

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

While subfossil Cladocera remains are considered to be a reliable proxy for tracking historical lake development, they have hardly been studied in large and deep lakes. In this study, subfossil Cladocera remains from Lake Garda in northern Italy were analyzed to track environmental changes since the Middle Ages. One core was retrieved from the largest sub-basin of Lake Garda (Brenzone, 350 m deep) and another two were retrieved from the profundal and littoral zones of a smaller sub-basin (Bardolino; 80 and 40 m deep, respectively). The species distribution of Cladocera remains in recent sediment layers was similar to that of the contemporary assemblages observed in the water column. The deepest sections of the three cores were characterized by species sensitive to water temperature, suggesting a lake response to the major climatic events that occurred during the late Holocene. A common and evident change in Cladocera assemblages was observed in the studied cores during the 1960s, when planktonic taxa such as *Daphnia* spp. and *Bosmina* spp. became the dominant group at the expense of littoral taxa. Despite the highly coherent general pattern within Cladocera assemblages, the cores showed minor differences, which were attributed to different morphological and hydrological features of the two basins forming Lake Garda. Multivariate analysis revealed a clear response of Cladocera to climate variability during periods of low lake nutrient levels (i.e. before the 1960s). This stands in contrast to conclusions drawn previously based on data for diatoms and pigments, which did not fully capture effects of climate change during these periods. Since the beginning of the lake's nutrient enrichment phase (i.e. after the 1960s), diatoms and Cladocera exhibited concomitant shifts in assemblage composition due to a pronounced increase and then a less pronounced decrease in nutrient concentrations. During this period, the response of the Cladocera to climate shifts was overridden by shifting nutrient levels. This work highlights the value of a multi-proxy approach for disentangling the multifaceted biological responses to multiple environmental stressors in large and deep lakes.

Key words: Cladocera; Paleoecology; Sediment; Climate change; Trophy; Lake Garda

Introduction

The application of new techniques in lake sediment studies has enabled rapid advances in the study of paleoecology 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 threat for many temperate lakes (Anderson et al. 2012), climate change has recently emerged as a crucial driver of lake ecological dynamics (Kernan et al. 2010). Climate-driven effects are particularly evident in remote and alpine regions, where the human impact is typically less pronounced. Conversely, in more anthropogenically-impacted lakes, climate and nutrient changes may have additive or synergistic effects on lake ecology, which makes it difficult to disentangle the effects of the two factors (Battarbee et al. 2012). Studies of sediment records, whose time span cover both the pre- and post-human disturbance periods, can partially overcome this difficulty (Kamenik et al. 2007; Lotter et al. 1997). 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 human and non-human stressors, such as nutrient levels and climate (Perga et al. 2015).

Cladocera (Crustacea) are a dominant component of both the pelagic and littoral zones in temperate lakes. They play a key role in the food web as they occupy a level between top-down regulators (fish and invertebrate predators) and bottom-up factors (nutrients and phytoplankton), and react strongly to changes in trophic conditions, temperature and water level (Jeppesen et al. 2001; Szeroczyńska 2006). Their chitinous bodies are well preserved in lake sediments and retain the necessary characteristics for species-level identification. Cladocera remains are therefore considered to be a good proxy for climate and environmental reconstruction (Alric et al. 2013; Frey 1986; Manca et al. 2007; Nevalainen et al. 2011; Sarmaja-Korjonen 2001; Zawisza and Szeroczyńska 2007). They have been widely

used to track historical changes in nutrient concentrations, either as the sole proxy or in combination with other paleolimnological proxies (Davidson et al. 2007; Jeppesen et al. 2001), pH (Jeziorski et al. 2008; Paterson 1994), 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 (Bigler et al. 2006; Kamenik et al. 2007; Lotter et al. 1997; 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. However, this study only partly discriminated between lake biological responses driven by climate change and nutrient enrichment.

This work reconstructs changes in the species distribution and abundance of subfossil Cladocera in Lake Garda (the largest Italian lake) during the late Holocene, and relates them to direct human impacts and climate change. Because physical and biological lake responses depend on the basin's size and depth, three cores were collected from the two basins of Lake Garda in order to i) analyze long-term differences in Cladocera species composition and abundance in different profundal and littoral

lake compartments, ii) relate these changes to historical limnological and climatic variability, and iii) compare the Cladocera results to data for other sediment biological proxies (such as diatoms, pigments and *Pediastrum* spp. abundance) to differentiate between the lake's responses to nutrient enrichment and climate change.

Study site

Lake Garda is the largest subalpine lake in Italy, with a surface area of 368 km² and a volume of 49 km³ (corresponding to 34% of Italian freshwater resources). 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, 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 (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 therefore 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 data are sporadic (Salmaso and Mosello 2010) but indicate that conditions in the lake were clearly

oligotrophic before 1975, with annual average total phosphorus (TP) concentrations below 10 µg L⁻¹ (Salmaso 2002). After a TP increase to around ~20 µg L⁻¹ in the deepest basin (Brenzone) between 1996 and 2005, the lake stabilized in an oligo-mesotrophic state with TP values around 18 µg L⁻¹ (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. TP concentrations for both basins prior to 1996 have been inferred by Milan et al. (2015) based on subfossil diatom (DITP) levels. Both basins exhibited a DITP baseline of 4-5 µg L⁻¹ until the 1960s, during which the concentrations increased following the monitoring trends. The lake's dissolved inorganic nitrogen (DIN) concentration has 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 increased steadily during the monitoring period, peaking in 2009 at >1400 mm³m⁻³ in both lake basins. Green algae, diatoms and cyanobacteria represent the dominant algal groups. The contemporary zooplankton of Lake Garda is largely dominated by copepods, but Cladocera and rotifers are significantly abundant from spring to autumn. Detailed paleolimnological and limnological data on diatom abundance, subfossil pigment distributions, and geochemistry are available in Milan et al. (2015), Salmaso (2010), Salmaso and Cerasino (2012) and Salmaso and Naselli-Flores (1999).

