

Effects of long term nutrient and climate variability on subfossil Cladocera in a deep, subalpine lake (Lake Garda, northern Italy)

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Abstract Albeit subfossil Cladocera remains are considered as a reliable proxy for tracking historical lake development, they have been scarcely studied in large subalpine lakes south of the Alps. In this study, subfossil Cladocera remains from Lake Garda in northern Italy were analyzed to track the lake's environmental changes since the Middle Ages. One core was retrieved from the largest sub-basin of Lake Garda (Brenzone, 350 m deep) and two cores were retrieved from the profundal and littoral zone of the smaller lake sub-basin (Bardolino, 80 and 40 m deep, respectively). The species distribution of Cladocera

remains in recent sediment layers was similar to that observed in contemporary water samples. The deepest sections of the three cores were characterized by species sensitive to water temperature, which suggest a key role of major climatic events in driving the lake response during the late Holocene. The most evident change in Cladocera assemblages in the studied cores was observed during the 1960s, when planktonic taxa such as *Daphnia* spp. and *Bosmina* spp. became dominant at the expense of littoral taxa. Despite the highly coherent general pattern of subfossil Cladocera, the cores showed a set of minor differences, which were attributed to different morphological and hydrological features of the two basins forming Lake Garda. Multivariate analysis revealed a clear relation of Cladocera to climate variability during periods of low lake nutrient levels (i.e. before the 1960s). This provides additional information on past ecological responses of Lake Garda, as contrast previous data from subfossil diatom and pigment analyses did not fully capture effects of climate change during the same period. Since the 1960s shifts in assemblage composition of Cladocera and diatoms were highly coherent, in response to nutrient increase and the following, less pronounced, decrease in nutrient concentrations. During the last five decades, the response of the Cladocera to climate variability appeared to be masked by nutrient change. This work points up the potential of the multi-proxy approach for disentangling the multifaceted biological

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responses to multiple environmental stressors in large and deep lakes.

Keywords Cladocera · Paleocology · Sediment · Climate change · Trophy · Lake Garda

Introduction

The application of innovative techniques for lake sediment studies has enabled rapid advances in the study of paleocology over the last two decades (Battarbee and Bennion 2011). The steadily growing number of paleoecological publications and citations since 1990 reflects the great utility of sediment records for reconstructing lake responses to eutrophication (Davidson and Jeppesen 2013). Although nutrient enrichment remains a key issue and a threat for many temperate lakes (Anderson et al. 2012), climate change has recently emerged as a crucial superimposed driver of lake ecological dynamics (Kernan et al. 2010). Climate-driven effects are particularly evident in remote and alpine regions, which are typically less affected by direct human impact. Conversely, in more anthropogenically impacted lakes, climate and nutrient changes may often produce additive or synergistic effects on lake ecology, which makes it difficult to disentangle the effects of the two factors (Battarbee et al. 2012). Sediment records spanning over both the pre- and post-human disturbance periods can partially overcome this difficulty (Lotter et al. 1997; Kamenik et al. 2007). However, only multi-proxy approaches can exploit the ability of different biological proxies to track different aspects of the overall lake ecological response to overlapped stressors, such as nutrient levels and climate (Perga et al. 2015).

Cladocera (Crustacea) represent a dominant component of both the pelagic and littoral zones in temperate lakes. They play a key role in the lake food web as they occupy a level between bottom-up factors (nutrients and phytoplankton) and top-down regulators (fish and invertebrate predators), and strongly react to changes in lake trophic conditions, temperature and water level (Jeppesen et al. 2001; Szeroczyńska 2006). Their chitinous bodies are well preserved in lake sediments and retain the characteristics necessary for species-level identification.

Cladocera remains are therefore considered to be a good proxy for environmental reconstruction (Frey 1986; Sarmaja-Korjonen 2001; Manca et al. 2007; Zawisza and Szeroczyńska 2007; Nevalainen et al. 2011; Alric et al. 2013). They have been widely used to track historical changes in lake nutrient concentrations, either alone or in combination with other paleolimnological proxies (Jeppesen et al. 2001; Davidson et al. 2007), pH (Paterson 1994; Jeziorski et al. 2008), and also to study historical changes in water levels (Korhola et al. 2000, 2005), submerged macrophyte distributions (Davidson et al. 2007) and food webs (Finney et al. 2000; Jeppesen et al. 2001).

Several studies on subfossil Cladocera have been conducted in Alpine lakes, including both mountain lakes (Lotter et al. 1997; Bigler et al. 2006; Kamenik et al. 2007; Nevalainen et al. 2011) and peri-alpine deep lakes such as Lakes Constance (Hofmann 1998), Geneva, Bourget, Annecy (Alric et al. 2013; Perga et al. 2015) and Zurich (Boucherle and Züllig 1983). However, of the large and deep lakes south of the Alps, which together account for over 80% of Italy's surface freshwater, only Lake Maggiore has been analyzed for subfossil Cladocera (Manca et al. 2007). A recent paleolimnological study focusing on diatom changes in Lake Garda since the Middle Ages (Milan et al. 2015) demonstrated the importance of climate variability as a driver of the lake's ecological dynamics at secular scale. However, this study could only partly discriminate between lake biological responses driven by nutrient enrichment and climate change, in relation to the ecological features of planktonic diatoms.

The present work aims at reconstructing changes in the species distribution and abundance of subfossil Cladocera in Lake Garda (the largest Italian lake) during the late Holocene, and at relating them to direct human impacts and climate change. Considering that physical and biological lake responses depend on lake physical properties, such as basin size and depth, three cores were collected from the two basins of Lake Garda in order to (1) analyze long-term differences in Cladocera species composition and abundance in different profundal and littoral lake compartments, (2) relate these changes to historical limnological and climatic variability, and (3) to discriminate between the lake's responses to nutrient enrichment and climate change through the comparison between Cladocera results and data from other sediment

biological proxies (i.e. diatoms, pigments and *Pediastrum* spp. abundance).

Study site

Lake Garda is the largest subalpine lake in Italy, with a surface area of 368 km² and a volume of 49 km³ (representing 34% of Italian freshwater resources; Fig. 1). Its catchment area (~2350 km²) ranges from 3556 m (Monte Presanella) down to 65 m a.s.l., with a mean altitude of ~1000 m a.s.l. Due to its small catchment/lake area ratio of 6:1 (Gerletti 1974), the theoretical lake water renewal time is ~27 years (Salmaso 2010). The main lake inflow, the River Sarca, flows through the catchment's mountainous northern portion, which includes the siliceous Adamello-Presanella mountain range, while the southern portion is dominated by sedimentary rocks (Sauro 1974). A submerged ridge divides the lake into a deeper western basin (Brenzone, 350 m depth) and a shallower eastern basin (Bardolino, 81 m depth), which accounts for 7% of the total lake volume

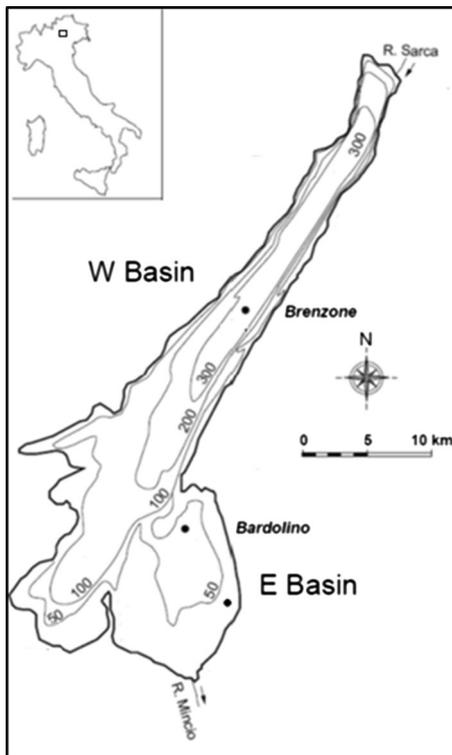


Fig. 1 Location and bathymetric map of Lake Garda. Dots indicate the coring points

(Salmaso 2010). Although Lake Garda is commonly classified as a warm monomictic lake, complete water mixing occurs only after particularly cold winters that reduce and homogenize the temperature of the water mass (holomixis).

