in relation to its large size and water volume (Gerletti 1974). On the other hand, the uncontrolled discharge of urban sewage water into the lake since the post-World War II economic boom caused a rapid increase in lake nutrient concentrations, which produced the first detected environmental perturbation in Lake Garda in the early 1960s. DI-TP and Car-TP were in good agreement, indicating that the lake reached mesotrophic conditions, which is also confirmed by historical information (Salmaso 2010). The increase in mesotraphentic colony-forming Fragilariaceae at the expenses of oligotrophic *Cyclotella* species was highly coherent with the response to moderate nutrient enrichment observed in small and large Alpine lakes (Bennion et al. 1995; Bigler et al. 2007; Jochimsen et al. 2013; Marchetto et al. 2004; Thies et al. 2012). In general, the *Cyclotella-Aulacoseira-Fragilaria* shift, recorded in both basins of Lake Garda, has been reported in numerous lake sediment records across the Northern Hemisphere (Rühland et al. 2015). The sub-fossil pigments exhibited higher concentrations during the nutrient enrichment period, thus suggesting an increase in lake productivity. In particular, the peak in zeaxanthin in the early 1990s indicated a moderate presence of cyanobacteria, which is in agreement with a progressive development of *Planktothrix rubescens* and with irregular small blooms of *Dolichospermum lemmermannii*, as recorded by limnological surveys (Salmaso 2000). The sub-fossil pigment concentrations exhibited similar trends in both basins, but higher values were recorded in the shallower Bardolino basin. Probably the great depth and the oxygenated conditions at the bottom of the Brenzone basin contributed to enhance the pigment degradation compared to the Bardolino basin (Leavitt 1993). Also limnological data revealed higher phytoplankton biovolume at Bardolino compared to Brenzone after the 1990s (Salmaso 2002), while recent surveys of lake surface chlorophyll concentrations based on remote sensing suggest similar annual productivity in both basins, but more pronounced seasonal variations in Bardolino (Bresciani et al. 2011). Although the diatom-based TP reconstruction showed highly comparable temporal trends in the two basins of Lake Garda, the TP values in the period 1996-2008 were slightly overestimated in the Bardolino basin. This could be explained with the different abundances between Bardolino and Brenzone cores of some species characterized by high TP-optimum in the NWEu-TP training set. The same phenomenon has been detected in other large and deep lakes, such as Maggiore and Mjoesa (Bennion et al. 2011a), where it was interpreted as a consequence of the under-representation of large and deep lakes in most of the currently used European diatom training-sets.

As outlined in the introduction, the discrimination between the effect of climate variability and nutrient enrichment is challenging, especially in nutrient-enriched lakes (Battarbee et al. 2012), where the ecological perturbations driven by climate variations are typically the result of complex interactions between physical, chemical and biological lake dynamics (Michelutti et al. 2015). In Lake Garda, both pigment and diatom-inferred TP concentrations increased at the beginning of the 21st century. Limnological data confirmed this observation by showing high epilimnetic TP concentrations and high phytoplankton biovolume in 2005 (Salmaso 2010). As no changes in nutrient concentrations were recorded in early-2000s, and since similar nutrient and phytoplankton pulses were observed in several temperate lakes within the Alpine region (Berthon et al. 2014; Tolotti et al. 2012; Tolotti pers. comm.), the registered nutrient pulse was interpreted as a response to recent climate variability. In fact, Salmaso and Cerasino (2012) demonstrated the importance of the winter air temperature in controlling nutrient availability and spring diatom growth through cascading processes involving deep water mixing in late winter. In large deep lakes with high nutrient segregation, warm summers combined with mild winters prevent deep circulation, causing a nutrient depletion in the epilimnion and a sequestration in the hypolimnion. In contrast, harsh winters promote the spring holomixis, which is able to fertilize the trophogenic water layers. Therefore, the sediment investigation of Lake Garda demonstrated that even large temperate lakes indirectly respond to climate variability.