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. Temperature data 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) were considered for the two coring points, respectively. Plots of mean winter and summer temperature at both meteorological stations are shown in the supplementary material (Fig. S1). Both meteorological stations exhibited similar temperature patterns, 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 19th century and the mid-1930s. After a drastic decrease in the 1940s, precipitation increased again to ~1000 mm until the 1990s, when another progressive decreasing trend began; this trend has persisted into the present. Spring and summer precipitation patterns followed the mean annual precipitation trend, but no particular trend is apparent in the winter and autumn precipitations.

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 at 0.5 cm intervals from 0 to 30 cm, and at 1 cm intervals from 31 cm down to the core bottom. Core chronologies were established using the CRS dating model (Appleby 2001) on the basis of direct gamma assay radiometric analyses (²¹⁰Pb, ²²⁶Ra, ¹³⁷Cs and ²⁴¹Am) conducted at ENSIS Ltd-University college London, UK. Two vegetal remains found in the Brenzone core were dated using ¹⁴C 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 (on a % dry weight basis) for the Bar1-11 and LitG1-13 cores, which both exhibited well resolved fluctuations and comparable peaks. Detailed information on the core parallelization is presented in the supplementary material (Fig. S2), 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, subfossil diatoms (Milan et al. 2015), and Cladocera, whereas the littoral core from Bardolino (LitG1-13) was analyzed for Cladocera only. Pigment and diatom data for the Bar1-11 core were assigned to the LitG1-13 core based on core parallelization. The cores' astaxanthin profiles were used as a proxy for invertebrate abundance (Leavitt 1993). The lake phosphorus concentrations used in this work were independently inferred from total concentrations of subfossil carotenoids and subfossil diatoms, which were analyzed in the cores Bren1-09 and Bar1-11 (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 100-400x magnification and all of the visible Cladocera remains (headshield, shell, postabdomen, postabdominal claws, mandible, caudal furca) and ephippia were counted. Three to six slides were counted for each sample with the aim of 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 their extreme scarcity of Cladocera remains. The number of individuals represented by the counted Cladocera remains was determined according to Frey (1986), and the taxonomic identification of those individuals was based on Flössner (2000), Margaritora (1983), and Szeroczyńska and Sarmaja-Korjonen (2007). The ecological preferences of the species were defined as in Flössner (2000), Frey (1986) and more recent publications (Kamenik et al. 2007; Zawisza and Szeroczyńska 2007), while the classification of individual species as “arctic”, “sub-arctic”, or “north-temperate” was based on the distribution of Cladocera in high-latitude sites (Harmsworth 1968). Cell-wall remains of the coccal green algae *Pediastrum* were also counted and identified at the species level (Komárek and Jankovská 2001). All of the

counted subfossil remains were expressed in terms of 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, often supported by optional partitioning method, were added to the main zones in order to facilitate core comparisons. The planktonic:littoral ratio was calculated as an informative 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 & Curtis dissimilarity matrix (Legendre and Legendre 1998) based on the double square root of Cladocera densities. Stress values and sample configurations from the NMDS analyses with increasing numbers of dimensions were compared in order to determine how many dimensions needed to be considered. Vector and surface fitting analyses were applied to the sample scores on the NMDS configuration, and computed using R 3.2.3 (R Core Team 2014), *vegan* package version 2.3-0 (Oksanen et al. 2015). Only variables with significant correlation ($p < 0.05$) are shown in

the NMDS plots. The aim of these analyses was to i) identify major drivers of long-term changes in the Cladocera assemblages of Lake Garda, and ii) integrate the Cladocera results with existing information from studies on sediment diatoms in the same cores (Milan et al. 2015). To the latter end, vector fitting, which identifies the maximum correlation between a sample distribution within the ordination space and selected variables, was applied to a range of limnological, sedimentary and climatic variables. Surface fitting was performed to correlate selected variables with gradients in species composition, and it fits a smooth surface for one selected variable, plotting the results on the ordination diagrams. The temporal variations of sample scores on the first subfossil Cladocera NMDS dimensions were analyzed and compared to the corresponding scores from the diatom NMDS for 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 determined to have been deposited in the year 1856 AD (supplementary material Fig. S2). 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 (supplementary material Fig. S2).