Chemical and biological data have been collected monthly from the Brenzone basin since 1996, while data from the Bardolino basin are available only for the period 1996–2008. Pre-1996 limnological data are sporadic (Salmaso and Mosello 2010) but indicate clearly oligotrophic lake conditions before 1975, with annual average total phosphorus (TP) concentrations below 10 µg L⁻¹ (Salmaso 2002). After a TP increase up to ~20 µg L⁻¹ between 1996 and 2005 (Brenzone basin), stable an oligo-mesotrophic status with TP values around 18 µg L⁻¹ established since the mid-2000s (Salmaso 2010). The trends in phosphorus concentration in the Bardolino basin are broadly similar to those in the Brenzone basin, although the Bardolino TP increased to ~13 µg L⁻¹ between 1999 and 2005 before falling to 8 µg L⁻¹ in 2008. Lake TP concentrations before 1996 have been inferred for both basins by Milan et al. (2015) based on subfossil diatoms (Diatom Inferred Total Phosphorus—DITP) levels. Both basins exhibited a DITP baseline of 4–5 µg L⁻¹ until the 1960s, when concentrations increased in good agreement with monitoring trends. The concentration of dissolved inorganic nitrogen (DIN) varied between ~180 and 260 µg N L⁻¹ since 1996, while epilimnetic silica contents ranged from 0.3 to 0.5 mg Si L⁻¹ and exhibited seasonal patterns similar to those of TP (Salmaso 2010).

The mean annual phytoplankton biovolume of Lake Garda steadily increased during the monitoring period, peaking at >1400 mm³ m⁻³ in both lake basins in 2009. Green algae, diatoms and cyanobacteria steadily represented the dominant algal groups. The contemporary zooplankton is largely dominated by copepods, but Cladocera and rotifers are significantly abundant from spring to autumn. Detailed paleolimnological data on subfossil diatoms, pigments, and geochemistry are available in Milan et al. (2015), limnological data on chemistry and planktonic assemblages in Salmaso and Naselli-Flores (1999), Salmaso (2010), Salmaso and Cerasino (2012).

Observational climate data for Lake Garda are available from the HISTALP data set (Auer et al. 2007), which includes homogenized monthly mean air temperatures and precipitations from the

meteorological stations of Torbole—Riva del Garda (~20 km away from the coring point at the Brenzone basin, data available since AD 1760) and Villafranca (~25 km away from the deepest point of the Bardolino basin, data available since AD 1788) (ESM1). Both meteorological stations exhibited similar temperature patterns during the last 250 years, but Villafranca showed more gradual decadal shifts in mean summer and winter air temperatures compared to Torbole-Riva del Garda. The mean annual precipitation oscillated around 1000 mm between the end of the nineteenth century and the mid-1930s. After a drastic decrease to values around 600 mm in the early 1940s, precipitation increased again to ~1000 mm until the 1990s, when another progressive decreasing trend with values around 850 mm began and is still persisting. Spring and summer precipitation patterns followed the mean annual precipitation trend, while winter and autumn precipitations do not show evident trends.

Materials and methods

Coring and sediment analyses

Three short sediment cores were collected with a gravity corer (UWITEC, Austria): Bren1-09 from the deepest point of the Brenzone basin (45°42′06″N, 10°43′30″E, collected in October 2009), Bar1-11 from the deepest point of the Bardolino basin (45°32′58″N, 10°40′34″E; collected in January 2011), and LitG1-13 from the littoral zone of the Bardolino basin (45°29′14″N, 10°40′52″E; water depth 39 m; collected in February 2013). Each core was vertically extruded and sliced in the laboratory. In order to allow a better comparison of sediment records and limnological data, the upper 30 cm of each core was sliced at 0.5-cm intervals, while the deeper intervals (30 cm to core bottom) were sectioned at 1-cm intervals. Core chronologies were established using the CRS dating model (Appleby 2001) on the basis of direct gamma assay radiometric analyses (^{210}Pb , ^{226}Ra , ^{137}Cs and ^{241}Am) conducted at ENSIS Ltd-University college London, UK. Two vegetal remains found in the Brenzone core were dated using ^{14}C at the Poznan Radiocarbon Laboratory, Poland. Further details on the core radioisotopic dating process are available in Milan et al. (2015). The chronology of the littoral core

was obtained by parallelizing the depth profiles of sediment percent dry weight of the Bar1-11 and LitG1-13 cores, which both exhibited well resolved fluctuations and comparable peaks. Detailed information on the core parallelization is available in the supplementary material (ESM2), while age-depth models and sedimentation rates for the two profundal cores (Bren1-09 and Bar1-11) are available in Milan et al. (2015).

The two profundal cores were analyzed for radionuclides, spheroidal carbonaceous particles (SCPs), water and inorganic content, algal and bacterial photosynthetic pigments and subfossil diatoms (Milan et al. 2015), and Cladocera. The littoral core from Bardolino (LitG1-13) was only analyzed for Cladocera. Pigment and diatom data for the Bar1-11 core were assigned to the LitG1-13 core based on core parallelization. The astaxanthin profiles were used as a proxy for invertebrate abundance (Leavitt 1993). The lake phosphorus concentrations used in this work were independently inferred for the two profundal cores (Bren1-09 and Bar1-11) based on total concentrations of subfossil carotenoids and on subfossil diatoms (Milan et al. 2015).

Subfossil Cladocera analyses

Cladocera remains were analyzed using the methods described by Frey (1986) and Szeroczyńska and Sarmaja-Korjonen (2007). About 2 cm³ of wet sediment were heated in KOH (10%), filtered through a 40 μm mesh, and finally treated with HCl (10%) to eliminate carbonates from the sediment. The residue was then brought to a total volume of 10 mL with distilled water, and stained with a glycerol-safranin mixture. Subsamples of 0.1 ml were transferred to slides for microscopic examination. Each slide was examined using a light microscope (LEICA DM2500) at 100–400× magnification and all the detectable Cladocera remains (headshield, shell, postabdomen, postabdominal claws, mandible, caudal furca), and ehippia were counted. Three to six slides were counted for each sample aiming at achieving a minimum of 100 Cladocera individuals per sample (Kurek et al. 2010). This minimum was not reached for some samples from the profundal cores, due to the extreme scarcity of Cladocera remains. The number of individuals represented by the counted Cladocera remains was determined according to Frey (1986). The

taxonomic identification was based on Margaritora (1983), Flössner (2000), and Szeroczyńska and Sarmaja-Korjonen (2007). The ecological preferences of the species were defined as in Flössner (2000), Frey (1986) and more recent publications (Korhola 1990; Kamenik et al. 2007; Zawisza and Szeroczyńska 2007). Cell-wall remains of the coccal green algae *Pediastrum* were also counted and identified at the species level (Komárek and Jankovská 2001). All the counted subfossil remains were expressed as numbers of individuals per cm³ of fresh sediment.

Data analysis

Stratigraphic plots were drawn with C2 version 1.7.2 (Juggins 2007). Homogenous Cladocera zones within the cores were identified using the optimal partitioning method as implemented in ZONE (Lotter and Juggins 1991). The number of significant zones was established using comparison to the broken stick model (Bennett 1996). Additional subzones based on Cladocera abundance, often supported by optimal sum squares partitioning method, were added to the main zones in order to facilitate core comparisons. The planktonic:littoral ratio was calculated as an indicator of Cladocera response to environmental factors such as nutrient availability and water level fluctuations (Sarmaja-Korjonen 2001), while the log₂-based Shannon Index (Shannon and Weaver 1949) was used to evaluate the diversity of the Cladocera assemblages.