The study of diatoms and sub-fossil pigments revealed a direct response of phytoplankton to nutrient increase, while the effect of temperature on the algal community is indirect, as it is mediated by changes in lake thermal stratification, deep circulation and mobilization of nutrients from the enriched hypolimnion. In Paper **II** Cladocera remains were analyzed as a proxy for temperature changes. The two profundal sediment records of Lake Garda revealed the presence at the core bottom of species preferring warm water temperatures, such as *Bythotrephes longimanus*, *Camptocercus rectirostris* and *Pleuroxus* spp.. This suggested that the deepest sections of both cores were deposited during the warm MCA (De Jong et al. 2013). The littoral core was too short to include records from this period. On the other hand, all three Garda cores cover the LIA. In the corresponding sediment layers, Cladocera remains were mainly composed of the so called "arctic", "subarctic" and "north temperate" species (Harmsworth 1968), while the total Cladocera abundances were very low (75 ind cm^{-3}). The subsequent increase in Cladocera remains indicated the establishment of warmer conditions. Similarly to what was observed for diatoms, a change in Cladocera assemblage composition was initiated by nutrient enrichment after the 1960s-1970s. In particular, the ratio between planktonic and littoral Cladocera exhibited an increase in planktonic species in all cores studied. Indicators of lake nutrient enrichment, such as Bosminidae and the *Daphnia longispina* group (Boucherle and Züllig 1983), dominated the Cladocera community and were responsible for the general increase in total Cladocera concentrations. During the 1990s the eutrophic indicator *Bosmina longirostris* showed a peak, which corresponded to the increase in total phosphorus concentrations registered by limnological studies (Salmaso and Mosello 2010). Moreover, in the same period, cyanobacteria blooms were recorded in Lake Garda, and Cladocera assemblages mirrored these events with higher values of *Chydorus sphaericus*. This species is often associated with cyanobacteria (Korhola 1990), as also observed in Lake Maggiore a few years earlier (Manca et al. 2007). The increase in *Bosmina (E.) coregoni* at the expense of *B. longirostris* during the 2000s indicated a return to lower trophic conditions, which is coherent with changes in other biological proxies, with decadal limnological data (Salmaso 2010), and with a general recovery of nutrient enrichment in deep subalpine and alpine lakes (Alric et al. 2013; Bigler et al. 2006; Manca et al. 2007).

The results of statistical analyses highlighted the importance of the annual air temperature in driving Cladocera species in Lake Garda, and identified the particular driving role of winter temperatures, especially on the deeper Brenzone basin. Cladocera data from both cores collected from the Bardolino basin showed a significant correlation also with spring and summer temperatures, indicating a major sensitivity to short-term temperature oscillations during the vegetative period. Moreover, the Cladocera NMDS sample scores showed fluctuations during the MCA and the LIA in both lake basins. Cladocera assemblages showed a gradual change during the warming stage after the LIA, thus anticipating the drastic shift of diatoms. This slow change at the beginning of the 19th century, when the anthropogenic impact on Lake Garda was still low, suggested that Cladocera are significantly affected by climate variability, especially under low nutrient conditions.

Similar to what was observed in Lake Garda, Paper **III** showed that the ecological development of Lake Ledro during the last few centuries can be divided into two major stages. Before the 1960s the lake dynamics were mainly controlled by the hydrological variability. Lake Ledro was oligotrophic and cold, and phytoplankton concentrations were low. Sub-fossil pigments and diatoms, together with pigment- and diatom-inferred pH and TP values, suggested very stable ecological lake conditions during this period. The dominant species *Cyclotella delicatula* corroborated the oligotrophic conditions. At the beginning of the 19th century diatom assemblages showed a drastic decrease in the abundance of planktonic taxa, which were almost completely replaced by benthic taxa mainly belonging to the genera *Fragilaria* and *Amphora*. As a consequence of this change, the DI-TP showed a pulse, in relation to the high TP optima associated to these typically tolerant species in the training-sets used in this study. This temporary regime shift represents the beginning of the successive reorganization of the planktonic diatoms. Before the 1960s Cladocera assemblages were steadily dominated by *B. longirostris*, thus suggesting a high lake nutrient level (Korosi et al. 2013), which was in disagreement with the TP reconstructions based on both pigments and diatoms. However, higher concentrations of this species were recorded in concomitance with major hydrological events, which likely were responsible for the transport of large amount of nutrients from the catchment into the lake. The presence of *Acroperus harpae* and *Alona affinis*, which are considered as "arctic" and "subarctic" species (Harmsworth 1968), supports the hypothesis that Lake Ledro was cold during the LIA. The major flood event registered at the beginning of the 19th century was reflected not only by a decrease in planktonic diatoms, but also by lower Cladocera concentrations and by the development of species preferring turbid water and/or species associated with detritus. The reorganization after this event was rapid for planktonic diatoms, but longer and slower for Cladocera. In general, the cold and wet weather conditions recorded during the LIA were responsible for the pronounced hydrological variability in Lake Ledro.

