Original Research Article

Littoral macrobenthic invertebrates of two high-altitude lakes in the Alps: A small-scale analysis

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ABSTRACT

High-altitude lakes have been studied intensively over the last decades both in terms of their biota and general physicochemical patterns and the importance of detailed knowledge of the small scale-regional fauna was underlined. In the present study the littoral macrobenthic invertebrate communities of two alpine lakes placed in the same geographical context (Upper and Lower Balma Lakes, Cottian Alps, Italy) were seasonally characterized, and the observed diversity was investigated in relation to site-specific abiotic features. During the ice-free period, two seasonal sampling campaigns (summer and autumn) were carried out at each lake along the littoral zones: macrobenthic invertebrate were collected and abiotic features (main physicochemical water parameters and main substrates) were monitored. PERMANOVA test disclosed significant differences in the invertebrate assemblages between the lakes, and SIMPER test highlighted that the observed dissimilarity was mainly related to Oligochaeta and Chironomidae taxa. The application of the RDA and VPA showed that differences were mainly due to littoral substrate composition and nutrient concentrations (i.e., phosphorus and nitrates) which could heavily affect both the structures and densities of the macrobenthic invertebrate communities in these environments. Findings from this study improve the knowledge about the macrobenthic invertebrate assemblages in the western area of the European Alps, highlighting the role of site-specific factors in small scales studies, and the importance of detailed knowledge of their local variability. The better understanding of the variation patterns in regional/ subregional biodiversity and their drivers are of pivotal importance and have implications in the decision of conservation policies.

1. Introduction

High-altitude lakes are characterized by harsh environmental conditions, supporting highly specific aquatic communities (Füreder et al., 2006; Bartels et al., 2021) and are well-known as indicators of global environmental change and "early warning systems" for the whole mountain environment (MOLAR Water Chemistry Group, 1999; Čiamporova-Zaťovičová et al., 2010; Moser et al., 2019). Indeed, although high-altitude lakes are generally much less influenced by human activity than other habitats, global and local anthropogenic threats can alter their natural environment. The most serious are water ex-

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ploitation (Tiberti et al., 2019), alien species introduction (Cantonati et al., 2021), climate change (Rogora et al., 2018), and long-range atmospheric transport of contaminants (Camarero et al., 2009; Pastorino et al., 2020a; Pastorino et al., 2022). Due to their small area, depth, and small catchment area, high-altitude lakes are extremely sensitive to effects of global, regional, and local scale impacts (Kopáček et al. 2002). The aquatic organisms of such areas are adapted to the severe conditions that characterize alpine water bodies such as persistent low temperatures, high exposure to high URV doses associated with the high altitude, common ultraoligo- or oligotrophic status, and usually acidic pH. High-altitude adaptation means irreversible, evolved physiological responses to high-altitude environments, associated with behavioral and genetic changes (Pastorino et al., 2019). During most of the year (from October–November to June–July), snow and ice cover the lakes, blocking sunlight from penetrating the underlying water column (Felip et al., 2002). Without the penetration of light, photosynthesis cannot take place and the lakes remain in darkness (Felip et al., 2002), becoming heterotrophic systems isolated from the surrounding area until the ice cover breaks. The ice-free season lasts for a few months, generally from mid-June to late October. During this brief period of ideal conditions, aquatic organisms can complete their life cycle before the snow covers the lakes again.