A non-metric multidimensional scaling (NMDS, Kruskal and Wish 1978) was performed separately for each core to identify patterns in the temporal development of Cladocera assemblages. NMDS was applied to a Bray and Curtis dissimilarity matrix (Legendre and Legendre 1998) based on double square rooted Cladocera densities. Data were transformed in order to reduce the variance and to downweight the most abundant taxa. Stress values and sample configurations of NMDS analyses with increasing numbers of dimensions were compared in order to identify the minimum number of dimensions to be considered. Vector and surface fitting analyses were applied to the sample scores on the final NMDS configurations (ESM3), and computed using R 3.2.3 (R Core Team 2014), function *ordisurf* in *vegan* package version 2.3–0 (Oksanen et al. 2015). The vector fitting, which identifies the maximum correlation between a sample distribution within the ordination space and selected

variables, was applied to a range of independent limnological, sedimentary and climatic variables. Surface fitting, which correlate a selected variable with gradients in species composition, was performed to fit a smooth surface for lake TP to the NMDS ordination diagrams. Only variables with significant correlation ($p < 0.05$) are shown in the NMDS plots. The aims of these analyses were to (1) identify major drivers of long-term changes in the Cladocera assemblages of Lake Garda, and (2) integrate the Cladocera results with existing information from studies on sediment diatoms in the same cores (Milan et al. 2015). The temporal variations of sample scores on the first subfossil Cladocera NMDS dimensions were compared to the corresponding scores from the diatom NMDS previously performed on the two profundal cores (Milan et al. 2015).

Results

Sediment coring and analyses

The radiocarbon analyses dated the Brenzone core bottom (48.5 cm depth) to 1418 ± 30 AD, while the ²¹⁰Pb analyses of the Bardolino core dated its 31.5 cm layer to the year 1842 ± 30 AD. Parallelization between the Bar1-11 and LitG1-13 cores made it possible to date the latter down to the 35.5 cm layer, which was deposited in the year 1856 AD (ESM2). The depth profiles of dry weight of LitG1-13 and Bar1-11 were very similar ($r = 0.94$, $p < 0.001$), but the absolute values for the LitG1-13 core were generally greater than those for Bar1-11 (ESM2).

The core from the Brenzone lake basin (Bren1-09, Fig. 2a) was not laminated and consisted of a clastic section extending from the core bottom up to ~26 cm depth, a transitional mixed layer of ~4 cm, and by an organic section zone from ~22 cm to the core surface. The content of organic was correspondently low before the 1970s (~15 cm; <15% of the dry weight), while it ranged between 20 and 30% in the topmost 15 cm (Fig. 2a). The entire lengths of the Bar1-11 and LitG1-13 cores consisted of a homogeneous, not varved grey sediment, with no darker gyttja layer on the top. Nevertheless, the organic content of Bar1-11 oscillated cyclically from 10 to 30% and peaked in the early 1990s (Fig. 2b). The average organic matter content of LitG1-13 was steady around 5% in the

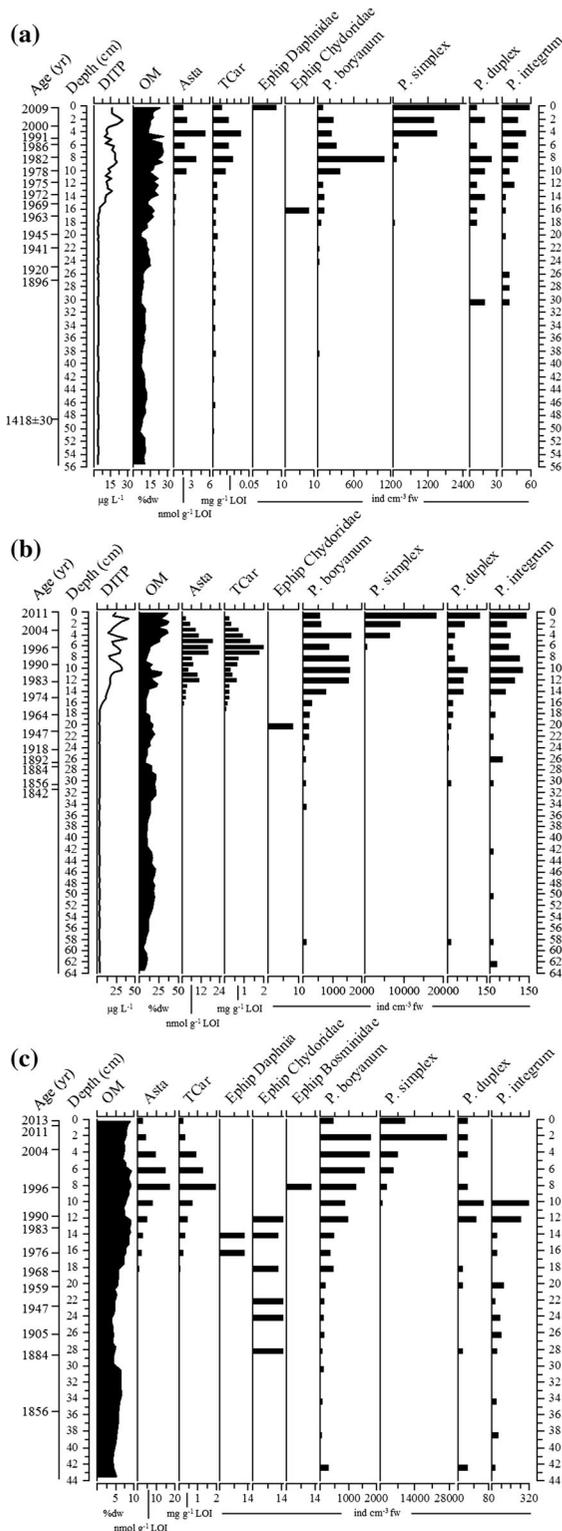


Fig. 2 Depth profiles of lithological and biological proxies in the **a** Brenzone, **b** Bardolino and **c** littoral cores. *DITP* diatoms inferred total phosphorus, *OM* organic matter, *Asta* astaxanthin, *TCar* total carotenoids concentrations, *Ehip Daphniae* Ehippiium of Daphnidae, *Ehip Chydoridae* Ehippiium of Chydoridae, *Ehip Bosminidae* Ehippiium of Bosminidae, *P. boryanum* *Pediastrum boryanum*, *P. simplex* *Pediastrum simplex*, *P. duplex* *Pediastrum duplex*, *P. integrum* *Pediastrum integrum*

lower core sections and increased up to $\sim 8\%$ since the mid-1960s (Fig. 2c).

The DITP concentrations inferred from both the profundal cores were very low prior to the 1960s ($3\text{--}5 \mu\text{g L}^{-1}$), while they rose up to $26 \mu\text{g L}^{-1}$ in the early 2000s in Bren1-09 (Fig. 1a) and $\sim 40 \mu\text{g L}^{-1}$ in the late 2000s in Bar1-11 (Fig. 2b). The total carotenoid concentrations (TCar) in Bren1-09 remained very low until the mid-1940s, when they started to steadily increase until the early 1990s (Milan et al. 2015). Bar1-11 exhibited higher TCar concentrations than Bren1-09, with increasing values from the 1990s to the early 2000s. The three cores showed very similar astaxanthin depth-profiles, which suggested an increase in zooplankton density in all three cores since the late 1960s, with a peak just after the mid-1990s (Fig. 2). Both the cores collected in the shallower Bardolino basin had higher astaxanthin concentrations than the Brenzone core (Fig. 2b, c).

