Changes in midge assemblages (Diptera Chironomidae) in an alpine lake from the Italian Western Alps: the role and importance of fish introduction

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Abstract Introduction of exotic species is one of the major human impacts for decline in autochthonous biodiversity. In freshwater lentic environments, alien fish introduction heavily shapes macrobenthic invertebrate structure, with special regard for Chironomidae (Diptera) community. These effects could be more evident in alpine lakes whose environments are considered as remote and undisturbed, but extremely susceptible and vulnerable to both natural and anthropogenic impacts. In this context, modern and subfossil chironomid assemblages were studied in a high-altitude lake (Balma Lake, Piedmont, Italy) which was not previously investigated and subject to brook trout introduction for recreational fishing at the end of the twentieth century (after 1970). Seasonal samplings (summer, autumn) were performed in five littoral and three deep sites, while a core sample was extracted from the deepest point of the lake. The analysis highlighted significant differences in subfossil chironomid communities before and after fish introduction and between subfossil and modern communities, with notable decrease in recent diversities. Dissimilarities were mainly related to *Corynocera oliveri*, *Zavrelimyia*, *Micropsectra*, *Metriocnemus,* and *Heterotrissocladius marcidus* type. Therefore, this study highlights the importance and effects of anthropic environmental events, such as fish introduction, in climate reconstructions and their interpretation, especially regarding the last 100/200-year period, when human environmental issues have become more significant.

Keywords Chironomidae assemblages · Paleolimnology · Environmental impacts · Alien fish

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Introduction

According to Pechlaner (1971), alpine lakes, or "high-mountain lakes", are considered among the most remote and undisturbed aquatic environments (Čiamporova-Zat'ovičová et al., 2010), showing peculiar features and harsh conditions, leading to low species diversity and relatively simple community structures with only few dominant but well-adapted species (Rott, 1988; Starkweather, 1990; Bretschko, 1995;

Füreder et al., 2006). Despite their remote position, their small size, and the high turnover of surface waters, alpine catchments are extremely receptive and vulnerable to natural and anthropogenic impacts (Fjellheim et al., 2009), both on a local (water abstraction, tourism, introduction of exotic species) and global scale (long range transport of toxic pollutants and radioactive nuclides, acid rain, global climatic changes) (Psenner & Schmidt, 1992; Psenner & Catalan, 1994; Camarero et al., 1995a, b; McGregor et al., 1995; Battarbee et al., 2002; Maiolini et al., 2006; Magnea et al., 2013). Due to these features, alpine lakes are considered as indicators of global environmental change and "early warning systems" for the whole mountain environment (MOLAR Water Chemistry Group, 1999; Čiamporova-Zat'ovičová et al., 2010). Moreover, they represent "natural laboratories" for ecological assessment, in order to investigate global changes in water quality and biodiversity (Rogora et al., 2008; Rossetti et al., 2008) and to assess the large-scale effects of anthropogenic activities (Battarbee et al., 2009; Williams et al., 2019).

Among anthropic pressures, the introduction of alien species is one of the major reasons for the worldwide decline in biodiversity (e.g., Vitousek et al., 1996; Mooney & Cleland, 2001; Raposeiro et al., 2017), being considered one of the main threats to global autochthonous biodiversity in susceptible and vulnerable environments such as alpine lakes (Knapp et al., 2001; Schabetsberger et al., 2009; Tiberti et al., 2014, 2016). Although these environments were generally fishless due to the natural migration barriers (Pechlaner, 1966, 1984; Fritts & Rodda, 1998; Sax et al., 2002; Gurevitch & Padilla, 2004), they have been frequently stocked with salmonids for recreational purposes (Tiberti et al., 2014; Sánchez-Hernández et al., 2015), causing consequences on food web structures and ecosystem functions. Predatory fish introduction implies dramatic ecological consequences and cascading effects down to lower trophic levels (Carpenter et al., 1985; Pace et al., 1999; Findlay et al., 2005; Bystrom et al., 2007; Milardi et al., 2016; Raposeiro et al., 2017), modifying the physical structure of the ecosystem (Zambrano et al., 2001; Dibble & Kovalenko, 2009; Weber & Brown, 2009), both in zooplankton and macrobenthic invertebrate communities (McNaught et al., 1999; Tiberti et al., 2014; Sánchez-Hernández et al., 2015). Fish presence plays a central role in shaping the structure of most aquatic insect communities in lakes, sometimes greater than the role of lake origin or physiography (Bendell & McNicol, 1987; Binckley & Resetarits, 2005; Schilling et al., 2009; Raposeiro et al., 2017).

Chironomids communities are a pivotal element in aquatic food webs, constituting 50% of secondary production in lakes, and being an important prey item for fish (Armitage, 1995). Midge assemblages are potentially affected by different features and ecological processes, although temperature is considered as the main factor (Füreder et al., 2006; Brooks et al., 2007; Moller Pillot, 2009a). Due to their larval chitinous head capsules, subfossil chironomids are frequently abundant and well-preserved in-lake sediments, becoming an excellent material for paleolimnological investigations (Walker, 1995; Heiri & Lotter, 2003; Lencioni & Lazzara, 2004; Skov et al., 2010; Cao et al., 2014; Raposeiro et al., 2017). Both climate (temperature) and in-lake processes (trophic state) interact in shaping past chironomid communities (Brodersen & Anderson, 2002; Millet et al., 2014; Williams et al., 2016); influence of temperature on chironomid assemblages may be exceeded by other environmental factors, such as anthropogenic changes in the catchment (Millet et al., 2014; Williams et al., 2016). Among them, salmonid introduction is one of the main drivers for possible changes in midge assemblages, enhancing those generated by higher temperatures due to global warming (Williams et al., 2016). High relative abundance, high amounts of protein, high digestibility, and the fact that chironomid larvae act as growth promoters in fish diets make them an important dietary component of freshwater fish fauna (Armitage, 1995; Porinchu & MacDonald, 2003). Fish introduction could also have an indirect effect through nutrient release via excretion, affecting type and ratio of nutrients within the systems and leading to changes in primary production rates and to a shift in the predator/grazer ratios (Raposeiro et al., 2017).

In this context, it was deemed of interest to assess the temporal changes within the chironomid assemblages in a high-altitude lake located in the Italian Alps (Balma Lake, Piedmont, Italy) which was not previously investigated and subject to brook trout introduction for recreational fishing at the end of the twentieth century. The aims of the present study were

(i) to characterize the modern chironomid community in Balma Lake, both the littoral and the bottom/profundal habitats, (ii) to characterize the chironomid paleo-community through paleolimnological analyses, and finally (iii) to asses changes in chironomid community in relation to the fish introduction. We hypnotized these changes to be related to environmental impacts (climate change and introduction of alien fish), and we focused on brook trout stocking as a complementary factor, interacting with other features in shaping chironomid communities. This study represents the first attempt to investigate the consequences of fish introduction on macrobenthic invertebrate communities of alpine lakes through paleolimnological analyses for the European Alps area.

Methods

Study area

Balma Lake is a glacial origin lake located in the Cottian Alps (Municipality of Coazze, Piedmont, Northwest Italy) at 2216 m above sea level (a.s.l.) (Fig. 1a, b). It is included in the Special Area of Conservation (SAC) and Special Protection Area (SPA) IT1110006 “Orsiera Rocciavrè” and in the Orsiera Rocciavrè Natural Park. The main core of its catchment is composed of ophiolite metamorphic bedrock and the landscape is dominated by rocky outcrops, ridges, and severe mountain walls. The lake is located within the alpine belt, above the tree line; ice cover generally lasts from late October to late May/early June. Due to these characteristics, Balma Lake represents a typical example of an alpine lentic environment (sensu Füreder et al., 2006, Fjellheim et al., 2009). No previous studies or data about the hydrochemistry or macrobenthic invertebrate community composition of this lake are still available.

A morphometric and bathymetric characterization of the lake was carried out during early summer 2017 through aerial photography and bathymetric surveys, using flying and floating drones (Fig. 1c). Surveys were performed with the support of GeoStudio RC (Giaveno, Italy), which provided the tools and performed the data processing. Balma Lake is circle shaped, with a perimeter equal to 414 m, while the surface area is 1.21 ha and reaches a maximum depth of 6.42 m. A small inlet is located at the western shore, dividing into three small branches before entering the lake. Although a true outlet is not evident, at the eastern side, the Rio Balma originates by water filtration through the sediments delimiting the basin (Fig. 1b, c). The main source of human impact for this area from the last four decades of twentieth century is mainly represented by long distance transport of pollutants from the plain, grazing activities, and fishing. Balma Lake was formerly fishless, but brook trout *Salvelinus fontinalis* Mitchill, 1814 was introduced for recreational fishing during the last decades of the twentieth century since the seventies (Forneris & Palmegiano, 1986; Delmastro, 1987; Balma et al., 1992; CREST, 2000; Rizzetti et al., 2001). Information regarding brook trout present and/or past densities or population structure for Balma Lake is still unavailable.