The core from the Brenzone lake basin

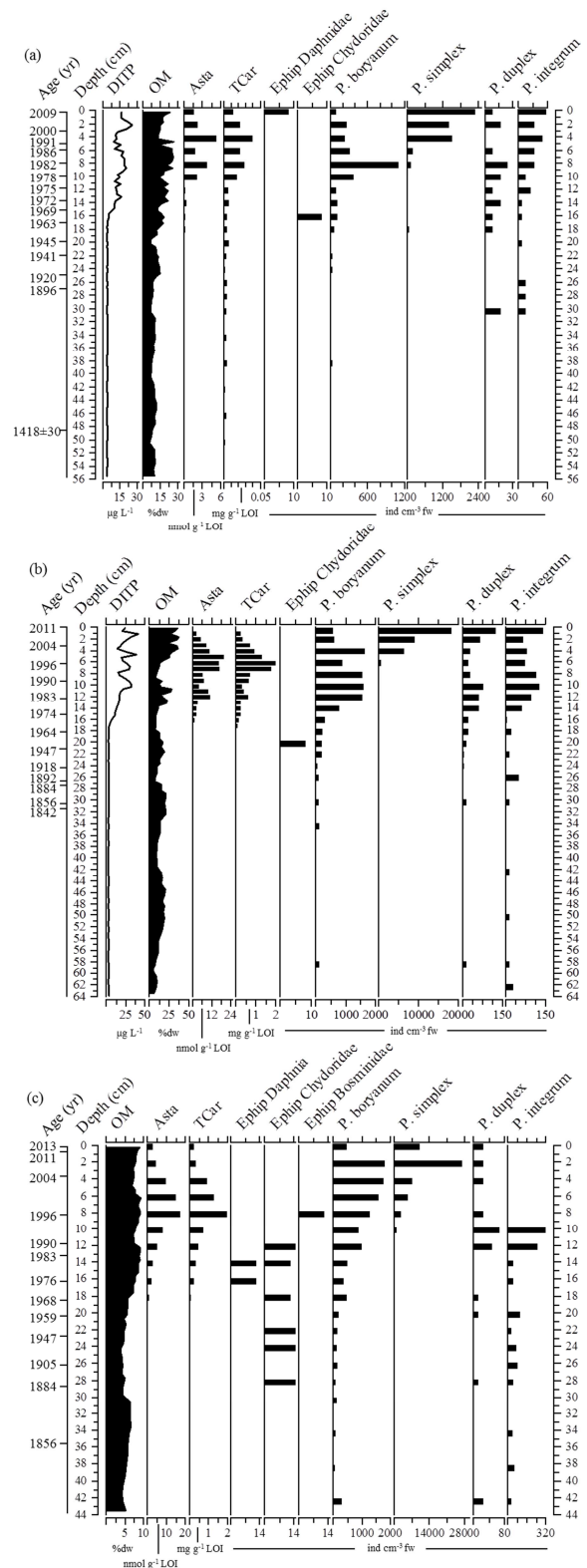


Fig. 1 Depth profiles of geochemical and biological proxies in the (a) Brenzone, (b) Bardolino and (c) littoral cores. DITP: Diatoms inferred total phosphorus; OM: organic matter; Asta: astaxanthin; TCar: Total carotenoids concentrations.

(Bren1-09, Fig. 1a) was characterized by low levels of organic matter before the 1970s (<15% of the dry weight), although its organic content ranged from 20 to 30% in the

topmost 15 cm (Fig. 1a). Visual inspection revealed a grey mineral zone extending from the bottom to ~26 cm and an organic zone from ~22 cm to the most recent layers, which were subdivided by a transitional mixed layer of ~4 cm. The entire lengths of the Bar1-11 and LitG1-13 cores consisted of a homogeneous grey sediment, with no darker gyttja layer on the top. Nevertheless, the organic content of Bar1-11 varied cyclically from 10% to 30% and peaked in the early 1990s (Fig. 1b). The average organic matter content of LitG1-13 was steady at ~5% of the dry weight in the lower parts of the core bottom core section and increased up to ~8% since the mid-1960s (Fig. 1c).

The DITP concentrations for both profundal cores were very low prior to the 1960s (3-5 $\mu\text{g L}^{-1}$) but then rose to 26.0 $\mu\text{g L}^{-1}$ in the early 2000s in Bren1-09 (Fig. 1a) and ~40 $\mu\text{g L}^{-1}$ in the late 2000s in Bar1-11 (Fig. 1b). The total carotenoid concentrations (TCar) in Bren1-09 remained very low until the mid-1940s, when they started to increase steadily until the early 1990s (Milan et al. 2015). Bar1-11 and LitG1-13 exhibited higher TCar concentrations than Bren1-09, with increasing values from the 1990s to the early 2000s. The three cores had very similar astaxanthin depth-profiles, which suggested that the zooplankton density had increased in all three cores since the late 1960s and peaked some time after the mid-1990s (Fig. 1). The core collected in the shallower Bardolino basin had higher astaxanthin concentrations than the cores from the Brenzone basin (Fig. 1b-c).

Subfossil Cladocera

The subfossil Cladocera assemblages for the Brenzone, Bardolino, and littoral cores featured 32 taxa (6 planktonic and 26 littoral;

see Fig. 2a), 35 taxa (7 planktonic and 28 littoral; see Fig. 2b), and 37 taxa (6 planktonic and 31 littoral; see Fig. 2c), respectively. The identified taxa belonged to the families Leptodoridae, Daphniidae, Bosminidae, Chydoridae and Cercopagidae. Despite the high number of recorded taxa, the specimen densities (Tot Cladocera) were generally low (Fig. 3). Nevertheless, the sediment records were in good agreement with modern pelagic Cladocera assemblages in terms of both species composition and relative abundance (Table S1). Two significant Cladocera zones (BR-C1 and BR-C2) could be identified in the Brenzone core (Fig. 2a), and BR-C1 was subdivided into two subzones (BR-C1a and BR-C1b) based on the total Cladocera concentration. 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 (Korhola 1990), *Acroperus harpae* (Baird) and *Alona affinis* Leydig, were also present, as were *Alona quadrangularis* (O. F. Müller), *Leydigia leydigi* (Schödler) and *Chydorus piger* (Sars), which are associated with the lake water-sediment interface (Korhola 1990). BR-C1b (52-19 cm depth) exhibited a 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 (Korhola 1990). Only a few individuals of the pelagic *Bosmina (E) coregoni* Baird and *Bosmina (E) longispina* Leydig, and the littoral species *A. harpae* were identified in the sample at 34 cm depth (Fig. 2a). The zone BR-C2 (19-0 cm depth) exhibited a drastic increase in *Bosmina* spp., *Byth. longimanus*,