Subfossil Cladocera

The subfossil Cladocera assemblages for the Brenzone, Bardolino, and littoral cores featured 32 taxa (6 planktonic and 26 littoral; Fig. 3a), 35 taxa (7 planktonic and 28 littoral; Fig. 3b), and 37 taxa (6 planktonic and 31 littoral; Fig. 3c), respectively. The identified taxa belonged to the families Leptodoridae, Daphniidae, Bosminidae, Chydoridae and Cercopagiidae. Despite the numerous taxa identified, the total Cladocera densities (Tot Cladocera) were generally low (Fig. 4). Nevertheless, the most abundant species identified in the section of the pelagic cores which have been deposited during the last two decades coincide with the dominant species recorded in the lake plankton since the 1990s (ESM4). Two significant Cladocera zones (BR-C1 and BR-C2) could be identified in the Brenzone core (Fig. 3a). BR-C1 was further subdivided into two subzones (BR-C1a and BR-C1b) based on the total Cladocera concentration

(Fig. 4). BR-C1a (55–52 cm depth) was characterized by the presence of only a few planktonic species, such as *Bosmina* spp. Seligo and the zooplanktivorous *Bythotrephes longimanus* Leydig. Two species associated with littoral macrophytes, i.e. *Acroperus harpae* (Baird) and *Alona affinis* Leydig, were also present, as well as *Alona quadrangularis* (O. F. Müller), *Leydigia leydigi* (Schödler) and *Chydorus piger* (Sars), which are associated with the lake water–sediment interface. BR-C1b (52–19 cm depth) exhibited lower number of planktonic species and increasing abundances of littoral species that prefer either turbid waters, such as *A. rectangula* Sars and *Chydorus sphaericus* (O. F. Müller), or the water–sediment interface, such as *Monospilus dispar* Sars and *Pleuroxus* spp. Baird. Only a few individuals of the pelagic *Bosmina* (*E. coregoni* Baird and *Bosmina* (*E. longispina* Leydig, and of the littoral species *A. harpae* were identified in the sample at 34 cm depth (Fig. 3a). The zone BR-C2 (19–0 cm depth) exhibited substantially higher density in *Bosmina* spp., *Byth. longimanus*, and in the planktonic *Daphnia longispina* (O. F. Müller) group since the early 1960s. The zooplanktivorous *Leptodora kindtii* (Focke) appeared in the topmost core layers. Among the littoral group, *A. quadrangularis* increased. *Alonella nana* Baird, *Disparalona rostrata* Koch and *Pleuroxus trigonellus* (O. F. Müller), which are associated with lake sediments, appeared for the first time in this zone.

The Bardolino core revealed two significant Cladocera zones: BA-C1 to BA-C2 (Fig. 3b), with zone BA-C1 further divided into four subzones based on Cladocera abundance. BA-C1a (64–56 cm depth) was characterized by several littoral species associated with macrophytes, such as *A. harpae*, *Alona* spp. Baird and *Alonella* spp. Sars, and by clear water species such as *C. piger*, *D. rostrata* and *Eurycercus* spp. Baird. *Camptocercus rectirostris* Schödler and *Pleuroxus* spp. Baird, which are considered to be indicators of warm water temperature, were also present. *Chydorus globosus* (Baird) was found exclusively within this zone, while Bosminidae were quite scarce (Fig. 3b). Although the littoral species identified in the previous zone disappeared in BA-C1b (56–41 cm depth), *Alona quadrangularis* rapidly increased and became the dominant species within this zone. BA-C1c (41–27 cm depth) showed a decrease in *A. quadrangularis* and *A. rectangula*, and *Alonella exigua* Lilljeborg, as well as higher abundance of *C. piger*.

The planktonic *Bosmina longirostris* (O. F. Müller) was also first recorded in this zone. Compared to Zone BA-C1c, zone BA-C1d (27–15 cm depth) was characterized by the presence of *Acroperus elongatus* (Sars) together with *Alona costata* Sars, *A. guttata* Sars and *Alonella excisa* Fischer. Moreover, *A. quadrangularis* and *M. dispar* increased, while *C. rectirostris* and *D. rostrata* reappeared after having been absent in BA-C1b-c. *Leydigia* spp. Kurz, which prefers lake sediments, appeared for the first time in the early 1900s. Bosminidae were still present at low levels, while *D. longispina* started to become more abundant. In BA-C2 (15–0 cm depth) *B. longirostris* peaked in the early 1970s, while *B. (E) coregoni*, *B. (E) longispina*, *Byth. longimanus* and *D. longispina* group peaked in the early 2000s (4.5 cm). *L. kindtii* also increased in the upper layers. Despite the overall decrease in the abundance littoral species, *A. harpae*, *A. quadrangularis*, *C. sphaericus* and *Leydigia acanthocercoides* (Fischer), which are indicators of turbid waters, were present in high levels during the early 1990s (8 cm). In addition, *Pleuroxus uncinatus* Baird, a species usually associated with sediments, was encountered exclusively in this zone.

The littoral core was divided into three significant zones: LIT-C1, which was further divided into two subzones, LIT-C2 and LIT-C3 (Fig. 3c) due to Cladocera abundance. The deepest zone, LIT-C1a (44–32 cm depth), was dominated by a small number of littoral species, especially *A. excisa* and *Eurycercus* spp. The planktonic *B. (E) longispina* was also present, but scarcely abundant. The zone LIT-C1b (32–19 cm depth) exhibited increased abundances of littoral species and the sand-associated *Rhynchotalona falcata* (Sars). In LIT-C2 (19–7 cm depth), the abundance of *B. longirostris*, *B. (E) longispina* and *Byth. longimanus* increased rapidly, peaking in the 1980s (10–12 cm), while *B. (E) coregoni*, the *D. longispina* group, and *L. kindtii* reached higher densities in the mid-1990s (6 cm) in LIT-C3 (7–0 cm depth). All littoral species that were strongly represented in LIT-C1 and LIT-C2 decreased rapidly in abundance in LIT-C3 with the exception of *A. guttata*.

Total Cladocera abundance, planktonic to littoral ratio, and Shannon index for the three cores studied are presented in Fig. 4. Zone BR-C1 of the Brenzone core (Fig. 4a) had a similar proportion of planktonic and littoral species, and a very low total Cladocera abundance that reached its absolute minimum at a