Modern chironomid communities

Two sampling campaigns were carried out in 2017 during the ice-free period, in summer (early August) and at the beginning of the fall season (October). In order to check modern chironomid assemblages in Balma Lake, five sites and three points were chosen in the littoral and deep zones, respectively (Fig. 1c, Table 1).

Littoral sites were selected after recognizing the presence of different microhabitats and in agreement with the bathymetric surveys. Three subsamples were collected at each site with a Surber net (0.32 × 0.32 m, 250 µm mesh size), placing it from the shore to a water depth between 25 and 60 cm, considering the whole substrate composition (rocks, cobbles, sand, organic matter accumulation etc.). In order to standardize sampling effort, substrates were disturbed for at least 2 min (Füreder et al., 2006; Fjellheim et al., 2009). Deep sites were selected in agreement with bathymetric surveys and placed at different depths (4.3 m, 5.0 m and 6.4 m). Single samples were collected from each site using a Van Veen grab (2 l, 260 cm^2 sampling surface) and sieved through a net with a mesh size of 250 µm (Boggero et al., 2014). After collection, both littoral and deep samples were stored in situ with 70% ethanol solution and brought to the laboratory where they were examined using a stereomicroscope for sorting and chironomid larvae dissection. Permanent slides were

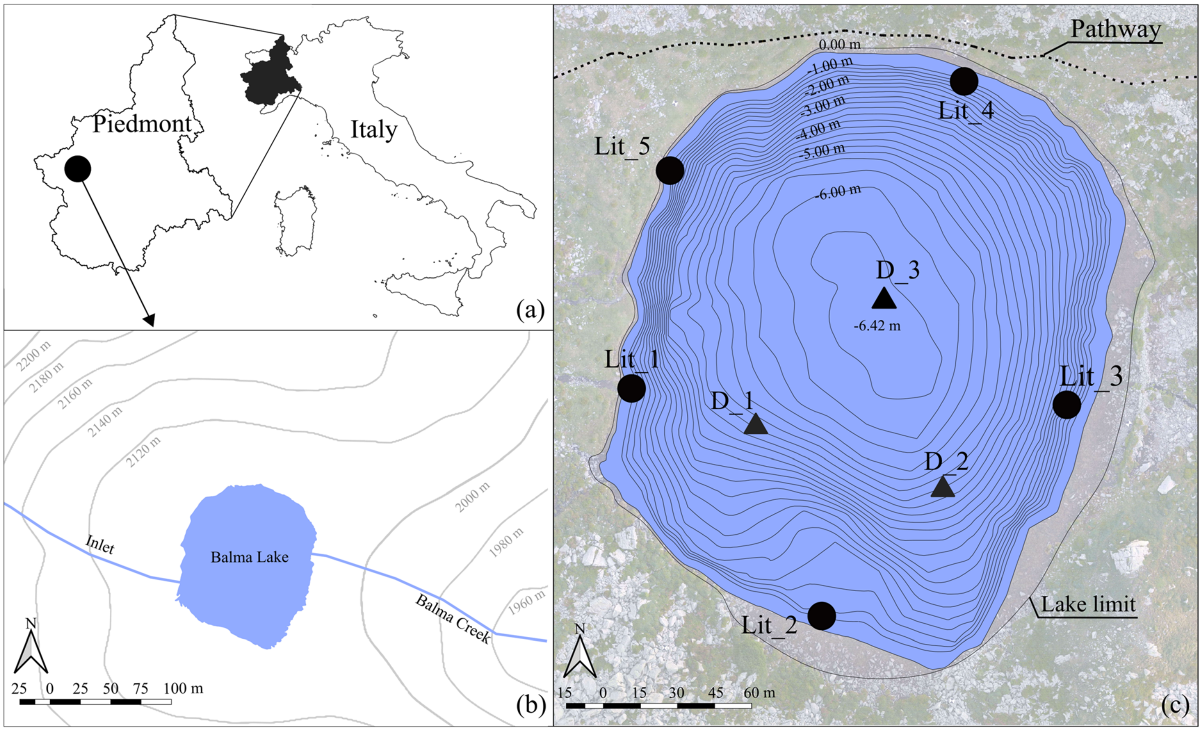

Fig. 1 Study area (**a**, **b**) and location of the sampling sites in Balma Lake (**c**)

Table 1 Geographical coordinates and short description of the Balma Lake sampling sites

Sampling site	Longitude	Latitude	Site description
Lit_1	07°10′49.22″	45°02′13.39″	Northwestern side near the inlet; bottom composed of gravel and cobbles; depth equal to 25 cm
Lit_2	07°10′51.26″	45°02′11.83′’	Southern side of the lake; bottom composed of submerged boulders; depth equal to 40 cm
Lit_3	07°10′53.78″	45°02′13.50″	Eastern side of the lake; bottom composed of boulders; depth equal to 40–60 cm
Lit_4	07°10′52.46″	45°02′15.76″	Northern side; bottom composed of cobbles; depth equal to 30 cm
Lit_5	07°10′49.63″	45°02′15.02″	Northwestern side; bottom composed of gravel and cobbles, with presence of organic material; depth equal to 30 cm
D_1	07°10′50.53″	45°02′13.24″	Southern portion of the lake, against the littoral site 1; bottom composed of sand and mud. Depth equal to 5.0 m
D_2	07°10′52.82″	45°02′12.82″	Southern portion of the lake, against the littoral site 3; bottom composed of sand and mud. Depth equal to 4.30 m
D_3	07°10′52.23″	45°02′14.28″	Center of the lake; bottom composed of sand and mud. Depth equal to 6.42 m

prepared with Faure, to allow taxonomic identification of specimens, which were identified at least to the generic level, following Oliver (1983), Pinder & Reiss (1983), Sæther (1983), Lencioni et al. (2007), Moller Pillot (2009a, b, c) and Andersen et al. (2013).

Core sampling and chironomid head capsule identification

A coring campaign was conducted in October 2017, using a 50 mm gravity Kajak-type sediment corer

(Kajak et al., 1965; Renberg, 1991; Brooks et al., 2007). One 30-cm-long core was extracted from the deepest sampling site (6.42 m) (site D_3 in Fig. 1c). The core was sealed in a sampling tube, brought to the laboratory, and stored at 4°C until further analyses. Subsamples were obtained by cutting the core in 14 transverse sections of 2 cm each. The choice of section thickness was dictated by the need to obtain a sample suitable for analysis with a minimum volume equal to 30 cm^3 of wet sediment, as indicated for mountain lakes due to their sedimentation rates (Heiri & Lotter, 2003; Heiri et al., 2003; Brooks et al., 2007).

Head capsule (HC) extraction was performed following the indications reported by Lang et al. (2003) and Brooks et al. (2007). Samples were deflocculated in distilled water warmed at 40°C for 20 min and then sieved using a 100 µm mesh. The sieve residues were then sorted and chironomid HC was extracted under the stereomicroscope (at least × 25 magnification). Samples were processed in order to obtain at least 100 HC for each section, to produce significant statistical results (Brooks et al., 2007). HC were then progressively dehydrated in 80% and 100% ethanol (5 min for each step) and mounted on microscope slides, ventral side up, in Euparal® essence.

The collected HC were identified to genus or species level (when possible) using an optical microscope at × 60 to × 100 magnification, following Oliver (1983), Pinder & Reiss (1983), Sæther (1983), Lencioni et al. (2007), Brooks et al. (2007), Moller Pillot (2009a, b, c) and Andersen et al. (2013). For each core section, three subsamples were considered in order to perform statistical analyses, with each subsample containing a higher number of HC than the minimum value ($n > 50$) for representative samples (Brooks et al., 2007).

Dating and core chemistry (organic matter and C/N ratio analysis)

In order to analyze total organic carbon (TOC, %) and total nitrogen (TN, %), 15 mg bulk samples were obtained for each section, manually powdered using an agatha mill, and oven-dried at 105°C for 24 h. Samples were acidified using HCl, increasing concentration up to 18%, and processed by frontal chromatography, using a CHN Analyzer (model ECS 4010 CHNSO, manufactured by Costech Analytical Technologies Inc., Valencia, California, U.S.A) (Hedges & Stern, 1984).

The total lead (Pb) concentration in the core sections was determined by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS). Following an adaptation from EPA Method 3052, freeze-dried samples were manually powdered using an agatha mill. Then, 0.300 g aliquots were solubilized with a mixture of 5 ml of nitric acid (69%) and 1 ml of hydrogen peroxide (30%) and subjected to microwave digestion for 55 min using a Multiwave PRO Anton Paar reaction system (Anton Paar, Graz, Austria). Then, samples were centrifuged to remove residue, transferred to flasks, and filled to the mark with MilliQ water (25 ml final volume). Samples were diluted to 1:20 and were analyzed by ICP-MS NEXION 350X (manufactured by Perkin Elmer, Waltham, Massachusetts, U.S.A.). Pb concentrations were determined using the calibration curve method obtained from the analysis of five standard solutions (range 0–100 ppb).