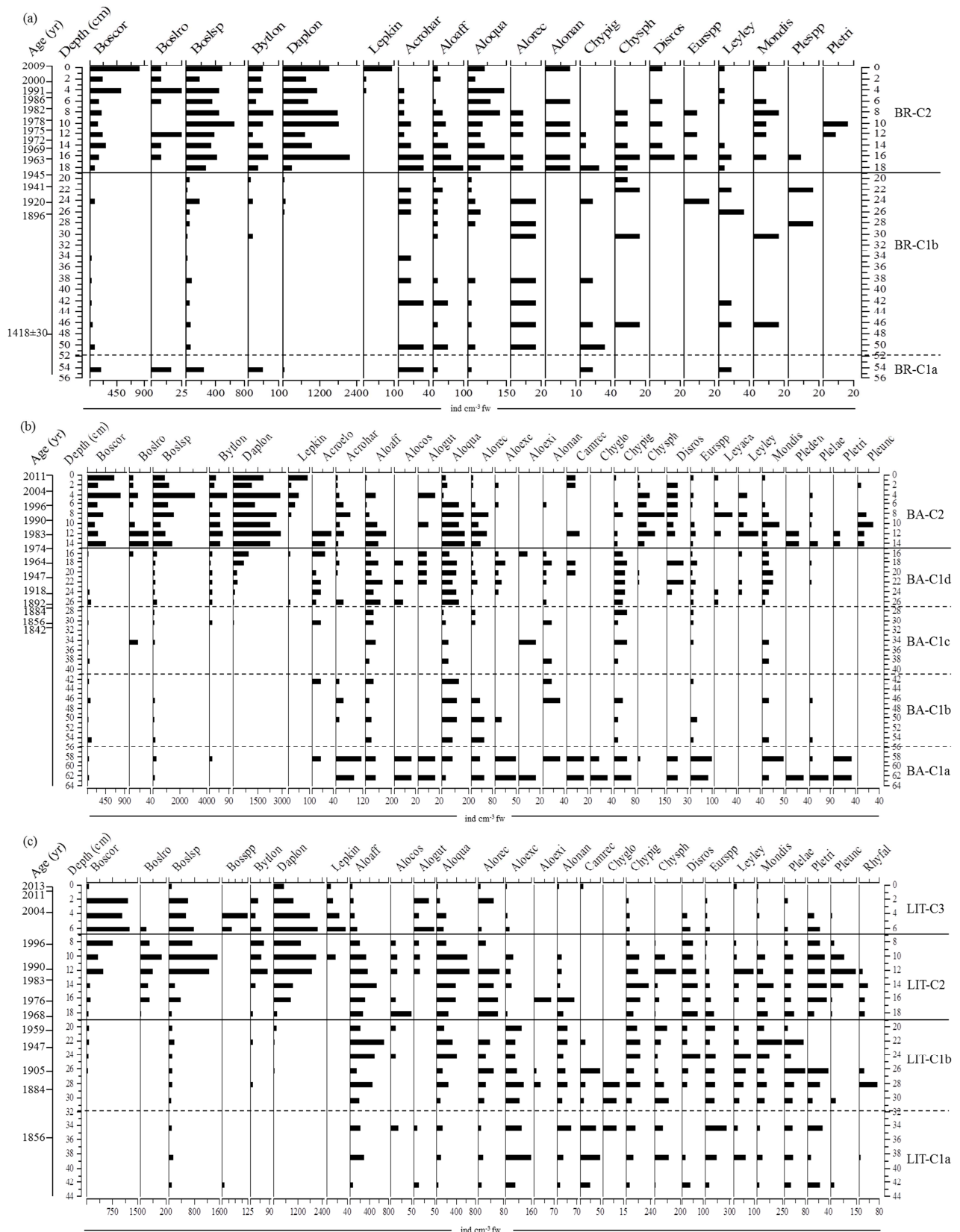


Fig. 2 Depth profiles of key Cladocera taxa in the (a) Brenzone, (b) Bardolino and (c) littoral cores. Species codes and full names of taxa are presented in Supplementary material Table S1.

and in planktonic *Daphnia longispina* (O. F. Müller) group since the early 1960s. The zooplanktivorous *Leptodora kindtii* (Focke)

appeared in the topmost layers. Among the littoral group, only *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 analysis of the Bardolino core revealed two significant zones: BA-C1 to BA-C2 (Fig. 2b), with zone BA-C1 being further divided into four subzones. 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 warmer water temperature, were also present. *Chydorus globosus* (Baird) was found exclusively within this zone, while Bosminidae were quite scarce (Fig. 2b). Many species decreased and disappeared in BA-C2b (56-41 cm depth), while *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.*

rectangularis, and *Alonella exigua* Lilljeborg and *C. piger* were recorded in this zone. The planktonic *Bosmina longirostris* (O. F. Müller) was recorded for the first time in this zone. 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* was very abundant at the beginning of the 1970s, while the *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*

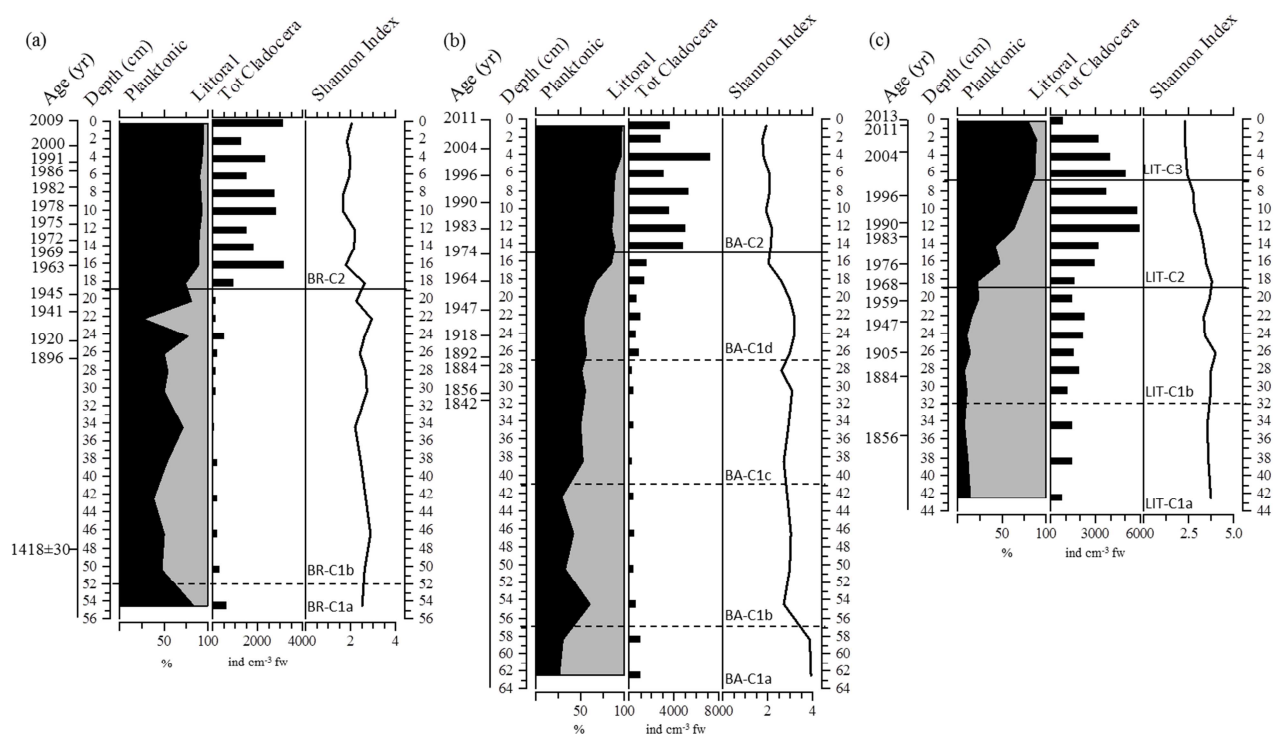


Fig. 3 Depth profiles of Cladocera results in the (a) Brenzone, (b) Bardolino and (c) littoral cores. Tot Cladocera: sum of all Cladocera specimens counted in each sample.