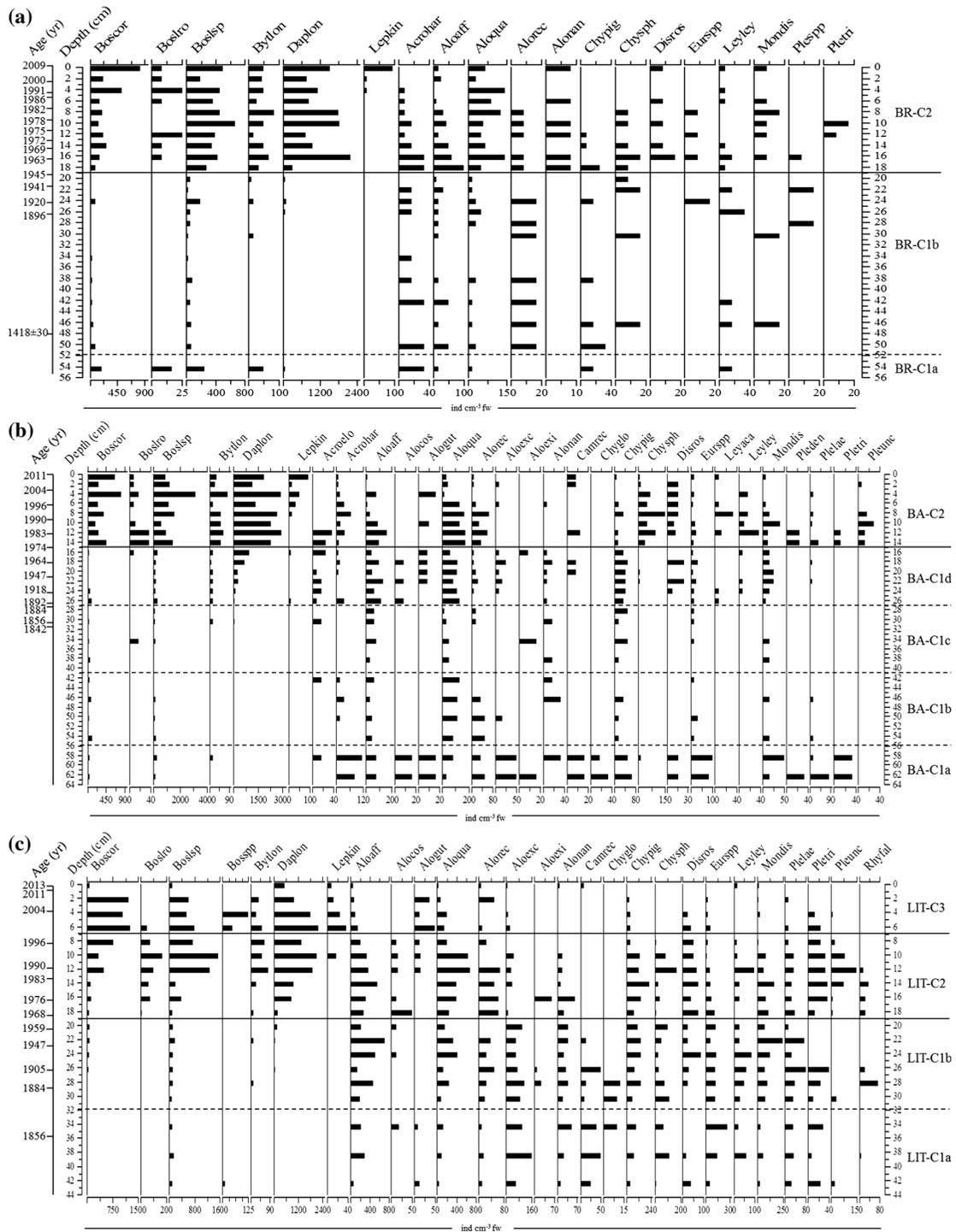


Fig. 3 Depth profiles of key Cladocera taxa in the **a** Brenzone, **b** Bardolino and **c** littoral cores. Species codes and full names of taxa are presented in Supplementary material Table S1

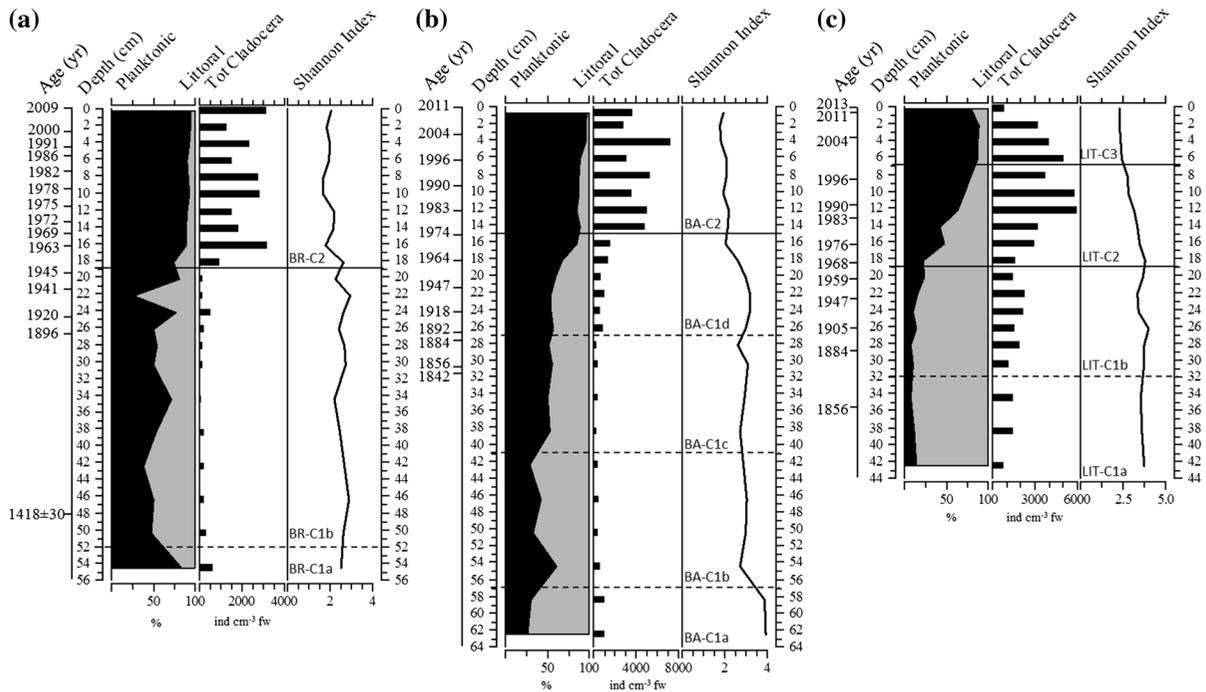


Fig. 4 Depth profiles of Cladocera results in the **a** Brenzone, **b** Bardolino and **c** littoral cores. *Tot Cladocera* sum of all Cladocera specimens counted in each sample

depth of around 34 cm. Conversely, zone BR-C2 was characterized by a pronounced increase in the total Cladocera abundance, which was accompanied by an increasing proportion of planktonic taxa (which peaked at 94%) and by lower Shannon Index. The Bardolino core (Fig. 4b) showed a comparable increase in the total Cladocera abundance since the 1960s (zone BA-C2), which was accompanied by reduced species diversity. However, secular changes in Cladocera diversity and in the proportion of planktonic taxa were more gradual. The littoral core (Fig. 4c) was characterized by a drastic shift from dominance of littoral species in the bottom core sections to planktonic species dominating in the late 1960s (zone LIT-C2). This shift was accompanied by a progressive decrease in species diversity, which persisted till present. Only a few ephippia of *Daphnia* and *Chydoridae* were identified in the two profundal cores, while they were more abundant in the littoral core, which also contained some Bosminidae ephippia in its bottom layers.

Well preserved coenobia of *Pediastrum* spp. were identified along the three cores, with only the Brenzone core showing very low abundance in its bottom

section. In general, the eutrophic *Pediastrum boryanum* (Turp) Menegh was recorded all over the three cores and was found to become gradually more abundant since the late 1940s, peaking in the early 1980s in Bren1-09 and in the 2000s in the two cores from the Bardolino basin. The most recent samples of all three cores were characterized by the presence of the oligotrophic *Pediastrum simplex* Meyen. The levels of *Pediastrum duplex* Meyen (an indicator of high nutrient concentrations) and the clear water *Pediastrum integrum* Nägeli increased since the 1960s in Bren1-09, whereas in Bar1-11 such changes occurred in the 1970s. These species were more abundant in LitG1-13 during the first half of the 1990s but then declined dramatically.