Usually, alpine lakes are oligotrophic or ultraoligotrophic water bodies, where altitude, lake size, and catchment characteristics determine the structure of the living communities (Catalan et al., 2006; Hamerlik et al., 2014; Hamerlik et al., 2017; Bartels et al., 2021). These communities are greatly influenced by water temperatures, pH, dissolved oxygen, nutrient concentrations, and ice-cover duration (Catalan et al., 2006; Hamerlik et al., 2014). Exposure to wind and consequent water movements in lakes can also have effect in shaping communities (Cazenave & Zanatta, 2016; Bartels et al., 2021). Moreover, substrate has been found as a pivotal factor in shaping macrobenthic invertebrate communities in alpine lakes as in other aquatic environments (Boggero et al., 2006; Bartels et al., 2021). However, alpine lakes community are also affected by human activities, and global changes have altered communities over time. Climate change affect the mountain biota directly owing to the increase in temperature and the variation of precipitation regimes and indirectly owing to the variation in abiotic components of the environment (e.g., glacial retreat) (Hoorn et al., 2018; Tito et al., 2020). Finally, presence of introduced fish is also a key factor, as non-native species introduced for fisheries have cascading consequences on alpine lakes communities, due to predation and alterations of the trophic conditions (Tiberti et al., 2014; Sánchez-Hernández et al., 2015; Milardi et al., 2016; Perilli et al., 2020; Cantonati et al., 2021). The combined pressures of global warming and human activities require a full understanding of the spatial and temporal variations in regional biodiversity patterns, their drivers, and concomitant implications for devising conservation policies (Zheng et al., 2021).

High-altitude lakes have been studied intensively over the last decades both in terms of their biota and general physicochemical patterns (Füreder et al., 2006; Fjellheim et al., 2009; Novikmec et al., 2015; Hamerlík et al., 2017). However, it was also underlined the importance of detailed knowledge of the regional fauna for an understanding of climate-driven processes on a broader geographical scale (Čiamporova-Zaťovičová et al., 2010). Alpine water bodies could have different characteristics within the same area (i.e., substrate types, depth, etc.) leading to high regional diversity of dwelling macroinvertebrates (Hamerlík et al. 2014). Analyses at fine spatial scale, regarding small areas, are still lacking (Sax & Gaines, 2003; Schröter et al., 2005; Anderson et al., 2011), but are necessary and useful to understand specific processes, providing useful findings for the realization of region-specific policies (Cheng et al., 2021). However, data will be also valuable to understand variation in local diversity which could be complicated by the introduction of new species by humans, variations in microclimate, and different eco-geomorphic controls inherent to different landscapes. Diversity in alpine or polar regions is more likely to be controlled by temperature than in disturbed landscapes where human impacts could reshape species distribution (Nascimbene & Spitale, 2017; Sabatini et al., 2017; Peters et al., 2019).

In this context, it was deemed of interest to investigate two alpine lakes, in order to improve the knowledge about the western alpine area, starting from local differences. Balma Lakes (Cottian Alps, northwest Italy) placed in the same area, represent an alpine system that could be considered as a natural laboratory to investigate the synergic action of environmental features in shaping alpine macrobenthic invertebrate littoral communities considering small/local-scale variability.

The aims of the present study were: (i) characterize the littoral macrobenthic invertebrate communities of the Balma Lakes system, (ii) investigate for differences in the littoral macrobenthic invertebrate assemblages between the lakes, (iii) investigate site-specific factors that contribute to shape the littoral macrobenthic invertebrate communities. Global, large-scale patterns of climate control the structure of the benthic invertebrate communities in lakes, but site-specific factors have the main role in shaping communities.

2. Materials and Methods

2.1. Study area

Balma Lakes are two high-altitude small water bodies *sensu* Catalan et al. (2006) located above the tree line in the Cottian Alps (Municipality of Coazze, Piedmont, northwestern Italy). The Lower Lake is located at 2101 m a.s.l. while the Upper Lake is placed 2212 m above sea level (Fig. 1a, b). Both lakes are of glacial origin and are included in the Special Area of Conservation (SAC) and Special Protection Area (SPA) IT1110006, called "Orsiera Rocciavrè" and in the Orsiera Rocciavrè Nature Park. In both lakes, ice cover generally lasts from late October to late May/early June.