The post-1960s period was mainly characterized by nutrient enrichment, which was caused by the development of tourism and intensive agriculture activities. The studied biological proxies agreed in indicating a shift in the planktonic community, thus identifying this period as a second major lake ecological stage. Mesotraphentic and later eutraphentic diatom taxa increased at the expense of oligotraphentic species. Subfossil pigments confirmed the increase in *P. rubescens* and in potentially toxic cyanobacteria, which was highlighted by limnological studies (Casellato 1990), while Cladocera showed a dominance of Bosminidae and *D. longispina* group. Even though the nutrient enrichment remained the main driver for lake development in this period, the sensitivity of the lake to hydrological variability remained recognizable. In fact, cold and rainy years at the end of the 1970s and 1990s were probably causing low pigment concentrations, while snowy winters in AD 2004 and 2005 could be the reason for the higher values of pigments, eutraphentic *Stephanodiscus parvus* and inferred lake TP concentrations. The latter event seem to confirm that snow precipitation and thawing are particularly efficient in mobilizing nutrients stored in the catchment soil (Simonneau et al. 2013).

The trophic reference conditions were straightforward identified as the lake status before the 1960s, when the lake showed stable oligotrophic conditions. On the other hand, the definition of the ecological reference conditions was more difficult as the lake was affected by multiple climatic and anthropogenic perturbations during the last few centuries. As the species composition in the different planktonic communities remained almost unaltered before the middle 19th century, reference conditions were reasonably identified prior to this time.

The geochemical analysis presented in Paper **IV** revealed an overall change in Lake Garda since the mid-20th century. The local scale impacts appeared to be reflected by small differences between the two basins. In contrast, the geochemical analysis of Lake Ledro showed continuous fluctuations along the entire core, which were interpreted as a result of the influence of the large catchment area on the lake system. The Pb concentrations in both lakes showed the typical trend recorded in the Alpine region, which reflects larger scale atmospheric deposition in northern Italy/southern Alps (Schwikowski et al. 2004; Thevenon et al. 2011). The increase in Pb content after the early 20th century marks the use of lead additives in gasoline, while the decrease in Pb concentrations after the peak during the 1970s-1980s mirrored the ban of lead additives and the implementing of measures for emission control. The past redox conditions were tracked using the Mn/Fe ratios, which suggested oxygenated bottom waters in the Brenzone basin after the 1960s as also recorded by limnological surveys (Salmaso and Mosello 2010). On the other hand, the ratio indicated anoxic conditions within the sediments (Koinig et al. 2003) also before the 1960s. Unfortunately, no information on oxygen concentrations at the lake bottom is available for the period before the regular lake monitoring. On the other hand, the 430:410 spectrophotometric absorbance ratios suggested a pronounced pigment degradation, likely due to the combined effects of the great depth of the deepest basin (Leavitt 1993) and the oxygenated conditions in the bottom water (Guilizzoni et al. 1992). Therefore, the anoxic conditions were probably restricted only to the sediment-water interface. The shallower basin exhibited a slight change in redox conditions after the 1990s, which corresponded to the lower oxygen concentration in the bottom water recorded at the beginning of the 1990s (Salmaso et al. 1994). In Lake Ledro no particular changes in redox conditions were observed (or at least not preserved) in the sediment core.

The Mn/Fe ratios were also used to assess the lake productivity. The values suggested an increase in aquatic productivity in Brenzone since the 1960s, which corroborates results obtained from both the diatoms and pigment analysis and the limnological evidence, which suggested higher algal biovolume since AD 1996 (Salmaso 2010). In contrast, in Bardolino basin the Mn/Fe ratios suggested a decrease in aquatic productivity, which disagreed with previous limnological and paleolimnological data from (Salmaso 2002). A similar situation was observed in Lake Bourget (France), where a comparable period of oxygen depletion was explained as the consequence of an increase in organic matter accumulating in the lake sediments during the eutrophication period (Giguet-Covex et al. 2010).