(Fischer), which are indicators of turbid water (Korhola 1990), 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. 2c). The deepest zone, LIT-C1a (44-32 cm depth), was dominated by a small number of littoral species, with *A. excisa* and *Eurycercus* spp. being most abundant. The planktonic *B. (E) longispina* was also present, but scarcely abundant. The zone LIT-C1b (32-19 cm depth) exhibited increased levels 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*. The total abundance of Cladocera, the planktonic to littoral ratio, and Shannon indices for the three studied cores are presented in Fig. 3. Zone BR-C1 of the Brenzone core (Fig. 3a) had a similar proportion of planktonic and littoral species, and a very low total Cladocera abundance that reached its absolute minimum at a 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 a lower Shannon Index. The Bardolino core (Fig. 3b) showed a similar

increase in the total Cladocera abundance since the 1960s (zone BA-C2), which was accompanied by reduced species diversity, but secular changes in Cladocera diversity and the proportion of planktonic taxa were more gradual. The littoral core (Fig. 3c) was characterized by a drastic shift from littoral species being dominant 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 that has persisted into the present. Only few ephippia of *Daphnia* and *Chydoridae* were identified in the two profundal cores but they were more abundant in the littoral core, which also contained some ephippia of the Bosminidae 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 the 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 have been increasing since the 1960s in Bren1-09 whereas in Bar1-11 such changes have only been observed since 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 samples deposited before and after core-specific threshold periods (Fig. 4), i.e. the early-1950s (sample 33) for Bren1-09 (Fig.

4a), the early-1960s (sample 37) for Bar1-11 (Fig. 4b), and the mid-1970s (samples 29-33) for LitG1-13 (Fig. 4c). This pattern was confirmed by plotting the sample scores on the first NMDS dimension against the

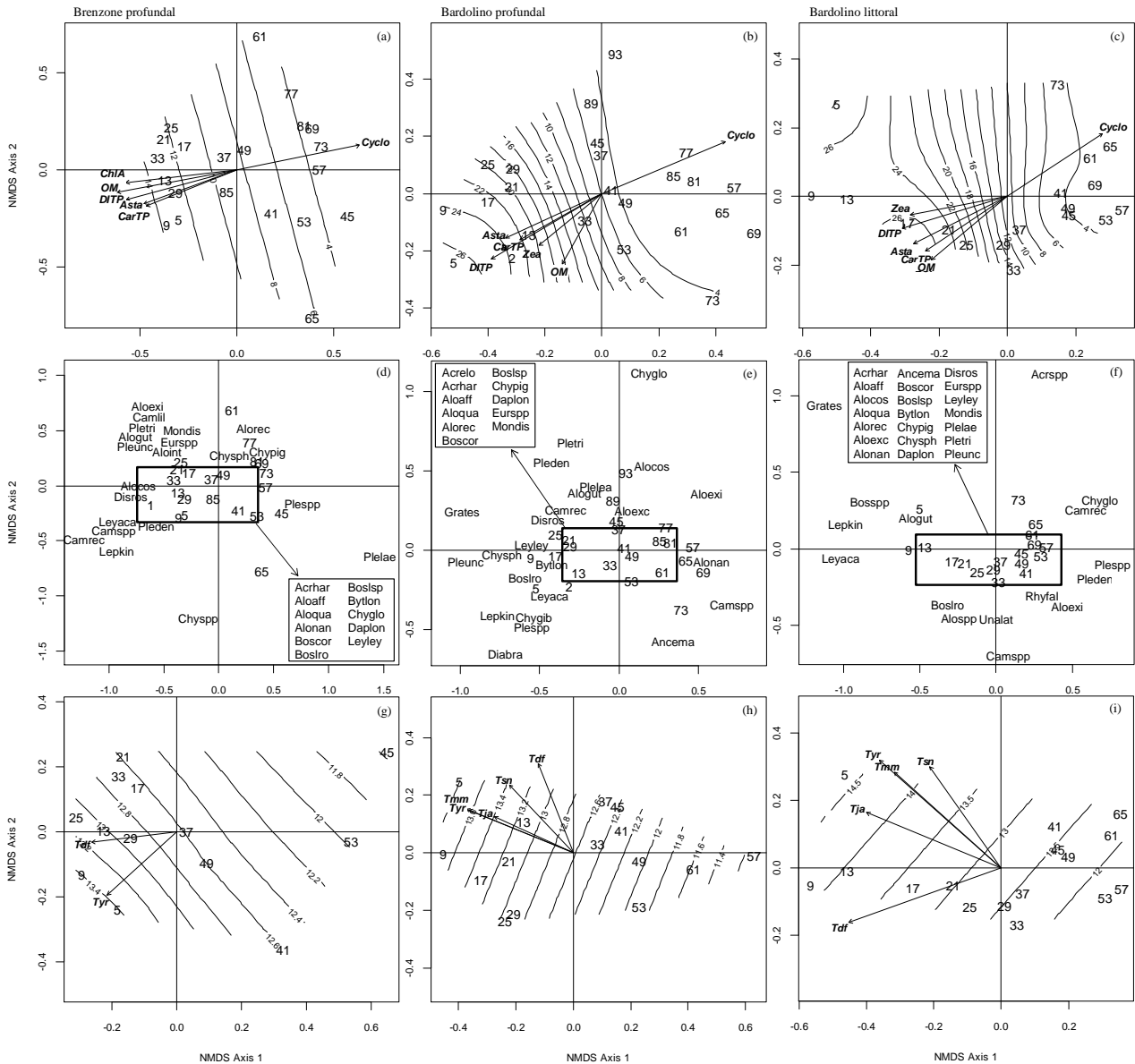


Fig. 4 Cladocera NMDS ordination relative to cores from Brenzone and Bardolino basins. Numbers in the plot refer to core samples. (a-b-c) Vector and surface fitting for trophic variables. The vectors' orientations show the direction of most rapid change for each environmental variable, while their lengths 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-e-f) Species distributions on the samples in the ordination space. Species codes and full names of taxa are presented in Supplementary material Table S1. (g-h-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_{yr} - mean annual air temperature; T_{df} - winter air temperature (December-January-February); T_{mm} - spring air temperature (March-April-May); T_{ja} - summer air temperature (June-July-August); T_{sn} - autumn air temperature (September-October-November).