Beginning of Pb emissions could be dated back to 6000–8000 years ago due to human use (Needleman, 1999), being the first indication regarding the atmospheric deposition of Pb pollution dated between 2000 and 1500 BC (Renberg et al., 2000). However, atmospheric Pb pollution dramatically increased during the past century reaching a maximum peak in the 1970s with increased fossil fuel combustion and the extensive use of leaded petrol (Nizich et al., 2000; Renberg et al., 2000; Brännvall et al., 2001; Siver & Wonziak, 2001). Due to the deposition of atmospheric pollution, Pb concentration increased over time also in soils, where concentration trends follow the history of atmospheric ones (Renberg et al., 2000). During the peak in the early 1970s, the deposition rate was about 15 mg m^{-2} $year^{-1}$ (approximately 1000-fold the background rate) and it declined to about 1 m^{-2} -$year^{-1}$ (Rühling et al., 1996; Renberg et al., 2001) after implementation of stricter emission standards and the introduction of lead-free petrol (Brännvall et al., 2001; Renberg et al., 2001; Siver & Wonziak, 2001). Since the 1970s, Pb emissions decreased by 60-70% (Pacyna et al., 2007), showing a strong declining trend in Italy as well (ISPRA, 2009). In this context, indirect dating of the top core sections could be hypothesized through comparisons with other studies in European lakes. As the highest peak in total Pb concentrations within core sediments is reported during the 1970s (Brännvall et al., 2001; Renberg et al., 2001; Arnaud et al., 2003, 2004; Spadini et al., 2003; Camarero et al., 2009b; Nedjai et al., 2011), the detection of this peak in the top sections of the Balma Lake core could allow the identification of the core section corresponding to the 1970s period, when fish was introduced in the Piedmont area alpine lakes.

Due to the absence of vegetal and fossil remain, and other organic matter remains, two bulk sediment samples (chosen at 8–10 cm and 20–22 cm core depth) were used for AMS ^{14}C carbon analyses which were performed at the Poznan Radiocarbon Laboratory (Poznan, Poland). The calibration of the radiocarbon dates was based on the IntCal13 dataset (Reimer et al., 2013). Balma age-depth model was obtained using RStudio Package Clam 2.3.2 (Blaauw, 2010) through smooth interpolation function, using ^{14}C AMS dates and total Pb peak as non-carbon datum.

Results of age-depth modeling were used to define four age groups for further analyses, with particular attention to the period including fish introduction.

Physical and chemical parameters

At each sampling site (both littoral and deep) and during both sampling campaigns, the main chemical and physical features were monitored. Water temperature (°C), dissolved oxygen (% and mg l^{-1}), conductivity (µs cm^{-1}), and pH were recorded using field meters HI 9033 conductivity meter, HI 9125 pH/ORP meter, and HI 9147 oximeter; all instruments were manufactured by Hanna Instruments Inc. Woonsocket, RI, USA). In deep sites, depth and transparency were assessed using a graduated rope and the Secchi disk. Water samples were collected at each site in sterile containers paying attention to avoid inclusion of sediment particles and then were brought to the laboratory. Here, concentrations of NH_4^+ (mg l^{-1}), NO_3^- (mg l^{-1}) and P (mg l^{-1}) were measured. Concentration of NO_3^- was quantified measuring the absorbance at 525 nm, obtained by an adaptation of the cadmium reduction method (APHA et al., 1998); concentration of NH_4^+ was achieved by an adaptation of the Nessler method, measuring the absorbance at 420 nm (ASTM, 2015); finally, PO_4^{3-} concentration was obtained with an adaptation of the ascorbic acid method (APHA et al., 1998) measuring absorbance at 610 nm. Analyses were carried out using a multiparameter spectrophotometer (HI83200-02, manufactured by Hanna Instruments Inc. Woonsocket, RI, USA) and three replicates were done for each parameter.

Statistical analyses

Non-metric Multidimensional Scaling (NMDS) was performed to summarize variations among age-grouped sections, with particular regard to the period of fish introduction. For this purpose, HC densities in each sample (HC g^{-1}) were considered, data were transformed prior to analyses ($\log(x + 1)$) in order to reduce the influence of very abundant taxa (Clarke & Gorley, 2006), and a resemblance matrix was subsequently obtained using the Bray–Curtis measure. One-way PERMANOVA (Anderson, 2001; McArdle & Anderson, 2001) was performed to check significant differences in chironomid assemblages among identified age groups, which were pairwise compared. Multivariate homogeneity of group dispersions was checked using PERMDISP (Anderson, 2006) before PERMANOVA application. SIMPER analysis (Clarke, 1993) was applied to the data matrix to identify the main taxa contributing the most to observed significant differences highlighted by the PERMANOVA. Fish presence, Pb concentrations, and C/N ratio were studied in relation to changes in chironomid assemblages within the core samples. The contribution of each variable to chironomid variability was estimated through variance partitioning based on a CCA and plotted as a Venn diagram. Significance of each feature and relative interactions were checked using the Monte Carlo permutation test, with 999 permutations. Finally, dominance and Shannon–Wiener diversity were calculated for each subsample section and Kruskal–Wallis test was used to check for significant differences among values observed for different section groups. The Conover–Iman test (Conover & Iman, 1979; Conover, 1999) was used as post hoc test, to assess the presence of significant differences between groups. All analyses were performed using RStudio version 3.4.3. Figures are produced with RStudio and processed with software Inkscape version 0.92.

Results

Chemical and physical features

Mean values of chemical and physical features measured at Balma Lake during the study period are reported in Table 2. Temperatures are lower in autumn (around 8°C) than summer (around 15°C) in agreement with seasonal trends, and no thermal stratification was observed in the water column. Conductivity showed values around 18 µS cm^{-1} and good oxygenation levels were detected, as a condition of oversaturation was frequently observed, especially in autumn. pH values ranged between 6.64 in summer and 7.69 in autumn. Nutrients generally showed low levels, with similar values in both sampling seasons for NH_4^+ (around 0.09 mg l^{-1}) and P (0.01 mg l^{-1}). Water transparency was slightly higher in summer (3.25 m) than autumn (2.80 m), but in both seasons the Secchi disk did not remain visible until the lake bottom was reached.

Modern chironomid assemblages

Samplings allowed to collect 4415 macrobenthic invertebrates: 4321 in the littoral zone and 94 in the deep sites. Diptera Chironomidae was the most abundant taxon, representing 67.2% of the whole littoral community, while 27.2% were Oligochaeta and remaining taxa showed percentages lower than 2.8%. No chironomid specimens were observed in the deep sites, where only Oligochaeta were found. Therefore, following description regards only the littoral area.

Twelve chironomid taxa were recorded in the Balma Lake (Table 3), belonging to the subfamilies Orthocladiinae (31.2% of the whole littoral community), Tanypodinae (17.9%), and Chironominae (Tribe Tanytarsini 17.6%). Subfamilies Diamesinae (0.05%) and Prodiamesinae (< 0.1%) were less abundant, being observed only in summer and almost exclusively in the inlet zone (site Lit_1 in Fig. 1c). The photographs of the most abundant chironomid taxa collected in the littoral zone are presented in Fig. 2a–d.

Tanypodinae was represented by *Zavrelimyia* Fittkau, 1962 and *Macropelopia* Thienemann, 1916; *Zavrelimyia* was the most abundant, being observed in the whole littoral zone in both seasons, except for the site Lit_1 in summer. Subfamily Orthocladiinae showed the highest number of taxa (equal to 6), being mainly represented by *Psectrocladius psilopterus* group, detected in all littoral sites in both seasons, while other taxa were generally less abundant and observed mostly in the inlet area, except for *Cricotopus/Orthocladius* van der Wulp, 1874 in autumn. Two genera belonging to Tanytarsini tribe were observed: *Paratanytarsus austriacus* Kieffer, 1924 was the most frequent, being found along the whole littoral zone, and *Micropsectra* Kieffer, 1908, that was only recorded near the inlet (site Lit_1, in Fig. 1c) (Table 3).