Numerical analysis

The NMDS analyses of Cladocera data from the three cores showed an evident separation of sediment samples deposited before and after core-specific threshold periods (Fig. 5), i.e. the early-1950s (sample 33) for Bren1-09 (Fig. 5a), the early 1960s (sample 37) for Bar1-11 (Fig. 5b), and the mid-1970s (samples

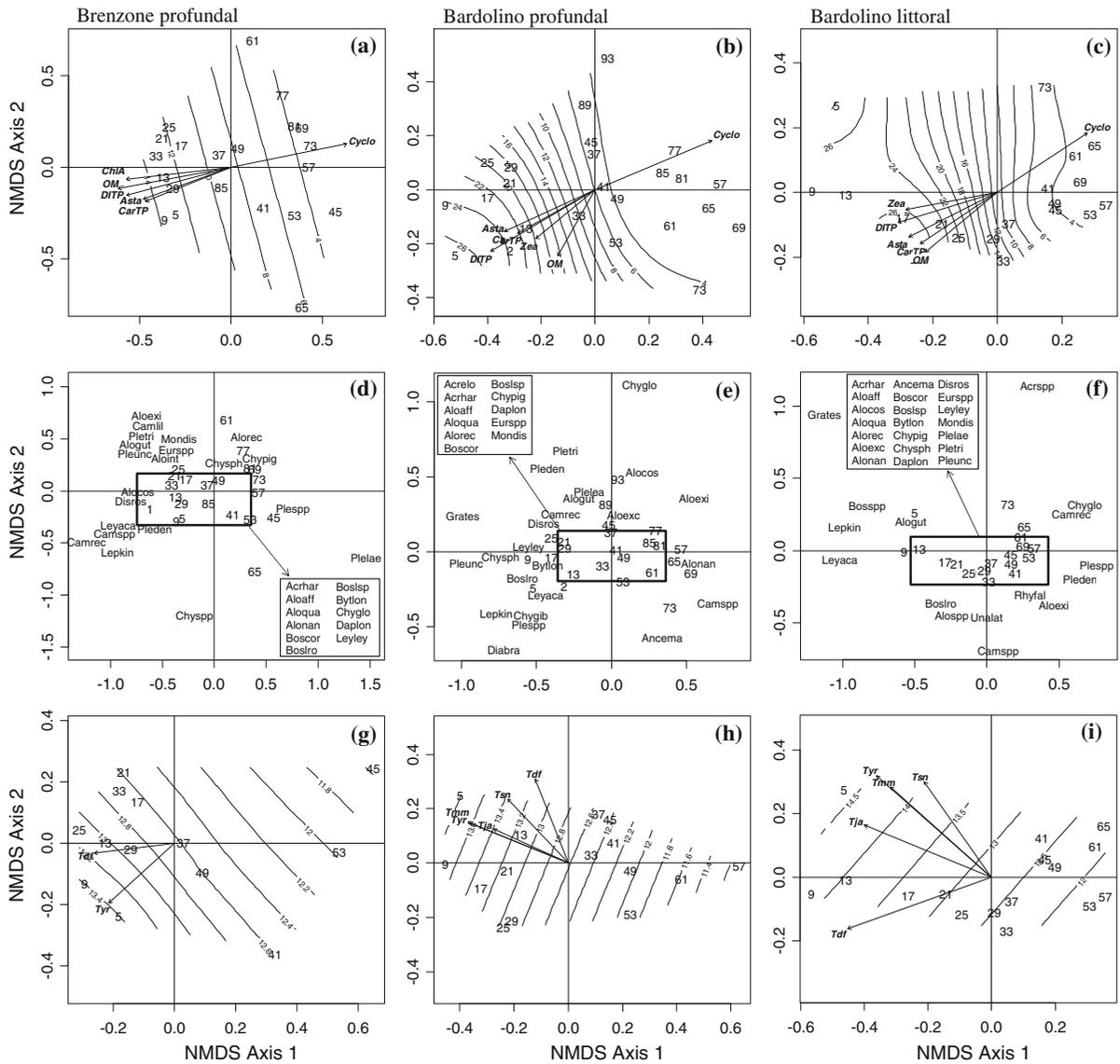


Fig. 5 Cladocera NMDS ordination relative to cores from Brenzone and Bardolino basins. Numbers in the plot refer to core samples. **a–c** Vector and surface fitting for trophic variables. The vectors’ orientations show the direction of most rapid change for each environmental variable, while their lengths indicate the correlation between the environmental variable and the sample ordination. The surface fitting is based on correlations with DITP, and the numbers on the surface lines are DITP values. **d–f** Species distributions on the samples in the ordination space. Species codes and full names of taxa are presented in Supplementary material ESM4. **g–i** Vector and surface fitting for climate variables. The surface fitting is based on correlations with the mean annual air temperature; the

numbers on the surface lines are measured temperatures. Results are shown for the following variables, all of which exhibited significant ($p < 0.05$) correlations: *OM* organic matter; *DITP* TP concentrations reconstructed from sediment diatom abundance data, *CarTP* TP concentration reconstructed from total carotenoids levels, *ChlA* chlorophyll a, *Asta* astaxanthin, *Zea* zeaxanthin (a proxy for colony-forming cyanobacteria), *Cyclo* *Cyclotella* spp. abundance, T_y mean annual air temperature, T_{df} winter air temperature (December–January–February), T_{nm} spring air temperature (March–April–May), T_{ja} summer air temperature (June–July–August), T_{sn} autumn air temperature (September–October–November)

29–33) for LitG1-13 (Fig. 5c). This pattern was confirmed by plotting the sample scores on the first NMDS dimension against the chronology of the sediment cores collected from the deepest points of the two lake basins (Fig. 6). The sample scores on the second NMDS dimension did not show any clear trend for any of the studied cores and will therefore not be discussed further.

The vector fitting revealed a strong relationship between Cladocera species composition and the lake TP concentrations as inferred from subfossil pigment (CarTP) and diatom (DITP), with the TP arrows pointing toward the recent core samples characterized by higher TP concentrations, and the oldest samples being associated with low TP levels and higher relative abundances of *Cyclotella* spp. (Fig. 5a–c). The surface fittings outlined a strong correlation between Cladocera species composition and abundance and DITP and independent trophic-related variables, such as sediment organic content. The Cladocera species distributions (Fig. 5d–f) clearly reveal the association of Bosminidae with the most recent core sections and of Chydoridae with the bottom part of the Bren1-09 core (Fig. 5d). The recent samples of Bar1-11, which were dominated by planktonic species, were grouped in the lower left quadrant of the NMDS ordination (Fig. 5e), while the majority of the species recorded in LitG1-13 (Fig. 5f) were concentrated in the central part of the ordination spaces, which indicated a weaker relationship between species and samples of different ages. Vector and surface fitting of climate variables, which was limited to ^{210}Pb -dated core sections (Fig. 5g–i), showed a linear relationship between Cladocera and mean

annual temperature in the three cores. In addition, the recent samples of the Brenzone core were correlated with mean winter temperatures, while the correspondent samples from both Bardolino cores were correlated with all four seasonal mean air temperatures.

The Cladocera sample scores on the first NMDS dimensions fluctuated within the negative range in the sections deposited before 1960s of both the profundal cores (Fig. 6). The most pronounced and rapid increase started in the Brenzone core in the early 1940s and was followed by a period of scarce variability till recent years (Fig. 6a), while changes in the Bardolino basin were smaller and more gradual, especially in the littoral core, but continued also after the 1960s (Fig. 6b). In contrast to Cladocera, diatom sample scores exhibited little variability prior to the 1960s, and very pronounced and rapid change since then in both profundal cores (Fig. 6c, d).

Discussion

The investigation of the three sediment records collected from the two main sub-basins of Lake Garda confirmed that sedimentary Cladocera remains reflect the species composition and abundance of modern pelagic Cladocera assemblages in this lake. This confirms the potential of this biological proxies for palaeoecological reconstructions, as previously reported in several studies on different lake types (Nykänen et al. 2009; Alric and Perga 2011). In addition, the littoral sediment record enabled an assessment of the long term development of littoral