chronology of the sediment cores collected from the deepest points of the two lake basins (Fig. 5). 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 pigment (CarTP) and diatom (DITP) levels, 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. 4a-c). The surface fittings indicate that the Cladocera levels are strongly and linearly related to DITP and other independent trophic-related variables. The species distributions (Fig. 4d-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. 4d). The recent samples of Bar1-11, which were dominated by planktonic species, were grouped in the lower left quadrant. (Fig. 4e). The majority of the species recorded in LitG1-13 (Fig. 4f) are concentrated in the central part of the plot, which indicates a weaker relationship between species and samples of different ages. Vector and surface fitting of climate variables, which was limited to ²¹⁰Pb-dated core sections (Fig. 4g-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 samples from both Bardolino cores were correlated with all four seasonal mean air temperatures.

The Cladocera sample scores on the first NMDS dimensions showed pronounced fluctuations in the sections deposited before 1960s of both the profundal cores. The most pronounced and rapid increase started in the Brenzone score in the early 1940s and was followed by a period of scarce variability till recent years (Fig. 5a). Changes in the Bardolino basin were slower, especially in the littoral core, but continued into the post-1960s period (Fig. 5b). In contrast to Cladocera, diatom sample scores in both the profundal cores exhibited very little variability prior to the 1960s, and very pronounced and rapid change since then (Fig. 5c-d).

Discussion

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 (Frey 1986), as previously reported in several studies on different lake types (Alric and Perga 2011; Nykänen et al.

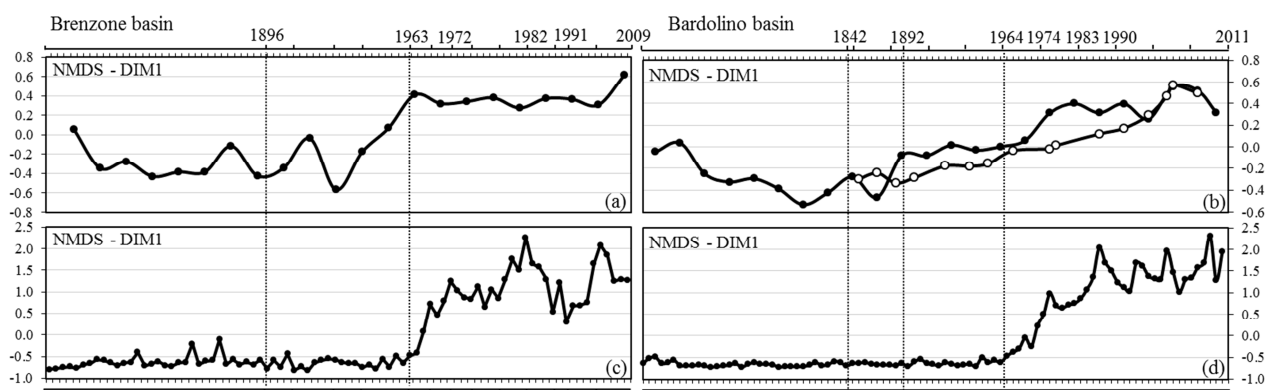


Fig. 5 Cladocera (a-b) and diatoms (c-d) sample scores on the first NMDS dimension for the Brenzone and Bardolino cores. Filled circles represent profundal cores; empty circles represent the littoral core.

2009). In addition, sediment records enabled an assessment of the long term development of littoral assemblage compositions. 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 (Manca et al. 2007; Salmaso and Naselli-Flores 1999). The subfossil Cladocera from our 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 homogeneity of environmental trends and ecological dynamics within Lake Garda (Salmaso 2002).

Despite the similar general patterns of secular Cladocera variability, there were subtle differences in the species composition and abundance data for 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 geochemical and algal proxies examined in a previous work (Milan et al. 2015) reinforce this hypothesis. The core from the Brenzone basin showed a higher deposition 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 both the submerged ridge separating the two main lake sub-basins and to the scarcity of inlets discharging into this lake basin (Gerletti 1974). The sediment organic content profiles of 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 lower organic content and subfossil pigment concentrations recorded in the Brenzone core do not reflect differences in the two basins' productivities, which are very similar (Salmaso 2002). Instead, the differences in these two variables are probably related to the more rapid degradation of settling organic material in this very deep 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 first distinct Cladocera zone was identified in the deep sections of the profundal cores collected from the two lake basins, i.e. the sections between the bottom and a depth of ~50 cm, 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 had a high abundance of littoral species, especially clear water- and macrophyte-associated species, which might indicate a macrophyte-dominated stage. Species reflecting warm water temperatures has been also detected in this deep section of the profundal cores, including *Byth. longimanus* in Brenzone and *C. rectirostris* and *Pleuroxus* spp. in Bardolino. The combination of these observations suggests that the deepest layers were deposited during the Medieval Climatic Anomaly (MCA) (De Jong et al. 2013). No such deep section characterized by species indicative of high nutrient levels and warmer conditions has been detected in the shorter littoral core.