The Upper Lake is S-shaped, with two sub-basins separated by a shallow mid-section. The lake perimeter is
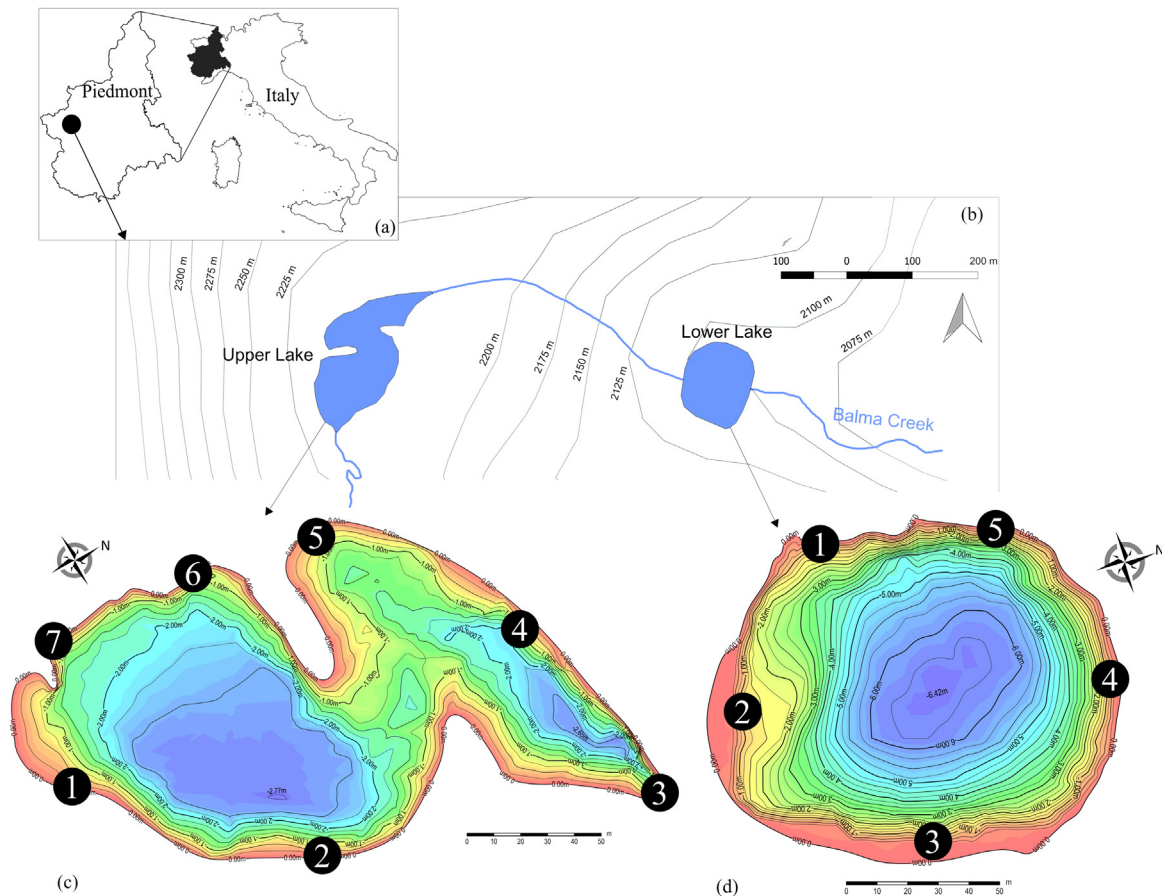
Fig. 1. Study area (a, b) and location of the sediment core sampling station in Upper (c) and Lower (d) Balma Lakes. Numbers reported on the littoral areas (c, d) represent the sampling sites.

774 m, with a 1.82 ha surface area and 2.77 m maximum depth. The lake is placed in a catchment core composed of ophiolite metamorphic bedrock and the landscape is dominated by rocky outcrops, ridges, and mountain walls. A little meadow area is placed at the southern side of the lake, where a small inlet flows. The outlet origins from the northeastern corner of the lake, flowing downstream where it becomes the inlet of the Lower Lake (Fig. 1b, c).