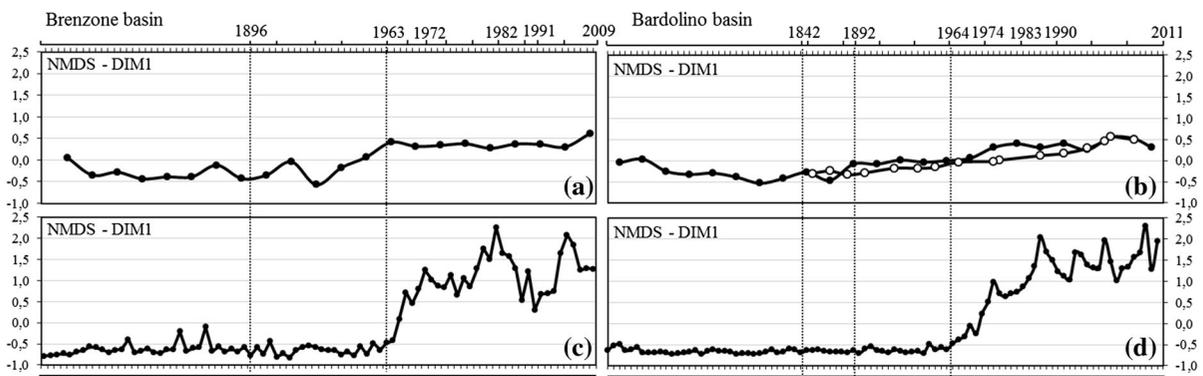


Fig. 6 Cladocera (a, b) and diatoms (c, d) sample scores on the first NMDS dimension for the Brenzone and Bardolino cores. Filled circles represent profundal cores; empty circles represent the littoral core

assemblage compositions of Lake Garda. Littoral taxa usually occur only sporadically in pelagic net samples from peri-alpine lakes with moderate productivity, which are typically dominated by copepods and rotifers (Salmaso and Naselli-Flores 1999; Manca et al. 2007). On the other side, the littoral zone of large lakes is still very poorly considered by limnological surveys, despite it is usually exposed to multiple human stressors (Spitale et al. 2011).

The subfossil Cladocera from the three cores showed highly coherent changes in species composition and abundance in both the section deposited during the last ~150 years, i.e. after the maximum of the Little Ice Age (LIA), and in the deepest, non-dated sections. This coherence confirms the overall homogeneity of environmental trends and ecological dynamics within Lake Garda (Salmaso 2002). However, a deeper observation revealed subtle differences in the secular variability of Cladocera species composition and abundance in the three sediment records, which have been interpreted as responses to morphological and habitat differences between the two major lake basins. The depth profiles of the lithological and algal proxies examined in a previous work (Milan et al. 2015) support this interpretation. The core from the Brenzone basin showed a higher proportion of minerogenic material, which has been attributed to the solid material discharged by the lake's main inlet, the River Sarca, into the deepest basin. On the other hand, the lower mineral content of the two cores collected from the shallower Bardolino basin is probably related to the separation from the main lake basins (through the submerged ridge) and to the scarcity of inlets discharging into this lake basin (Gerletti 1974). The sediment profiles of organic content in the three studied cores suggest that the productivity of Lake Garda has increased over the last ~50 years, which was confirmed by the increase in subfossil pigment concentrations since the 1960s–1970s (Milan et al. 2015). The different organic content and pigment concentrations in the two cores does not seem to be attributable to different productivity of the two basins, which has been demonstrated to be in the same order of magnitude (Milan et al. 2015). These differences may be probably related to the higher degradation rate of settling organic material and pigments in the very deep Brenzone basin, which is oxygenated down to the bottom. Similarly, the scarcity of Cladocera species and individuals in the Brenzone core may be related to

the pronounced depth-related degradation of settling biological remains and to dilution by the higher levels of minerogenic sediment in this lake basin.

The deep sections (between the bottom and a ~50 cm depth) of the profundal cores collected from the two lake basins identified the first distinct subfossil Cladocera zone, Bosminidae, which are considered to be indicators of high lake nutrient levels (Hofmann 1998; Korosi et al. 2013), were particularly abundant in this zone of the Brenzone core. The deepest section of the Bardolino core showed higher abundance of littoral species, especially clear water- and macrophyte-associated species, which might indicate a past macrophyte-dominated stage. Species reflecting warm water temperatures were also detected in this deep section of both the profundal cores, including *Byth. longimanus* in Brenzone and *C. rectirostris* and *Pleuroxus* spp. in Bardolino, together with species preferring low water temperatures, such as *A. harpae* and *A. affinis*. The combination of these apparently contrasting observations suggests that the deepest layers of both profundal cores were deposited during the transition from the Medieval Climatic Anomaly (MCA) and the LIA (De Jong et al. 2013). The length of the littoral core was insufficient to reach this transitional stage.

The sections BR-C1b, BA-C1b-c and LIT-C1 of the three cores were characterized by low Cladocera abundances and a pronounced change in species composition. In particular, several species typical of high latitude lakes or considered to be indicators of cold waters increased in abundance or appeared for the first time. Some of these species, such as *A. harpae* and *A. affinis*, are often considered to be early immigrants after ice retreat (Kamenik et al. 2007). The presence of species associated with lower water temperatures in these sections of both the profundal and littoral cores suggests that the corresponding layers were deposited during a prolonged cold period, such as the LIA, which in the Alpine region culminated between 1750 and 1850 (De Jong et al. 2013). Comparable effects of the cold stage between the eighteenth and nineteenth century have also been identified in Lake Orta and Lake Ledro (Manca and Comoli 1995; Milan et al. 2016), while historical archives indicate that there were several particularly cold events in this period that were accompanied by total or extended ice cover on Lake Garda, for example in 1709 and 1869 (Sauro 2001). The years following the LIA culmination,

which could be dated based on lead isotopes, were characterized by the increase in Cladocera abundance in all three cores and in species associated with warm water, such as *C. rectirostris* and *Pleuroxus* spp. (Szeroczyńska pers. commun.). The apparent slower shift towards species indicating warmer conditions, which has been observed in the pelagic zone of the Bardolino basin, should be interpreted as an artefact of the simultaneous increase of the pelagic species, which strongly affect the final abundance of the less abundant species, including those indicating warmer conditions.

In all three cores, a much more rapid increase in the abundance of planktonic species occurred after the 1960s–1970s, as exemplified by the dramatic increase in the P:L ratios of all three cores. Bosminidae and the *D. longispina* group, which are all considered to be indicators of lake nutrient enrichment (Boucherle and Züllig 1983), dominated the Cladocera community during these decades, and were responsible for the general increase in total Cladocera abundance. The peak abundances of *B. longirostris* coincided with the increased phosphorus load from the lake catchment during the 1970s and again the 1990s, which has been confirmed by both limnological (Salmaso and Mosello 2010) and paleolimnological studies (Milan et al. 2015). The increasing lake nutrient level in the early 1990s also coincided with the first record of cyanobacterial blooms on Lake Garda (Salmaso 2010). The Cladocera assemblages mirrored these blooms with peak abundances of *C. sphaericus*, which is often associated with blue-green algae (Korhola 1990). Manca et al. (2007) highlighted the same species association in Lake Maggiore only a few years earlier than in Lake Garda.

Increasing abundances of *D. longispina* group and *Byth. longimanus* are considered indicative also of decreasing fish predation on the zooplanktonic community (Zawisza and Szeroczyńska 2007). Unfortunately, information on fish species composition (Oppi 1989) and capture data (Gandolfi, pers. commun.) are rather incomplete and scattered for Lake Garda, so they have a very limited quantitative value. Moreover, fishing practices and species capture patterns in Lake Garda have changed substantially in recent decades because of changing commercial preferences (Oppi 1989). Nevertheless, in lakes with increased nutrient level it has been commonly observed that after a first general increase in fish biomass since the mid-1950s, a

process of selection for species better fitted to the new conditions (primarily, cyprinids, perches and pikes) occurred and was accompanied by a decrease in the abundance of planktivorous fish since the 1970s (Oppi 1989). The appearance of the zooplanktivorous *L. kindtii* since the 1990s in all of the studied cores agrees with the recent observations of a further decrease in total fish biomass (Gandolfi, unpublished data).

The sediment layers deposited during the 2000s revealed further changes in the Cladocera composition of Lake Garda, with the abundance of the *B. (E) coregoni* group increasing in all three cores at the expense of *B. longirostris*. This shift within the Bosminidae suggests a return to lower trophic conditions, as observed in other recovering deep subalpine and alpine lakes (Bigler et al. 2006; Manca et al. 2007; Alric et al. 2013). Both decadal limnological data (Salmaso 2010) and paleolimnological reconstructions based on subfossil diatoms and photosynthetic pigments (Milan et al. 2015) revealed a stabilization of lake TP concentrations at oligo-mesotrophic level after the mid-2000s, which has been attributed to improvements in waste water treatment since the late 1990s (Sauro 2001).