Dating and core geochemistry

The results of Pb determination are reported in Fig. 3a. Between 18–20 and 28–30 cm core sections, Pb concentrations showed lower values than in other samples, ranging from 6.54 to 8.56 µg g^{-1}. Values started to increase from a depth of 16-18 cm and remained fairly constant until a depth of 8–10 cm (16.27–19.04 µg g^{-1}); at 4–6 cm, the increase

Table 2 Mean values and standard deviations of chemical and physical features measured at Balma Lake during the two sampling campaigns (summer and autumn)

	Summer	Autumn
Temperature (°C)	15.62 ± 0.77	8.24 ± 0.53
Dissolved oxygen concentration (mg l^{-1})	7.40 ± 0.89	7.29 ± 1.92
Oxygen saturation (%)	92.88 ± 8.52	104.75 ± 2.43
pH	6.64 ± 0.28	7.69 ± 0.12
Conductivity (µS cm^{-1})	18.29 ± 1.12	18.56 ± 1.05
Secchi Disk (m)	3.25	2.80
NH_4^+ (mg l^{-1})	0.09 ± 0.07	0.09 ± 0.05
NO_3^- (mg l^{-1})	8.55 ± 2.73	6.82 ± 2.14
P (mg l^{-1})	0.01 ± 0.01	0.01 ± 0.01

Table 3 Composition of the littoral chironomid communities and densities (ind m^{-1}) observed in Balma Lake (subfamilies are presented in bold)

Taxon	Summer					Autumn				
	Lit_1	Lit_2	Lit_3	Lit_4	Lit_5	Lit_1	Lit_2	Lit_3	Lit_4	Lit_5
Tanypodinae										
Macropelopia Thienemann, 1916			3.3	3.3	153.3	50.0	13.3	13.3		66.7
Zavrelimyia Fittkau, 1962		150.0	510.0	186.7	183.3	183.3	413.3	173.3	260.0	210.0
Chironominae										
Tanytarsini										
Micropsectra Kieffer, 1908	33.3					123.3				
Paratanytarsus austriacus Kieffer, 1924	150.0	250.0	153.3	106.7	286.7	123.3	573.3	226.7	430.0	73.3
Diamesinae										
Pseudodiamesa Goetghebuer, 1939	50.0				26.7					
Prodiamesinae										
Prodiamesa olivacea Meigen, 1818	3.3									
Orthocladiinae										
Chaetocladius Kieffer, 1911	3.3									
Cricotopus/Orthocladius	26.7						186.7	66.7	116.7	
Hydrobaenus Fries, 1830	280.0	16.7				16.7				
Metriocnemus van der Wulp, 1874	3.3									
Parorthocladius Thienemann, 1935						86.7				
Psectrocladius psilopterus group	76.7	133.3	120.0	350.0	126.7	423.3	586.7	593.3	956.7	300.0

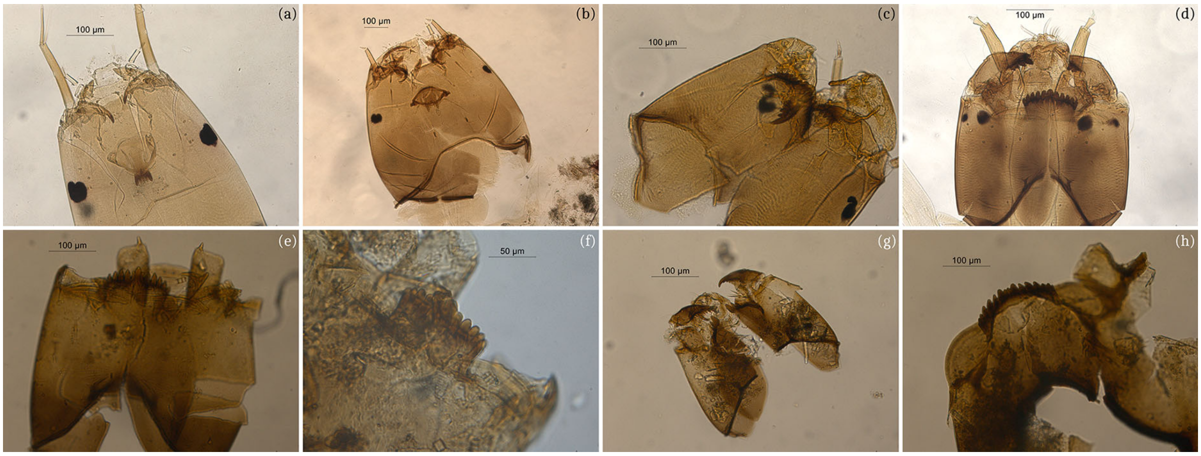

Fig. 2 Photos of chironomid head capsules belonging to the main taxa observed in the Balma Lake littoral community (**a** *Zavrelimyia*; **b** *Macropelopia*; **c** *Psectrocladius*; and **d** *Paratanytarsus austriacus*) and core samples (**e** *Micropsectra*; **f** *Corynocera*; **g** *Heterotrissocladius marcidus* type; and **h** *Metriocnemus*)

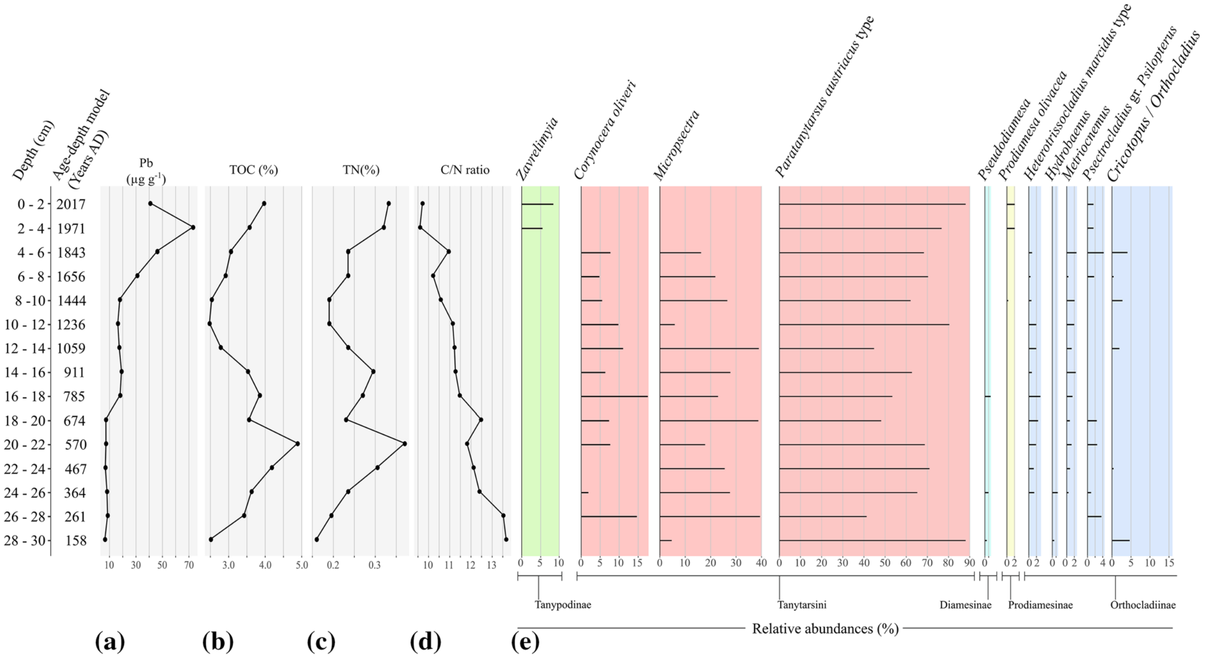

Fig. 3 **a** Total lead (Pb) (µg g^{-1}); **b** organic carbon (TOC) (%), **c** nitrogen (TN) (%), **d** C/N ratio, and **e** relative abundances of chironomid taxa from Balma Lake are also showed

becomes more evident (30.95 µg g^{-1}). Finally, Pb concentration reached a peak in the 2–4 cm section (73.07 µg g^{-1}) and then decreased at the surface samples (40.75 µg g^{-1}). No evidence of sediment movement after sedimentation emerged during the sample analysis. The measured Pb data have been compared with others different Pb analyses recorded on cores collected in nearby lake basins (Arnaud et al., 2003, 2004; Camarero et al., 2009b; Nedjai et al., 2011) and other European lakes (Brännvall et al., 2001; Renberg et al., 2001; Spadini et al., 2003). Also considering data published by ISPRA (2009), which showed a decrease in Pb emissions in Italy after 1990, it was possible to estimate that the observed peak could be likely related to the 1970s.

The results of radiocarbon analyses allowed to date the 8–10 cm and 20–22 cm samples (Table 4). These data and the information obtained from Pb determination were used to date core sections. Ages obtained from the smooth interpolation model are reported in Fig. 3, while the graphical representation of the used age-depth model is reported in Fig. 4. Accumulation rate for the Balma Lake was equal to 0.018 cm $year^{-1}$.

TOC (Fig. 3b) and TN (Fig. 3c) values increase with the rising depth in the core sediment of Balma Lake ranging from 2% to 4.84% and 0.13% to 0.36%, respectively. Both TOC and TN reach higher values at 18–20 cm. The corresponding C/N ratios gradually increased downcore from 9.7 to 13.6 (Fig. 3d).