The Lower Lake was already described in previous studies (Pastorino et al., 2020b; Perilli et al., 2020; Cantonati et al., 2021). It is circular shaped with a perimeter equal to 414 m; the surface area is 1.21 ha, and the maximum depth is 6.42 m. The main catchment core has same composition described for the Upper Lake, and the landscape is dominated by same elements observed above, with the exception of the meadow, that is absent near the Lower Lake. The small inlet is located at the western shore dividing into three small branches before entering the lake. Although a true outlet is not evident, the Balma Creek originates from water filtration through the sediments at the eastern side of the basin (Fig. 1b, d).

The most relevant anthropogenic impacts in the Balma Lakes area over the last four decades of the 20th century are represented by the long-distance airborne transport of pollutants from the urban areas in the plain, grazing activities, and fishing (Pastorino et al., 2020a, 2020b; Perilli et al., 2020; Cantonati et al., 2021). Although the Balma Lakes were originally fishless, the brook trout *Salvelinus fontinalis* Mitchill, 1814 was introduced for recreational fishing in the 1970s (Balma et al., 1992; Pastorino et al., 2020b; Perilli et al., 2020; Cantonati et al., 2021). The brook trout population of the Lower Lake is well structured, with individuals in age classes from 0+ to 4+ and an average fish biomass equal to 17.9 g m^{-2} (Pastorino et al., 2020b). Brook trout population likely affected macrobenthic invertebrate communities over time, due to predation and (Perilli et al., 2020), diatom assemblages (Cantonati et al., 2021) in the Lower Lake, due to predation and affecting nutrient concentrations. The brook trout population of the Upper Lake was never studied before, but high densities were observed during the present study. Excretion

2.2. Physicochemical features

During each sampling event, values of the main physicochemical water parameters were recorded. Water temperature (°C), dissolved oxygen (mg L^{-1}), oxygen saturation

(%), pH (unit) and conductivity (μS cm^{-1}) were recorded using field meters (HI 9033 conductivity meter, HI 9125 pH/ORP meter, HI 9147 oximeter; all instruments were manufactured by Hanna Instruments Inc., Woonsocket, RI, USA). Three values were collected at each site in both lakes, during each sampling campaign. The Secchi disk was used from a boat in the deepest point of both lakes, to check water transparency. Water samples (1 L; three replicates) were also collected at each site in sterile containers paying attention to avoid inclusion of sediment particles, and then brought to the laboratory. Here, nitrate (NO_3^-) concentrations (mg L^{-1}) were quantified measuring the absorbance at 525 nm, obtained by an adaptation of the cadmium reduction method (APHA, AWWA, WEF, 1998); ammonium (NH_4^+) concentrations (mg L^{-1}) were measured by an adaptation of the Nessler method, measuring the absorbance at 420 nm (ASTM 2015); finally, the phosphorus (P) concentrations (mg L^{-1}) were obtained with an adaptation of the ascorbic acid method (APHA, AWWA, WEF, 1998) measuring absorbance at 610 nm. Analyses were carried out using a multi-parameter spectrophotometer (HI83200-02, manufactured by Hanna Instruments Inc. Woonsocket, RI, USA) and three technical replicates were done for each parameter.

2.3. Macrobenthic invertebrate samplings

Two sampling campaigns were carried out in 2017 (Lower Lake) and 2021 (Upper Lake), during the ice-free period, in summer (July/early August) and at the beginning of the fall season (October). Surveys were focused on the littoral areas of both lakes, defined as the nearshore habitat where enough light reaches the lake bottom to allow benthic primary producers to grow (Vander Zanden & Vadeboncoeur, 2020).