The central sections (BR-C1b, BA-C2 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 during this period. 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 culminated in the Alpine region between 1750 and 1850 (De Jong et al. 2013). Comparable effects of the cold stage between the 18th and 19th century have also been identified in Lake Maggiore (Manca and Comoli 1995), 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 were characterized by increasing Cladocera abundance in all three cores. Species associated with warmer water such as *Pleuroxus* spp. (Szeroczyńska pers. commun.) became more abundant, especially in the Brenzone basin and the littoral zone of Bardolino just after the end of the LIA. The shift towards species indicating warmer conditions was slower in the pelagic zone of the Bardolino basin, where *C. rectirostris* and *Pleuroxus* spp. were only observed in layers deposited during and after the late 1940s, suggesting a much slower recovery. Within a few years of the lake's temperature starting to recover, the abundance and diversity of littoral species also started increasing gradually. However, in all three cores, there was a much more rapid increase in the abundance of planktonic species 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 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 coexistence in Lake Maggiore a few years earlier than in Lake Garda.

Increasing abundances of *D. longispina* group and *Byth. longimanus* are also considered indicative 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. comm.) 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). Regardless, as is commonly observed in lakes with increased nutrient level, after a first general increase in fish biomass since the mid-1950s, there was a process of selection for species better fitted to the new conditions (primarily, cyprinids, perches and pikes) that

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 slight 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 (Alric et al. 2013; Bigler et al. 2006; Manca et al. 2007). 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 concentrations 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 present 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 several deep subalpine lakes, including Lake Bourget, Annecy, Geneva, Constance, Maggiore and Vrana (Alric et al. 2013; Manca et al. 2007; Schmidt et al. 2000). However, most of the available information on the ecological preferences of Cladocera with respect to nutrients originate from studies conducted on small alpine lakes (Bigler et al. 2006; Kamenik et al. 2007; Lotter et al. 1997; Nevalainen et al. 2011) or eastern European lakes (Korponai et al. 2011; Szeroczyńska 2006; Zawisza and Szeroczyńska 2007). In addition to being less numerous, previous studies on Cladocera ecology in large and deep subalpine lakes mainly focused on individual species or responses to ecological stressors other than TP and climate (see citations above), 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 this work provide information on the evolution of Cladocera assemblages since the Middle Ages, when the lake was not affected by anthropogenic eutrophication. However, an earlier analysis of three parallel sediment records spanning several centuries was not sufficient to enable unequivocal identification of a single principal driving factor in the evolution of the Cladocera species distribution because it did not provide evidence for possible changes in the roles of nutrients and climate over time, or for additive and/or synergic effects. Disentangling these two influences is complicated by the fact that organisms generally react to combinations of simultaneous changes in their

multidimensional ecological niches (Salmaso 2002), and also to the timing of climate warming in the Alpine region, which coincided closely with the onset of nutrient enrichment (Salmaso unpublished data).

The application of multivariate statistics helped us to overcome some of these limitations. NMDS analyses with vector and surface fittings on the profundal and littoral cores from Lake Garda revealed a highly coherent response of subfossil Cladocera to nutrient- and productivity-related factors such as CarTP and DITP, pigment concentrations, and the organic content of the sediment. Vector fitting based on climate variables indicated a dominant 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 also responded strongly to spring and summer temperatures. This largely confirms the results of recent investigations, which suggested a crucial influence of winter temperature on the depth of lake mixing during winter and the associated 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 suggested a general response 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 (Davidson et al. 2007; Korhola et al. 2000; 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. The Cladocera sample scores fluctuated strongly during the MCA and the LIA in both the profundal cores. After the culmination of the LIA, the Cladocera sample scores increased gradually in both lake basins, in a way that anticipated the major diatom changes that occurred during the 1960s in both lake basins as a result of nutrient enrichment caused by urban sewage emissions and intensive agriculture within the lake catchment (Milan et al. 2015). The lake shifted from oligotrophic to mesotrophic conditions during this period, and the diatom shift was accompanied by comparable shifts in pigment concentrations and *Pediastrum* species composition. As suggested by Milan et al. (2015), this confirms that diatoms respond directly to increasing nutrient inputs, but indirectly to climate variability. Changes in water temperature appear to indirectly modulate the productivity of deep temperate lakes by changing 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 helped to enhance the fertilization of Lake Garda that had been triggered by the increased nutrient load from the lake catchment. The species composition of

subfossil Cladocera began to change at the turn of the 19th century, when the anthropogenic impact on Lake Garda was still minimal and comparable to that of earlier centuries. The change was gradual and slow compared with the very rapid nutrient-driven diatom change, but the variability of subfossil Cladocera and diatoms became rather similar during the nutrient enrichment period. These observations suggest that climate variability represents the primary driver for the Cladocera assemblages, especially under low nutrient conditions. However, climate effects can be overridden under conditions of nutrient enrichment, when Cladocera appear to respond strongly to nutrient-driven bottom-up factors. The NMDS analysis confirmed the applicability of the subfossil Cladocera as a proxy for reconstructing past climate variability, and their reliability in reflecting the community response to temperature factors alone or in combination with nutrient changes in a large and deep lake.

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 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, to the profundal and littoral zones of large and deep lakes. The usefulness of Cladocera in tracking historical climate changes was clearly demonstrated by the data gathered for the lake's oligotrophic period (i.e. the period 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 analysis in Lake Garda complements previous results based on subfossil diatoms and pigments, and helped to disentangle lake responses to climate variability and nutrient enrichment. This highlights 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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Supplementary Material

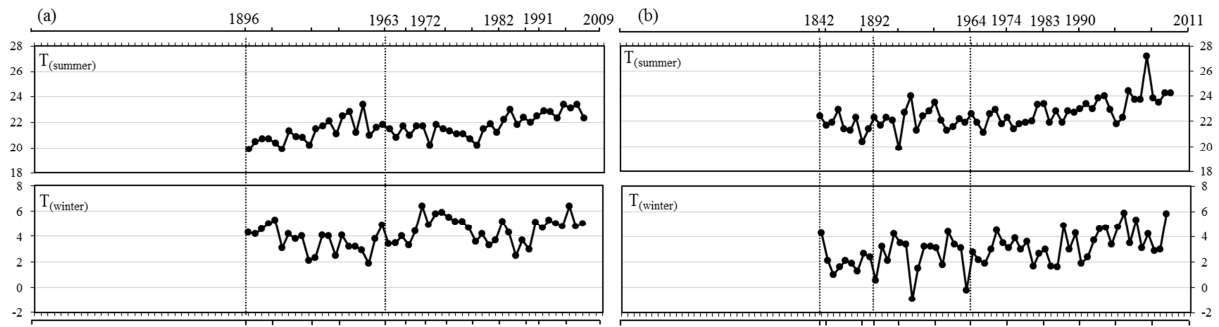


Fig. S1 Summer and winter mean air temperature (°C) profiles recorded at (a) Torbole-Riva del Garda since 1896 AD and (b) Villafranca since 1842 AD. (June-July-August) (December-January-February)