Subfossil chironomid assemblages

Analysis of the Balma Lake core samples allowed for the identification of 7480 chironomid HC distributed among 5 subfamilies and 11 genera. In agreement with

Table 4 Radiocarbon ages for samples of the Balma Lake core

Depth of sample	Lab code	14C age (years BP)	Calibrated 2σ age (year BP)
8–10	Poz-111888	800 ± 30	675–767
20–22	Poz-111887	1515 ± 30	1335–1423

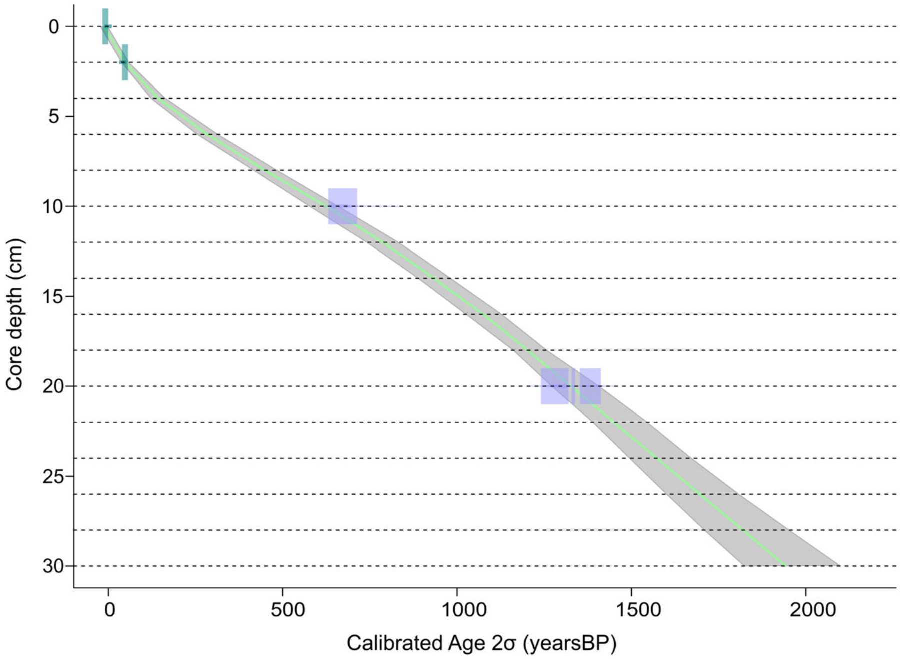

Fig. 4 Age-depth model based on smooth interpolation of best point calibration estimates from ^{14}C dates and non-carbon dates (Pb concentrations in core sections) obtained using he Clam 2.3.2 Package for RStudio. Gray area shows the 95% confidence intervals based on 1000 interactions; green line represents the best-fitted age. Age values extrapolated by the program are reported in Fig. 2

Brooks et al. (2007), the number of collected HCs was significant in all sections, being always higher both than the minimum ($n > 50$) and the recommended value ($n > 150$) for representative samples. Among the core sections, mean HC density was equal to 11.2 ± 4.5 HC g^{-1}. The total number of chironomid taxa observed along the core sections ranged from 4 to 8 detected in the top of the core (sections 0–2 and 2–4 cm) and 24–26 cm depth section (Fig. 3e) respectively. Relative abundances of the observed genera and species are reported in Fig. 3e, while photographs of the most abundant taxa are presented in Fig. 2e–h. *Paratanytarsus austriacus* type and *Micropsectra* were the most frequent (41.3–88.1% and 4.7–39.4% respectively), being found in all sections except at the top (depth of 0–2 and 2–4 cm) where *Micropsectra* was absent. *Corynocera oliveri* Lindeberg, 1970 showed frequencies ranging between 1.9 and 17.6%, being present in all sections except in the top, at a depth of 22–24 cm, and in the bottom of the core (depth of 28–30 cm). Along the core, subfamily Orthocladiinae showed the highest richness (5 genera): *Heterotrissocladius marcidus* type (0.4–3.5%) and *Metriocnemus* van der Wulp, 1874 (0.4–2.6%) were observed in 11 sections, found to be absent only in the top samples and in the bottom *Psectrocladius psilopterus* group (0.4–15.7%) and *Cricotopus/Orthocladius* group (0.9–4.27%) were observed within 8 and 6 sections, respectively. Among Tanypodinae, genus *Zavrelimyia* was detected only in the two top samples, being absent in the rest of the core. Other taxa showed relative abundances lower than 2% so they were not considered in statistical analyses.

Non-metric Multidimensional Scaling is shown in Fig. 5, where the main groups of subsamples are highlighted by ellipses. Groups including older sections (B: AD 1059–1656; C: AD 570–911; D: AD 158–467) generally showed a good overlap, while the upper group (A: AD 1843–2017) is slightly separated from the rest of samples and, in particular, from the top sections (0–2 and 2–4 sections, AD 1971–2017) related to fish introduction, which occurred during the seventies. These top sections were characterized by the exclusive presence of *Zavrelimyia* (absent elsewhere within the core) and by the absence of *Micropsectra*, *Corynocera oliveri*, *Heterotrissocladius marcidus,* and *Metriocnemus*, which were widely present across the other sections (Fig. 3e) as

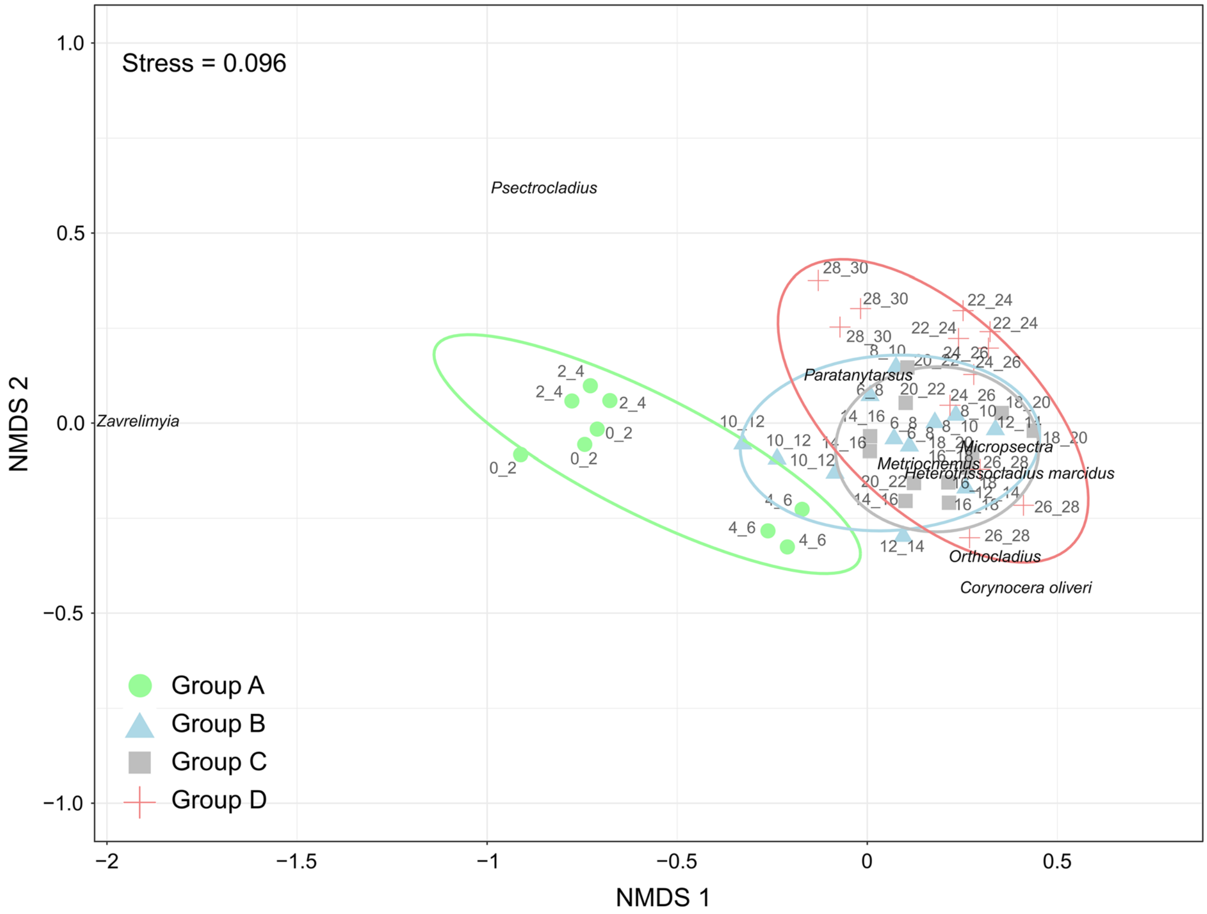

Fig. 5 Non-Metric Multidimensional Scaling (NMDS) performed on the chironomid data obtained from the sections of the Balma Lake core. Symbols represent subsamples of each section; numbers near the symbols indicate the section depth. The main chironomid taxa are also indicated

PERMDISP application did not show significant differences in the multivariate homogeneity of group dispersions ($F = 2.083$, $P = 0.117$). PERMANOVA comparisons (Table 5) allow for the detection of significant differences between the top group (A) and the other ones, while no significant difference was observed among the groups B, C, and D. SIMPER test highlighted that observed dissimilarity between communities ranged between 40.58 and 48.38% and were mainly related to *Corynocera oliveri*, *Zavrelimyia*, *Micropsectra*, *Metriocnemus*, *Psectrocladius psilopterus* group, and *Heterotrissocladius marcidus* (Table 5).