Five sampling sites were chosen in the littoral zone of the Lower Lake, while seven sites were chosen for the Upper Lake (Fig. 1c, d; Table 1). This choice was driven by the catchment characteristics, as the Lower Lake is round shaped with more uniform substrate and habitats, while the Upper Lake could be divided in two sub-basins and showed higher substrate variability than the Lower. In both lakes, sites were selected after walking the lake perimeter to check for the presence of different microhabitats. Samplings were carried out following the guidelines reported by Füreder et al. (2006) and Fjellheim et al. (2000; 2009). Three sub-samples were collected via kick sampling technique with a Surber sampler (32 cm × 32 cm, 250 µm mesh size), placing it from the shore to a water depth between 25 and 90 cm. All the present habitats (substrates), classified as boulders, rocks, cobbles, gravel, and mud/sand were considered and replicates were collected covering all the main habitat observed at each site. In order to standardize sampling effort, during each replicate collection, substrates were disturbed for 2 minutes (Füreder et al., 2006; Fjellheim et al., 2009). No aquatic vegetation was observed in the investigated lakes. After collection, samples were stored in situ with 70% ethanol solution and brought to the laboratory, where they were examined using a stereomicroscope (ORMA, TEKNO Series, model NB50T, led illumination, from × 8 to × 50 magni-

fications) for sorting, counting and taxonomical identification. Oligochaetes and chironomid larvae were mounted on slides and identified with an optical microscope (ORMA, BIOTEK, model K900BL, from × 60 to × 100 magnifications). Taxonomical identification was carried out at least to genus level, if possible. For each identified taxon, densities (ind m^{-2}) were calculated at each sampling site.

2.4. Statistical analysis

Due to the size of the sample ($n \leq 20$), the Wilcoxon non-parametric test was used to check for significant differences between seasonal values of chemical-physical data for each lake. The same test was used to check for differences between Upper and Lower Lake values in the analogous season.

In order to characterize the macrobenthic invertebrate assemblages, common community indices were calculated (mean number of observed genera, Shannon-Wiener index and Evenness). Seasonal values observed for the investigated lakes were compared using the Wilcoxon non-parametric test. The same test was used to check for differences between Upper and Lower Lake values in the analogous season. A similarity matrix was obtained from macrobenthic invertebrate littoral densities data using the Bray–Curtis measure and two-way PERMANOVA (Anderson, 2001; McArdle and Anderson, 2001) was performed to check for significant differences between communities of the investigated lakes (factors "Lake" and "Season"). Prior to analyses, data were transformed ($\log[x+1]$) to reduce the influence of very abundant taxa (Clarke and Gorley, 2006) and the multivariate homogeneity of group dispersions was checked using PERMDISP2 (Anderson, 2006). To minimize the influence of rare taxa, only organisms with relative abundances > 2% or present in at least two samples were included. SIMPER analysis (Clarke, 1993) was applied to the data matrix to identify the main taxa which most highly contributed to observed significant differences highlighted by the PERMANOVA. All analyses were performed using 9999 permutations.

Redundancy Analysis (RDA) (ter Braak and Smilauer, 1998; Legendre and Legendre, 1998) was used to investigate the relationship between macrobenthic invertebrates and environmental variables. RDA was chosen after applying the Detrended Correspondence Analysis (DCA), as the gradient lengths were <4 standard deviations (ter Braak and Smilauer, 2012). To avoid multicollinearity, a subset of environmental variables was chosen after correlation analyses. The Pearson product-moment correlation coefficient (r) was used for this purpose. If two or more variables were strongly correlated (threshold value r < |0.7| and $p<0.001$), they were excluded from the analysis (Dorman et al., 2013). As values of the Pearson correlation coefficient were always lower than the threshold value, all variables were included in the analysis. In addition, the habitats (substrates) observed and sampled at each site were also considered as a variable and classified in categories (1=mud/sand, 2=gravel, 3=gravel and cobbles, 4=cobbles, 5=boulders). The total explained variance within the macrobenthic invertebrate data was partitioned among three groups of variables associated with chemical-

Table 1

Geographical coordinates and short description of the Balma Lake sampling sites. Secchi disk data are reported. Description for the Lower Balma Lake sites are taken from Perilli et al. (2020).