The response of subfossil Cladocera to the lake's nutrient enrichment during the last few decades is highly coherent with changes in other sediment biological proxies. The abundance of *P. boryanum* and *P. duplex*, which are regarded as indicators of eutrophic conditions (Komárek and Jankovská 2001), increased rapidly after the 1960s–1970s, while the oligotrophic *P. simplex* was recorded in the uppermost layers of all three cores. Concentrations of total carotenoids and astaxanthin increased in all the three cores during the nutrient enrichment period, although their increase lagged somewhat behind that of Cladocera. In addition, subfossil diatom analysis revealed rapid and pronounced increases in the relative abundance of planktonic Fragilariaceae, *Stephanodiscus* spp. and *Aulacoseira* spp. after the early 1960s, at the expense of small *Cyclotella* spp. (Milan et al. 2015).

Comparable nutrient-driven changes in the subfossil Cladocera assemblages have been observed in other deep perialpine lakes, including Lake Bourget, Annecy, Geneva, Constance, Maggiore and Vrana (Schmidt et al. 2000; Manca et al. 2007; Alric et al. 2013). However, most of the available information on the ecological preferences of Cladocera with respect to nutrients originate from studies conducted on small

alpine lakes (Lotter et al. 1997; Bigler et al. 2006; Kamenik et al. 2007; Nevalainen et al. 2011) or central-eastern European lakes (Szeroczyńska 2006; Zawisza and Szeroczyńska 2007; Korponai et al. 2011). Beside being less numerous, previous studies on Cladocera ecology in large and deep perialpine lakes mainly focused on individual species or responses to ecological stressors other than TP and climate, or were based on sediment cores collected before the beginning of lake restoration measures (Boucherle and Züllig 1983; Hofmann 1998). In contrast, the sediment records from Lake Garda analyzed in the present work provide information on the evolution of Cladocera assemblages since the Middle Ages, when the lake was still not affected by anthropogenic nutrient enrichment. However, the interpretation of lake changes after the beginning of major human impact (=nutrient) is complicated by the superimposed climate effects which became evident during the last few decades. Previous sediment studies in Lake Garda focused on algal proxies provided some hints but were not sufficient to discriminate between nutrient and climate. Disentangling the effects of these two stressors is further complicated by the fact that organisms generally react to combinations of different environmental drivers in their multidimensional ecological niches (Salmaso 2002). In addition, timing of climate warming in the Alpine region largely coincided with the onset of nutrient enrichment (Perga et al. 2015).

The application of multivariate statistics helped us to overcome some of these limitations. In particular, NMDS analyses with vector and surface fittings on the profundal and littoral cores from Lake Garda confirmed the highly coherent response of subfossil Cladocera to nutrients and productivity-related factors such as pigment concentrations, and the organic content of the sediment. The Cladocera community response to the nutrient increasing conditions after the 1960s was underscored in the NMDS by the shift from littoral species to pelagic and nutrient-associated species, such as *Bosmina* spp. On the other side, the vector fitting of climate variables indicated a key role of annual air temperature in driving Cladocera of both the profundal and the littoral lake zones considered in this work. Winter mean air temperatures played an equally important role in Brenzone, while Cladocera of both the profundal and littoral cores from the Bardolino basin were also related to spring and

summer temperatures. This largely confirms the results of recent investigations, which suggested a crucial influence of winter air temperature on the depth of lake mixing in late winter and on the consequent nutrient fertilization of the water column during the following spring (Salmaso and Cerasino 2012). The morphology and shallower depth of the Bardolino basin probably makes it less thermally inert than the deepest basin, and thus more sensitive to short term temperature oscillations during the vegetative period. Although the NMDS analyses on subfossil Cladocera from the littoral core outlined a general pattern which is comparable to that of the two deep cores, most of the identified species were concentrated in the central part of the relative NMDS plot. This suggests that littoral Cladocera assemblages might respond to environmental factors not considered in this study, such as habitat factors. Studies on other lakes have shown that littoral species are particularly sensitive to water transparency, water level changes and submerged macrophytes (Korhola et al. 2000; Davidson et al. 2007; Korosi et al. 2013).

The comparative analysis of sample scores on the first Cladocera and diatoms NMDS dimensions, respectively, clearly suggested that the responses of these key planktonic assemblages to changes in nutrient levels and climate drivers occurred with different timings and magnitude. The previous study on subfossil diatoms (Milan et al. 2015) already showed a very stable situation before the 1960s and a major role of TP on diatoms composition after the 1960s. The lake shifted from oligotrophic to mesotrophic conditions during this period, and the rapid and pronounced shift from centric to pennate diatoms was accompanied by comparable shifts in pigment concentrations and *Pediastrum* species composition. As suggested by Milan et al. (2015), this indicate a direct response of diatoms to increasing nutrient inputs. Changes in water temperature are able to indirectly modulate the productivity of deep temperate lakes by affecting their thermal dynamics. In particular, the air temperature controls the extension of the winter deep circulation, which in its turn determines nutrient replenishment of the epilimnion during the next vegetative season, and the related phytoplankton productivity. Therefore, the cold winters of the 1960s and 1970s probably contributed to enhance the fertilization of Lake Garda that had been triggered by the increased nutrient load from the lake catchment.

This suggest an indirect response of primary producers to climate variability. Similar situation emerged from this study for subfossil Cladocera. The presence of cladoceran species reflecting warm water temperatures (*Byth. longimanus*, *C. rectirostris* and *Pleuroxus* spp.) in the same period suggested also a climatic effect during high nutrient conditions. The Cladocera sample scores clearly fluctuated also during the MCA and the LIA in both the profundal cores. Since the turn of the nineteenth century, i.e. when the anthropogenic impact on Lake Garda was still negligible and comparable to that of earlier centuries, the Cladocera sample scores gradually increased in both lake basins, in a way that anticipated the major nutrient driven diatom changes that occurred during the 1960s in both lake basins (Milan et al. 2015). The early Cladocera changes were gradual and slow compared with the very rapid nutrient-driven diatom change, while variability of subfossil Cladocera and diatoms became rather comparable during the nutrient enrichment period. These observations suggested that climate variability represented the primary driver for the Cladocera assemblages before the beginning of the anthropogenic nutrient enrichment. On the other hand, the correlation between Cladocera species composition, lake TP level and air temperature (as outlined by the multivariate statistics NMDS) suggested that climate warming likely contributed to magnify the effect of nutrient enrichment after the 1960s. These results confirmed the potential of subfossil Cladocera as a proxy for reconstructing community responses to climate variability (i.e. temperature) alone or in combination with nutrients in large and deep lakes.

Conclusion

Although subfossil Cladocera remains are considered to be reliable proxies for studying historical lake development, they have not been studied extensively in large and deep lakes. This paleolimnological study on both the profundal and littoral zone of Lake Garda extended and validated the existing information regarding the ecological preferences of Cladocera, which previously derived mainly from studies on small and shallow lakes. The usefulness of Cladocera in tracking historical climate changes was clearly demonstrated by the data gathered for the pre-human impact oligotrophic period (i.e. before the 1960s). The

MCA, the LIA and the recent warming stage were all clearly reflected by changes in Cladocera species composition, and by changes in the relative abundance of species indicating warmer or cooler temperatures. The Cladocera analyses at Lake Garda complements previous results based on subfossil diatoms and pigments, and helped to disentangle the role of nutrient enrichment and climate variability on lake ecological dynamics. This stresses the strength of multi-proxy and multi-core approaches as tools for obtaining a deeper understanding of the overall ecological processes that govern the development of complex, deep subalpine lakes.

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