Among the variables considered to explain the variation of chironomid assemblages through the core, fish presence, Pb concentration, and C/N ratio were significant (Table 6) and explained 44.8% of the total variation (Fig. 6). Among individual fractions, fish presence explained 20.8% of the variation, while C/N ratio and concentrations of Pb explained 2.2% and 1.1%, respectively. Among the interactions, the one between fish presence and C/N ratio explained 16.5% of the chironomid assemblage variation, while other interactions showed contributions lower than 5%.

The Shannon–Wiener diversity was significantly lower in the top group A (Fig. 7) (Kruskal–Wallis test: $H_{2,42} > 7.73$, P-level < 0.05; Conover–Iman test: $P < 0.05$ for all comparisons), while it was not different among the other groups. Dominance was significantly higher for the top group (A) (Kruskal–Wallis test: $H_{2,42} = 9.308$, P-level < 0.05; Conover–Iman test: $P < 0.05$ for all comparisons) in contrast with the other sections (Fig. 7).

Table 5 Results of PERMANOVA and SIMPER tests based on chironomid assemblages observed in the Balma Lake core sample during the time period identified by the indirect dating method

PERMANOVA	Df	Sum of squares	Mean squares	*F*	*p*-level	Overall average dissimilarity (%)	SIMPER results: significant taxa and relative percentage contribute to dissimilarity	
Global test	3	1.423	0.474	17.475	0.001			
	41	1.113	0.027					
	44	2.535						
A vs B	1	0.593	0.593	19.041	0.001	40.58	*Micropsectra*	36.54
	19	0.592	0.311				*Corynocera oliveri*	14.76
	20	1.185					*Psectrocladius* gr. *Psilopterus*	10.27
							Zavrelimyia	5.68
							Metriocnemus	3.45
							Heterotrissocladius marcidus type	3.07
A vs C	1	0.876	0.876	33.054	0.001	45.55	*Micropsectra*	37.30
	19	0.503	0.026				*Corynocera oliveri*	17.11
	20	1.379					*Psectrocladius* gr. *Psilopterus*	9.39
							Heterotrissocladius marcidus type	4.83
							Zavrelimyia	4.65
A vs D	1	0.880	0.880	21.946	0.001	48.38	*Micropsectra*	36.89
	19	0.762	0.040				*Psectrocladius* gr. *Psilopterus*	8.17
	20	1.642					*Corynocera oliveri*	8.08
							Zavrelimyia	4.15

Table 6 Results of variance partitioning for variables considered to explain the variation of chironomid assemblages and their interactions

Variable	r^2	Adjusted r^2		d.f.	*F*	*P*-level
Fish presence + Pb concentration + C/N ratio	0.486	0.449	Model	3	12.899	**0.001**
			Residual	41		
Fish presence	0.441	0.428	Model	43	33.925	**0.001**
			Residual	43		
Pb concentration	0.230	0.211	Model	43	12.824	**0.001**
			Residual	43		
C/N ratio	0.086	0.064	Model	43	4.041	**0.017**
			Residual	43		
Fish presence + C/N ratio	0.463	0.437	Model	1	29.467	**0.001**
			Residual	42		

Significances are shown in bold and calculated using Montecarlo test with 999 permutations (only interactions with contribution $> 5\%$ to the observed variance are shown)

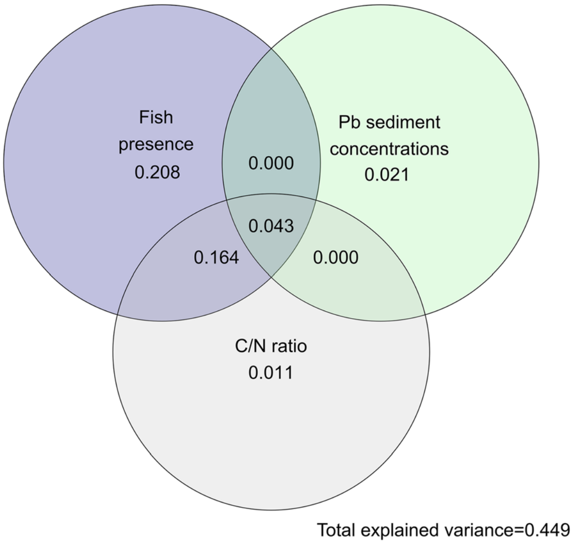

Fig. 6 Venn diagram summarizing the variance partitioning for the three significant variables (fish presence, Pb sediment concentration, and C/N ratio) considered to explain the variation of chironomid assemblages through the Balma Lake core

Discussion

High-altitude lakes are a precious landscape component of the Italian Alps, whose environments host few species with poorly structured trophic networks that respond rapidly to natural environmental changes and anthropogenic disturbances (Fjellheim et al., 2009). Among the human activities, the introduction of non-native species is one of the main threats (Vitousek et al., 1996; Mooney & Cleland, 2001; Raposeiro et al., 2017), in particular, for highly restricted, susceptible, and vulnerable environments such as alpine lakes (Fritts & Rodda, 1998; Sax et al., 2002; Gurevitch & Padilla, 2004).

Physical and chemical features

Temperature data revealed no vertical stratification in the water column, as expected due to shallow depth (6.42 m). The pH and conductivity values were in line with those reported in the literature for high-altitude environments (Kownacki et al., 2000; Boggero et al., 2006; Füreder et al., 2006; Fjellheim et al., 2009; Hamerlík et al., 2017), whose catchments are typical of granite and siliceous bedrock lakes, usually with pH values lower than 8 (Füreder et al., 2006) and extremely low values for conductivity ($< 50\ \mu S\ cm^{-1}$) (Boggero et al., 2006; Füreder et al., 2006). As expected, oxygenation levels were generally high (Tiberti et al., 2014) often showing oversaturation condition. Moreover, oxygenation

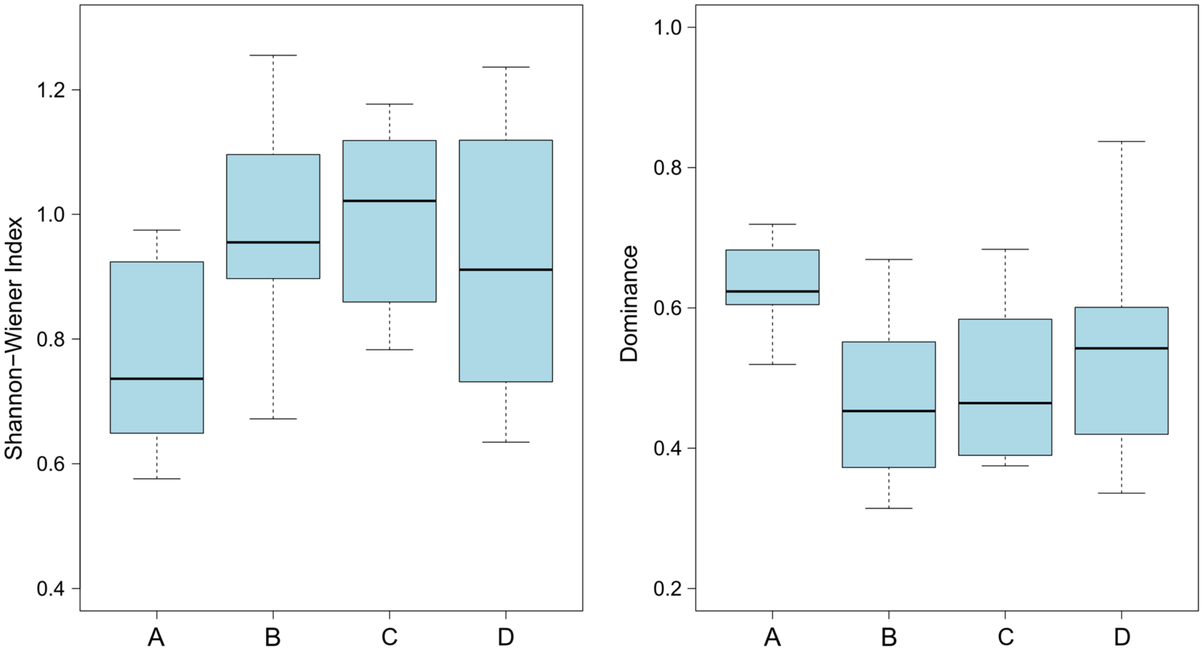

Fig. 7 Trends of Shannon–Wiener diversity and dominance calculated for the Balma Lake core section groups based on indirect dating

levels were always good in the whole water column during the monitoring periods. Nutrient levels (P, NO^{3-}, and NH^{4+}) were also in line with those generally associated with an oligotrophic condition (Boggero et al., 2006; Füreder et al., 2006; Fjellheim et al., 2009; Camarero et al., 2009a).