Lake	Sampling site	Longitude	Latitude	Site description	Secchi Disk
Upper	1	07°10′25.84"E	45° 02′11.57"N	Southeastern side of the lake, in front of the inlet mouth. Bottom mainly composed of mud and sand; depth equal to 50 cm.	1.1 ± 0.1 m
	2	07°10′29.11"E	45° 02′14.00"N	Eastern side of the lake; bottom composed of boulders; depth equal to 40 cm.	
	3	07°10′30.86"E	45° 02′17.93"N	North-eastern corner of the lake, in front of the outlet; the outlet flows downstream where it became the inlet of the Lower Lake; bottom composed of cobbles and boulders; depth equal to 90 cm.	
	4	07°10′26.85"E	45° 02′17.48"N	North-western corner of the lake; bottom mainly composed of boulders; depth equal to 45 cm.	
	5	07°10′24.29"E	45° 02′15.50"N	Western side of the lake, bottom composed by fine gravel; depth equal to 30 cm.	
	6	07°10′23.96"E	45° 02′14.30"N	Western side of the lake, bottom composed by gravel; depth equal to 25 cm.	
	7	07°10′24.04"E	45° 02′12.29"N	Southern side of the lake, shore in front of a giant boulder; bottom composed of mud and fine gravel; depth equal to 30 cm.	
Lower	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.	3.0 ± 0.3 m
	2	07°10′51.26"	45°02′11.83′′	Southern side of the lake; bottom composed of submerged boulders; depth equal to 40 cm.	
	3	07°10′53.78"	45°02′13.50"	Eastern side of the lake; bottom composed of boulders; depth equal to 40-60 cm.	
	4	07°10′52.46"	45°02′15.76"	Northern side; shore in front of the pathway; bottom composed of cobbles; depth equal to 30 cm.	
	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.	

physical (temperature, dissolved oxygen, pH, and conductivity), nutrients (NO_3^-, NH_4^+ and P) and substrate types using variation partitioning analysis (VPA) (Borcard et al., 1992). The contribution of each variable group was then plotted as a Venn diagram. The significance of each variable group and relative interactions were checked using the Monte Carlo permutation test with 9999 permutations. All analyses were performed using RStudio version 2021.9.0.351. Figures are produced with RStudio (R Core Team, 2021; RStudio Team, 2021) and processed with software Inkscape version 0.92.

3. Results

3.1. Physicochemical water parameters

Trends of physicochemical data are shown in Fig. 2. Generally, values significantly differed seasonally in both lakes (Wilcoxon nonparametric test, $p < 0.05$ for all comparisons), except for nutrients, which showed same levels in both lakes between the monitoring seasons (Wilcoxon nonparametric test, $p > 0.05$ for all comparisons). Values of all the examined physicochemical features measured in the analogous season (both in summer and in autumn) differed between the two lakes (Wilcoxon nonparametric test, $p < 0.05$ for all comparisons). Temperature and conductivity were always higher at the Lower Lake than in the Upper (Fig. 2a, d), while dissolved oxygen and pH showed different trends between summer and autumn (Fig. 2b, c). Interestingly, NH_4^+ and NO_3^- were significantly higher in the Lower basin than in the Upper, while P concentrations showed an opposite trend (Fig. 2e, f, g respectively). The highest depth where the Secchi disk was visible was reached in the Lower Lake (3.0 ± 0.3 m) while in the Upper Lake it disappeared at 1.1 ± 0.1 m (Table 1).

3.2. Macrobenthic invertebrate littoral communities

Values of community indices obtained in the Balma Lakes are shown in the Fig. 3. The densities of each collected genus were reported in the Table S1. Number of genera observed in both lakes did not differ significantly between the sampling seasons (Wilcoxon nonparametric test, $p > 0.16$ for all comparisons). Comparisons between Upper and Lower Lakes highlighted significant differences for all indices for the autumnal values (Wilcoxon nonparametric test, $p < 0.05$ for all comparisons). Summer values did not differ between the two lakes, except for Evenness (Wilcoxon nonparametric test, $p < 0.001$).