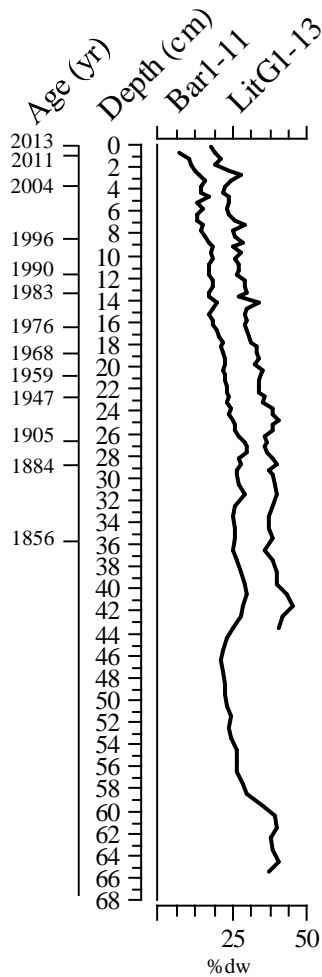


Fig. S2 Parallelization of the littoral core (LitG1-13) with the dry weight (DW) percentage of the Bardolino core (Bar1-11).

Table S1 Taxa identified in the three sediment cores (Bren1-09, Bar1-11, LitG1-13) and the water column (Salmaso and Naselli-Flores, 1999). The code is the species code used in the stratigraphic plots and NMDS analyses. The presence and absence of individual taxa are indicated by + and – symbols, respectively.

Code	Species	Author	Bren1-09	Bar1-11	LitG1-13	Lake
Acrelo	<i>Acroperus elongatus</i>	(Sars)	-	+	+	-
Acrhar	<i>Acroperus harpae</i>	(Baird)	+	+	+	-
Acrspp	<i>Acroperus spp.</i>	Baird	-	-	+	-
Aloaff	<i>Alona affinis</i>	Leydig	+	+	+	-
Alocos	<i>Alona costata</i>	Sars	+	+	+	-
Alogut	<i>Alona guttata</i>	Sars	+	+	+	-
Aloint	<i>Alona intermedia</i>	Sars	+	-	-	-
Aloqua	<i>Alona quadrangularis</i>	(O. F. Müller)	+	+	+	-
Alorec	<i>Alona rectangula</i>	Sars	+	+	+	-
Alospp	<i>Alona spp.</i>	Baird	-	-	+	-
Aloexc	<i>Alonella excisa</i>	Fischer	+	+	+	-
Aloexi	<i>Alonella exigua</i>	Lilljeborg	-	+	+	-
Alonan	<i>Alonella nana</i>	Baird	+	+	+	-
Ancema	<i>Anchistropus emarginatus</i>	Sars	-	+	+	-
Boscor	<i>Bosmina (E) coregoni</i> group	Baird	+	+	+	-
Boslro	<i>Bosmina longirostris</i> group	(O. F. Müller)	+	+	+	-
Boslspp	<i>Bosmina (E) longispina</i> group	Leydig	+	+	+	-
Bosspp	<i>Bosmina spp.</i>	Seligo	+	-	+	+
Bytton	<i>Bythotrephes longimanus</i>	Leydig	+	+	+	+
Camlil	<i>Camptocercus lilljeborgi</i>	Schödler	+	-	-	-
Camrec	<i>Camptocercus rectirostris</i>	Schödler	+	+	+	-
Camspp	<i>Camptocercus spp.</i>	Baird	+	+	+	-
Chygib	<i>Chydorus gibbus</i>	Sars	-	+	-	-
Chyglo	<i>Chydorus globosus</i>	(Baird)	+	+	+	-
Chypig	<i>Chydorus piger</i>	(Sars)	+	+	+	-
Chyspp	<i>Chydorus spp.</i>	Leach	+	-	-	+
Chysph	<i>Chydorus sphaericus</i>	(O. F. Müller)	+	+	+	-
Daplon	<i>Daphnia longispina</i> group	O. F. Müller	+	+	+	+
Diabra	<i>Diaphanosoma brachyurum</i>	Liévin	-	+	-	+
Disros	<i>Disparalona rostrata</i>	Koch	+	+	+	-
Eurspp	<i>Eurycercus spp.</i>	Baird	+	+	+	-
Grates	<i>Graptoleberis testudinaria</i>	(Fischer)	-	+	+	-
Lepkin	<i>Leptodora kindti</i>	(Focke)	+	+	+	+
Leyaca	<i>Leydigia acanthocercoides</i>	(Fischer)	+	+	+	-
Leyley	<i>Leydigia leydigi</i>	(Schödler)	+	+	+	-
Mondis	<i>Monospilus dispar</i>	Sars	+	+	+	-
Pleden	<i>Pleuroxus denticulatus</i>	Birge	+	+	+	-
Plelae	<i>Pleuroxus laevis</i>	Sars	+	+	+	-
Plespp	<i>Pleuroxus spp.</i>	Baird	+	+	+	-
Pletri	<i>Pleuroxus trigonellus</i>	(O. F. Müller)	+	+	+	-
Pleunc	<i>Pleuroxus uncinatus</i>	Baird	+	+	+	-
Polped	<i>Polyphemus pediculus</i>	(Linné)	-	-	-	+
Rhyfal	<i>Rhynchotalona falcata</i>	(Sars)	-	-	+	-
Sidcry	<i>Sida crystallina</i>	(O. F. Müller)	-	-	-	+
Unalat	<i>Unapertura latens</i>	Sarmaja-Korjonen	-	-	+	-