Levels of TN and TOC decreased from the surface down to about 10 cm, probably due to the mineralization of organic matter by bottom bacteria organisms, while the increasingly evident oscillation in strata between 14 and 20 cm was probably due to an inefficient recycling of organic matter. The observed increase in C/N ratios with depth is expected because of a bacterial preference for degrading nitrogen-rich compounds (Fenchel et al., 1998). However, values of C/N ratios around 10, as observed in the top sections, could indicate an origin of non-vascular aquatic plant, whereas values of about 20 or higher are related to a higher contribution of terrestrial organic matter (OM) (Klug et al., 2009). In the deeper sediments, C/N ratios are found to be quite uniform in value, suggesting that degradation processes are most intense because of higher proportion of terrestrial OM. Similar results were reached in oligotrophic Slovenian mountain lake sediments (Muri & Simčič, 2004) which found that respiratory activity was most intense in the upper 3–5 cm of the sediments, where autochthonous material and perhaps some labile terrestrial components were degraded than the deeper sediments, where terrestrial organic matter recorded increasing values.

Core dating

The Pb trend observed in the present study is in line with those reported in the literature for other catchments throughout Europe, especially the peak observed for the 2–4 core sections (Brännvall et al., 2001; Renberg et al., 2001; Arnaud et al., 2003, 2004; Spadini et al., 2003; Camarero et al., 2009b; Nedjai et al., 2011). This peak could be likely associated to the 1970s, when Pb deposition rate reached the highest level due to the increase in fossil fuel combustion and leaded gasoline use (Nizich et al., 2000; Renberg et al., 2000; Brännvall et al., 2001; Siver & Wonziak, 2001). This information allowed to identify the fish introduction period along the Balma Lake core sample, as fish were stocked in Piedmont Alpine lakes during the same decade (Forneris & Palmegiano, 1986; Delmastro, 1987; Balma et al., 1992; CREST, 2000; Rizzetti et al., 2001). The age-depth model obtained for the Balma Lake core is comparable to those reported in studies carried out in other European lakes (Table 7). In addition, the accumulation rate value observed for the Balma Lake is also in agreement with those reported in the literature. Although, accumulation rates from alpine lakes are generally very low (Appleby, 2000; Camarero et al., 2009b). These vary between different lakes (Bennet & Buck, 2016) showing wide ranges due to several factors such as catchment–lake relationship, erosion dynamics, and meteorological conditions (Arnaud et al., 2016).

Modern chironomid assemblages

The modern macrobenthic invertebrate community of Balma Lake is dominated by Diptera Chironomidae, which represent almost all of the whole observed community, as expected for high-altitude lakes (Bretschko, 1974; Kownacki et al., 2000; Füreder et al., 2006; Kownacki et al., 2006; Oertli et al., 2008; Fjellheim et al., 2009; Ciamporova-Zat'ovicová et al., 2010; Dumnicka et al., 2015; Novikmec et al., 2015). The chironomid littoral community was well diversified, with 12 taxa observed during the seasonal sampling campaigns. The most frequent taxa were *Zavrelimyia* and *Paratanytarsus austriacus*, which were observed in the whole littoral zone. These taxa are two of the most widespread chironomids in high-mountain lakes (Boggero et al., 2006; Füreder et al., 2006). In particular, *Paratanytarsus austriacus* could be one of the most abundant in these environments (Boggero et al., 2006; Hamerlík et al., 2017). Among the subfamily Orthocladiinae, *Psectrocladius psilopterus* group was observed in all sampling sites and is known to be commonly associated with low conductivity and phosphate poor freshwater environments (Moller Pillot, 2009c), as was observed in Balma Lake. Several taxa were present almost exclusively in the inlet area at the site 1 (Table 3). Among these genera, *Parorthocladius* Thienemann, 1935 is mostly associated with to small streams and shallow margin of standing waters (Brooks et al., 2007), while *Micropsectra* also inhabits spring water and small streams (Pinder & Reiss, 1983). On the other hand, *Micropsectra* is a cold stenothermic organism associated with oligotrophic conditions (Brooks et al., 2007), which could dominate oligotrophic environments (Frossard et al., 2013, 2014) and it is also widespread

Table 7 Results of age-depth modeling and mean sedimentation rate observed for the Balma Lake compared to the literature data for alpine and European lakes

Lake	Geographic area	Altitude (m a.s.l.)	Dating method	Core depth (cm)	Age obtained from age-depth model at 30 cm depth		Mean sedimentation rate (cm year^{-1})	References
					cal BP 2σ	BC/AD		
Balma	Cottian Alps, Italy	2100	AMS ^{14}C	30		158 AD	0.018	Present work
Toncek	Patagonia (Argentina)	1750	^{210}Pb and ^{137}Cs, tephrochronology	69		~ 1730 AD	0.105	Williams et al. (2019)
Mutterbergersee	Tyrol region, Austria	2483	^{210}Pb-CRS and AMS ^{14}C	33		1360 AD	0.046	Ilyashuk et al. (2019)
Marboré	Central Pyrenees, Spain	2612	AMS ^{14}C	700	400		0.061	Leunda et al. (2017)
Schwarzsee ob Sölden	Central Eastern Alps, Austria	2796	AMS ^{14}C	159	2200		–	Ilyashuk et al. (2011)
Lucern	Central Switzerland	434	^{137}Cs peak	163		1750 AD	0.070	Thevenon et al. (2011)
Meidsee	Southern Switzerland	2661		121	1800	200 AD	0.020	Thevenon et al. (2011, 2012)
Suorijaure 1	Sweden	962	Radiocarbon	400	600		0.470	Rubensdotter and Rosqvist (2009)
Suorijaure 2		962		110	2000		0.100	
Nero di Cornisello	Central Alps, Italy	2233	AMS ^{14}C	175	2500		0.011	Filippi et al. (2005)
Hinterburgsee	Central Switzerland	1515	AMS ^{14}C	900	1600		–	Heiri et al. (2003)

in alpine lakes (Boggero et al., 2006; Fjellheim et al., 2009; Novikmec et al., 2015; Hamerlík et al., 2017). It is a good water quality indicator, especially of oxygen-rich conditions (Oliver, 1971; Mousavi et al., 2002) and low total organic carbon content (Brodersen & Quinlan, 2006; Brooks et al., 2007). The limited presence of *Micropsectra* in Balma Lake modern community could likely be associated with the presence of allochthonous brook trout *Salvelinus fontinalis*, as species belonging to the genus *Micropsectra* were found to be highly sensitive to fish predation (Raposeiro et al., 2017). As was reported by Tiberti et al. (2016) in a study regarding the diet of *Salvelinus fontinalis* introduced in alpine lakes, the brook trout is an opportunistic predator which feeds commonly adopting visual predation strategy, and which in these environments shows a diet based mostly on small invertebrates such as chironomids, especially for small size specimens. In this context, high pigmentation or much visible color patterns could favor predation of certain taxa (Armitage, 1995; Perrine, 2017). This situation could regard the genus *Metriocnemus*, which shows alternate white and violet bands coloration pattern, and which was present only in the inlet site. The fish presence could also influence the extremely poor and reduced benthic community in the deep sites, consisting only of strictly endobenthic Oligochaeta. In fact, some burrowing taxa, such as Oligochaeta (but also Diptera larvae) could take advantage of the fish presence in high-altitude lakes (Carlisle & Hawkins, 1998; Knapp et al., 2001; Tiberti et al., 2014) and fish introductions could cause a severe decrease in macroinvertebrate abundances of the pelagic area, favoring the littoral zone (Milardi et al., 2016; Perrine, 2017). It is reasonable that macrobenthic invertebrates were pushed to a safer area in the littoral zone, were only small brook trout individuals can access.