Littoral communities observed in the Upper Lake were dominated by Oligochaeta on both seasons (50.0-22333.3 ind m^{-2}), except in sites 2, 3 and 4, where Hexapoda (193.3-4043.3 ind m^{-2}) were more abundant than Oligochaeta (Fig. 4a). The subfamily Tubificinae (especially species *Spirosperma ferox* and *Tubifex tubifex*) showed the highest densities, while subfamilies Naidinae (genus *Nais*) and Enchytreidae (genera *Cognettia, Cernosvitoviella* and *Mesenchytraeus*) were also abundant (Fig. 4c). Hexapoda were mainly Diptera Chironomidae, distributed among five subfamilies (Chironominae, Orthocladiinae, Tanypodinae, Prodiamesinae and Diamesinae) (Fig. 4e). *Paratanytarsus austriacus* (tribe Tanytarsini) showed the highest densities in both seasons, especially in autumn; *Cricotopus/Orthocladius* group was the most abundant among Orthocladiinae, followed by *Psectrocladius* and *Hydrobaenus*. The Tanypodinae *Zavrelimyia* showed also high densities. Among the other taxa, Bivalvia *Pisidium* was found in most of the sampling sites (13.3-1463.3 ind m^{-2}) (Table S1).

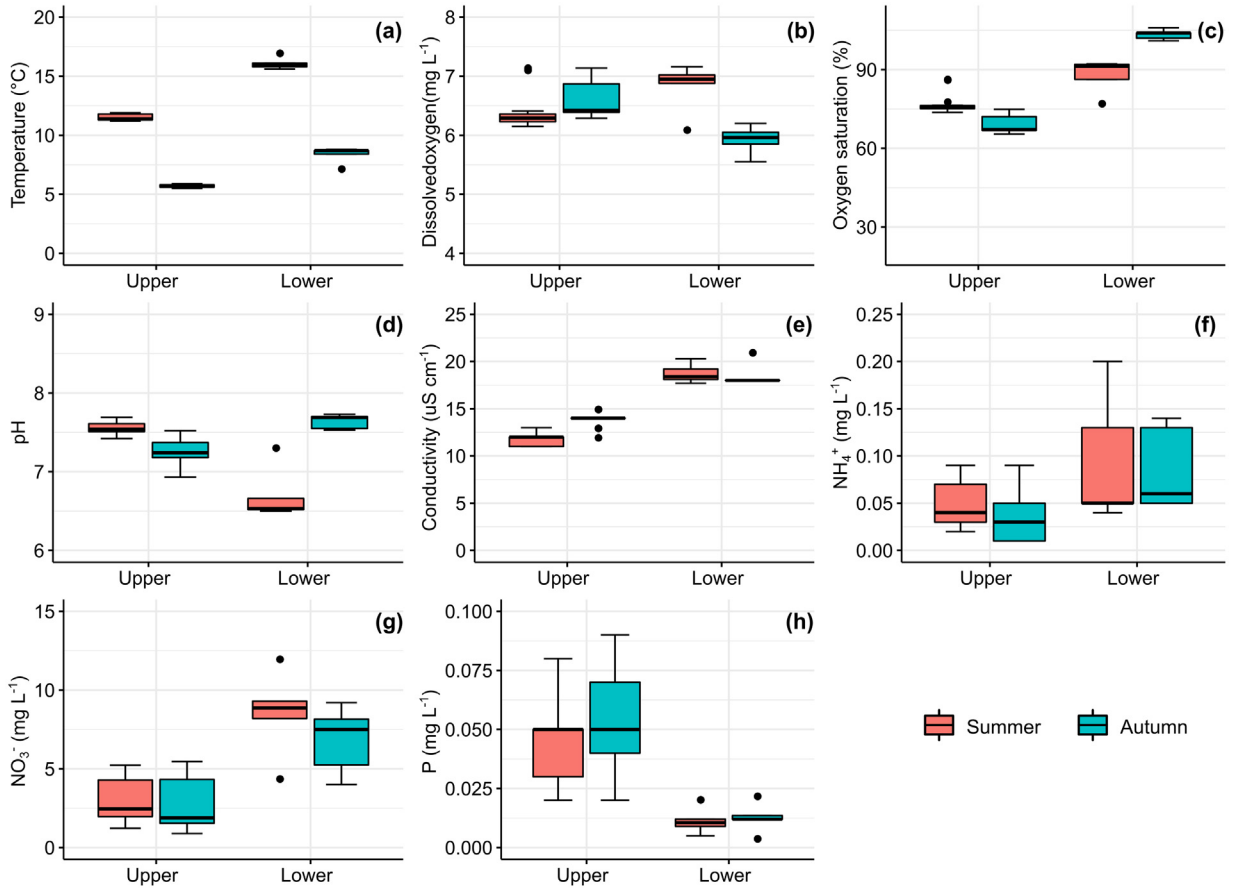In the Lower Lake densities were always lower than the Upper one, especially for the Oligochaeta group (53.3-