The lakes' productivities were also assessed using the P/Ti ratios. The increase revealed in all three cores after the 1960s indicated major lake productivity (Engstrom et al. 1985), which was confirmed by the increase in diatom and pigment concentrations in both lakes. The three studied cores showed an increase in deposited biogenic silica, as inferred by changes in Si/Al (Peinerud et al. 2001), from the beginning of the 1960s, which agreed with previous observations based on diatoms and pigment analysis. In the Brenzone basin Si/Al ratios and diatom concentration exhibited the same trend. On the other hand the Bardolino basin showed an opposite trend between diatoms and Si/Al ratios after the 1960s. In Lake Ledro Si/Al ratios exhibited a different trend compared to the one of total diatom concentrations, especially before the 1960s. On the other hand the Si/Al fluctuations were comparable to those observed for the large colonial pennate diatoms. The discrepancy between diatom concentrations and the Si/Al ratio might be explained by the different Si content in diatom taxa. In fact, the sole count of the valves without regard to the diatom cell size and their Si content could alter the Si/Al ratios, making an erroneous estimation of the diatom productivity inferred by Si/Al ratios (Peinerud et al. 2001).

Even though the River Sarca was strongly impacted by the establishment of dams and power plants since the 1930s, the Zr/Ti ratio, which is considered as a proxy for changes in grain size and in sediment origin, did not exhibit any variation in the Brenzone basin. On the other hand, K/Al indicated a shift in the quality of mineral matter, which could be related to these human activities. The weak change in Zr/Ti ratios after the 1960s in the Bardolino basin was not related to the hydropower establishment, as the underwater ridge limits the transport of mineral material originating from River Sarca to the Bardolino basin. In Lake Ledro the fluctuations in Zr/Ti ratios since the 1940s appeared to be influenced by the water-level regulation. Mineral matter quality, indicated by K/Al ratios, showed no significant changes after that period, but registered slight changes during the flood events.

Conclusions

This study confirmed the potential of paleolimnological investigations to place limnological monitoring data in a secular temporal context, which is particularly relevant considering that monitoring data in Central Europe are often restricted to periods when lakes already were nutrient enriched, or recovering from nutrient enrichment. Moreover, the study highlighted the ability of the multi-proxy and multi-site approach for obtaining an overall picture of the lake-catchment dynamics and temporal trajectories subjected to combined impacts induced by multiple human activities and climate change.

The sediment records of Lake Garda and Lake Ledro showed that since the 1960s the lakes experienced the typical nutrient enrichment trend observed in other deep subalpine lakes south and north of the Alps and, more generally, in many European temperate lakes. Moreover, the sediment analyses provided supplementary information on the lake's evolution over the last 10-15 years, corresponding to the most pronounced increase in air temperatures, which allowed interpreting results of recent surveys from a long-term lake evolution perspective. The large size and water volume of Lake Garda makes the lake relatively inert toward climate variability and minor changes in water chemistry, as pronounced shifts were recorded only after a considerable nutrient input. On the other hand, the study on Lake Ledro revealed the importance of a large catchment area in affecting the hydrological regime of a relatively small lake.

The main conclusions of each study are summarized below:

Paper **I**: The paleolimnological study of Lake Garda highlighted the importance of the multi-site approach in large lakes. The deeper Brenzone basin appeared more suitable for the reconstruction of lake TP level at secular scale, while the study of the shallower Bardolino basin provided information on local dynamics and disturbances. The long-term trophic evolution of Lake Garda is highly coherent with that of several large and deep Alpine lakes in relation to multiple anthropogenic activities. Moreover, the study stressed the necessity to include more large and deep lakes in the training-sets, in order improve the accuracy of TP inference models based on biological proxy organisms.

The identification of impacts of climate variability on the ecological lake evolution remains challenging, as it is tightly coupled on the lake's nutrient status. However, shifts in air temperature are able to control the thermal dynamics of deep lakes and, in particular, the thermal stability and a set of related factors, such as the interannual winter deep circulation pattern, which in turn can affect nutrient availability and nutrient uptake by phytoplankton.