Subfossil chironomid assemblages

Changes in subfossil community structure were observed and highlighted by NMDS analysis, variance partitioning, PERMANOVA, and SIMPER tests. Subfossil community observed across the core showed the presence of cold stenothermic taxa generally associated to oligotrophic conditions, such as *Micropsectra*, *Corynocera oliveri*, *Heterotrissocladius marcidus* type, and *Paratanytarsus austriacus* type. The presence of these organisms and the absence of the tribe Chironomini suggest that the Balma lake kept an oligotrophic status (Gandouin & Franquet, 2002) during time period identified by the age-depth model. Orthocladiinae was the subfamily with the greatest number of genera, including species which are well adapted to the low temperature characteristic of high-altitude environments (Bretschko, 1974; Wathne et al., 1995; Franceschini & Lencioni, 2002; Boggero et al., 2006; Füreder et al., 2006). Composition of the subfossil community was fairly constant along the core and changed dramatically in the top samples (0–4 cm), approximately since the fish introduction, when some taxa disappeared (*Micropsectra*, *Corynocera oliveri*, *Metriocnemus* and *Heterotrissocladius marcidus* type), *Zavrelimyia* appeared (Fig. 2; Table 5), and Shannon–Wiener diversity significantly decrease while the dominance increase. The presence of brook trout could have a severe impact on the abundances of macrobenthic invertebrate communities, as part of the diet (Tiberti et al., 2014). As top predator introduction alters chironomid assemblages, changes in diversity could occur, as observed by Raposeiro et al. (2017). Moreover, several authors observed a reduction in abundance, diversity, and richness associated with fish predators (Lammens & Hoogenboezem, 1991; Rieradevall et al., 1995; Miller & Crowl, 2006; Weber & Brown, 2009).

Micropsectra was found in all core sections except in the most recent and was the second taxon in terms of relative abundances (Fig. 3d). As mentioned above, *Micropsectra* is sensitive to fish predation (Raposeiro et al., 2017) and is also a prey item for third and fourth instars of *Zavrelimyia* (Moller Pillot, 2009a, c) which is a littoral dweller as observed in modern chironomid assemblages. *Zavrelimyia* preys also on *Heterotrissocladius marcidus*, which is also widespread in alpine lakes (Boggero et al., 2006; Novikmec et al., 2015; Hamerlík et al., 2017) and it is commonly associated with cold waters and oligotrophic conditions (Brooks et al., 2007; Moller Pillot, 2009c). However, it disappears from the core in the top sections and it is absent in the littoral community such as the cold stenothermic *Corynocera oliveri* (Brooks et al., 2007), which was the third taxon in terms of relative abundances observed along the core (Fig. 3d). Fish introduction could alter trophic webs (Sánchez-Hernández et al., 2015), with a decrease in the percentages for predator and/or detritivore taxa and an increase in grazer organisms (Raposeiro et al., 2017), in our case, respectively, represented by *Corynocera oliveri*, *Micropsectra*, *Heterotrissocladius marcidus* (Serra et al., 2017), and by *Paratanytarsus* (Heiri & Lotter, 2003). It also cannot be excluded that brook trout introduction affected nutrient load in the lake via excretion, as an increase in nutrient availability has consequences on primary production and on food webs (Du et al., 2015; Raposeiro et al., 2017). The results of the variance partitioning seem to support this consideration, as the interaction between fish presence and values of C/N ratio was significant and explained the 16.4% of the variation in chironomid assemblages. *Psectrocladius* is generally associated to macrophyte presence and/or productive lakes (Brodersen et al., 2001; Langdon et al., 2010; Axford et al., 2017) and it could respond to changes in macrophyte abundance or other aspects of lake productivity (Axford et al., 2017). Moreover, *Paratanytarsus austriacus* type is also often related to macrophyte presence (Buskens, 1987; Brodersen et al., 2001; Brooks et al., 2007). No aquatic macrophytes were observed at the Balma Lake during the present study, but the decreasing C/N ratio values in the top sections of the Balma core could be related to the presence of non-vascular aquatic vegetation (Klug et al., 2009), as suggested by datum obtained using the Secchi Disk, which remained visible at 3.25 m from the surface in summer and 2.80 m in autumn, never reaching the bottom. This could favor *Paratanytarsus* and Orthocladiinae taxa related to the presence of aquatic plants, algae, or submerged parts of macrophytes (*Psectrocladius* gr. *psilopterus*, *Cricotopus/Orthocladius* and *Parorthocladius*). Meanwhile, *Zavrelimyia* and *Paratanytarsus austriacus* are two of the most widespread chironomids in high-mountain lakes (Boggero et al., 2006; Füreder et al., 2006), the latter being observed along the entire core.

The most significant change in the chironomid composition of Balma Lake core was observed between 4–6 cm and 2–4 cm sections, with the disappearance of cold stenothermic taxa (*Corynocera oliveri*, *Micropsectra*, *Heterotrissocladius marcidus* type) and the appearance of *Zavrelimyia*. This taxon is adapted to warmer temperatures and shallow habitats and its presence could be related to an increase in the lake productivity, especially in the shallow zone, which could be associated to warm summers. A similar situation was observed in a paleolimnological study carried out in the Schwarzsee ob Sölden alpine lake (2792 m a.s.l.) in Austria by Ilyashuk et al. (2011). The trend observed in the Balma Lake could likely reflect an increase of water temperature, especially since the end of the nineteenth century and the beginning of the twentieth, in agreement with the overlapping period of the global warming. In the Alps, the temperature observed during the past several decades are two to three times higher than global linear warming (Beniston, 2006; Auer et al., 2007; Ilyashuk et al., 2011). Ilyashuk et al. (2019) highlighted a continuous warming trend since 1890 until 2010 for a high-altitude lake in Central Eastern Alps (1.1°C for 120 years), following broader-scale temperature variability in other European and Northern Hemisphere paleorecords. On the other hand, the temperature effect on the chironomid assemblages could be exceeded or amplified by other factors, such as human impacts. Millet et al. (2014) found that major shift in chironomid assemblages that occurred in a southwestern Greenland lake during the last decades of the twentieth century was due to anthropogenic changes (agriculture) independent of climate, while previous changes were mainly due to climate effect. In a lacustrine system of the Azores Islands, Raposeiro et al. (2017) showed that predator introductions (fish) differently affected chironomid taxa in addition to climate oscillations, volcanic eruptions, and anthropogenic activities, while communities seemed to be more triggered by climatic fluctuations during the fishless lake period. In a Patagonian lake, Williams et al. (2016) showed that the role of climate changes in shaping subfossil chironomid assemblages could be obscured by anthropogenic influences, including fish introductions. In this context, it is reasonable that brook trout introduction may have worked as a complementary factor, combined with other environmental impacts (mainly climate change), in shaping chironomid assemblages in Balma Lake.

This work presented herein highlights that, although climate changes remain the most important factor affecting variations of chironomid assemblages in alpine lakes, their effects could interact with anthropogenic influences, such as fish stocking (Williams et al., 2016). Moreover, if the introduction of alien fish was relatively contemporary with the period of global warming, it is difficult to separate individual factors as the main environmental driver for the changes in the chironomid assemblages (Williams et al., 2016).

Conclusions

The present study is an attempt to investigate the modern community and temporal changes within the chironomid assemblages in a high-altitude lake located in the Italian Alps (Balma Lake, Piedmont, Italy) which was not previously investigated and subject to brook trout introduction for recreational fishing at the beginning of 1970.

Main changes in the upper section (time period) of the Balma Lake core may be associated with the introduction of brook trout. Fish presence plays a pivotal role in shaping the community structure of most aquatic insects in lakes, and sometimes its role could be more incisive than lake origin or physiography (Bendell & McNicol, 1987; Binckley & Resetarits, 2005; Schilling et al., 2009). Chironomids are the available prey for introduced predators: studies elsewhere have shown predation by fish to be an important driver in shaping chironomid communities (Goyke & Hershey, 1992; Rieradevall et al., 1995; Mousavi et al., 2002; Milardi et al., 2016; Sayer et al., 2016). Climate change is the main factor affecting chironomid communities in alpine lakes, but interaction with other environmental impacts (as it is in this case the fish stockings) could lead to mixed effects. In this context, it could be difficult to establish which factor was the main environmental driver for the changes in past chironomid assemblages (Williams et al., 2016). Paleolimnological analyses are usually an important tool in climate reconstructions and are developed from transfer functions, based on the study of biological assemblages, such as chironomids and diatoms (Fritz et al., 1991; Heiri et al., 2014; Raposeiro et al., 2017). However, these analyses often assume that the main driver in their assemblages is climate fluctuations and does not take into consideration the consequences of alien fish introduction largely carried out for fishering purposes, especially during the twentieth century. These introductions could have significant effects on the results of climate reconstructions and their interpretation (Raposeiro et al., 2017). As alpine lakes play a pivotal role as indicators of global environmental change and "early warning systems", it is of primary importance to improve the knowledge about consequences of fish introduction in these lotic environments, in order to provide correct paleolimnological interpretations.

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