Paper **II**: The three sediment cores investigated from Lake Garda revealed comparable trends of Cladocera concentrations and assemblage composition in both the lake sub-basins, i.e. Brenzone and Bardolino. The study confirmed the reliability of Cladocera remains as a proxy to track past lake evolution. Moreover, Cladocera remains provided information on changes in the planktonic community related to important climate stages, such as MCA and LIA, under conditions of negligible nutrient enrichment. The comparison of Cladocera results with evidence based on other biological proxies reinforced the findings on lake responses to temperature, alone or in combination with nutrient changes.

Paper **III**: The multi-proxy approach adopted for the sediment study of Lake Ledro revealed concordant patterns in the temporal trends of biological proxies (diatoms, Cladocera, pigments), and allowed the identification of two major stages in the lake evolution. Before the 1960s the entire planktonic community responded to the hydrological variability in terms of quantitative changes and principally as a result of dilution processes. On the other hand, the period after the 1960s was characterized by taxonomical changes, which could be related to lake nutrient enrichment. Even though the study highlighted the comparable ability of the different biological proxies in tracking the lake hydrological and nutrient variability, diatoms appeared to be the best proxy for the identification of direct and indirect effects of water temperature changes in Lake Ledro. Despite the small size, Lake Ledro showed an unexpected weak direct response to climate variability, which was explained by the particular features of the lake catchment. The prevailing driving role of hydrological variability on the ecological conditions of this lake makes it possible to assert that the climate response of Lake Ledro is primarily indirect.

Paper **IV**: The geochemical study of Lake Garda and Lake Ledro confirmed the change in aquatic productivity of both lakes since the 1960s and the influence of local factors affecting changes in the two basins of Lake Garda. Moreover, it showed that considerable human activities on the River Sarca led to minor changes in the elements associated with mineral matter. On the other hand, the impact of hydroelectric exploitation, in particular the lake-level regulations, were registered in the sediments of Lake Ledro as continuous fluctuations in the element concentrations and ratios. The combined evidence of biological and geochemical proxies suggest that Lake Garda seems to be more affected by direct impacts of nutrient enrichment and/or climate than by hydroelectric exploitation. In contrast, small lakes with larger catchment areas, such as Lake Ledro, are influenced to a larger extent by the modifications occurring in the drainage basin, such changes related to hydrology and land-use.

Future prospects

The present study underlined the influence of individual, as well as combined, nutrient and climate variability on the lake ecological dynamics. At the same time it confirmed the complexity of disentangling these two major impacts. To improve this research in the future, the next steps would be:

- To apply the “resurrection ecology” technique to ephippia stored in the lake sediments in order to obtain vital individuals and to reconstruct the evolutionary genetic response of zooplankton populations at different stressors, including climate changes, toxic algal blooms and inputs of toxic substances (Jeppesen et al. 2001). Moreover, this technique would make it possible to study speciation and hybridization processes (Korhola and Rautio 2001), offering the opportunity to reconstruct past population abundances, population genetics, food web structure and microevolutionary variations (Jankowski and Straile 2003). Moreover few studies highlighted the development of specific hybrids in response to different lake perturbations. The presence of numerous Bosminidae hybrids in the sediment of Lake Ledro, made it difficult to arrange these species remains into numbers of individuals.

- It is well known that pelagic Cladocera change their body size and the length of mucro and antennule in response to limnological variations, such as nutrient enrichment and predation pressure (Korosi et al. 2008; Manca et al. 2007). Since the Cladocera community in Lakes Garda and Ledro showed a qualitative and quantitative response at nutrient enrichment and climate variability, the study of phenological shifts would add information on the Cladocera response at different stressors.

- Cladocera and diatoms analysis in Lakes Garda and Ledro showed particularly smoothed responses at climate variations, due to the major response to anthropogenic impacts. The analysis of sub-fossil Cladocera remains in remote, high altitude lakes located in the Southern Alps, where the climate-driven effects are particularly evident and the anthropogenic impact is typically low, could provide a better understanding of the Cladocera responses to temperature changes.

- Large and deep lakes south of the Alps not only present similar morphology, but have also been impacted by similar external factors during the last decades, e.g. hydroelectric exploitation. The geochemical approach based on WD-XRF analysis would add information on the lake-catchment dynamics of Italian subalpine lakes among which only Lake Maggiore has been extensively studied. This could allow the individuation of coherence or differences in the long term development of these lakes.

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