Fig. 2. Boxplots of water temperature (a), dissolved oxygen concentration (b) and saturation (c), pH (d) conductivity (e) NH_4^+ (f), NO_3^- (g) and P (h) observed in the Balma Lakes during the study period.
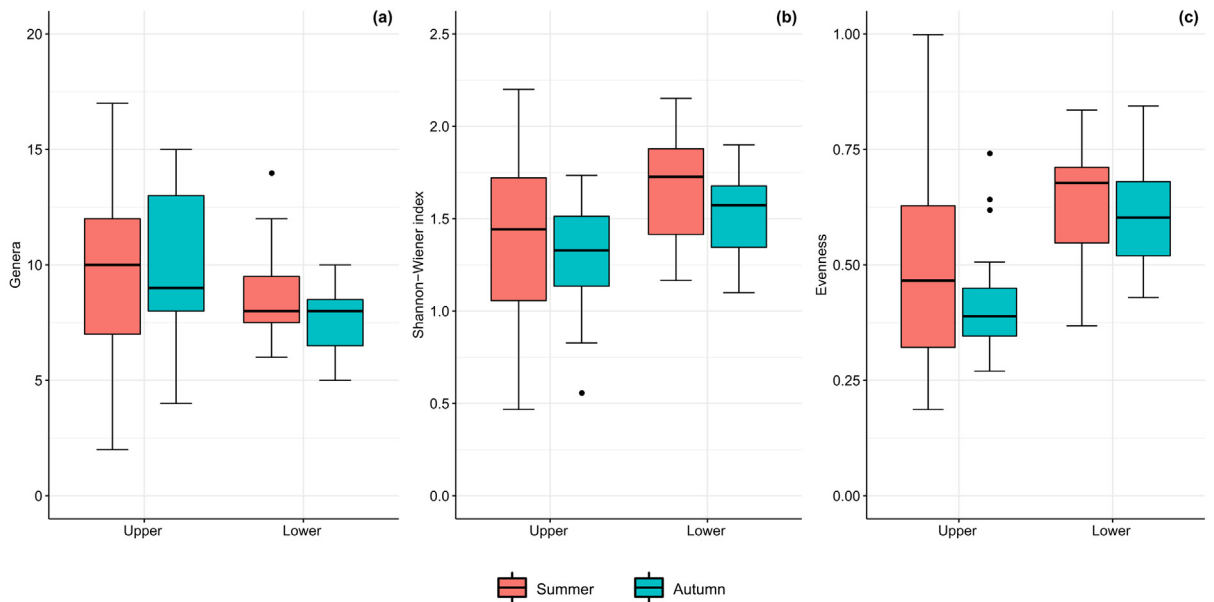

Fig. 3. Number of genera (a), Shannon-Wiener index and Evenness observed for the macrobenthic invertebrate assemblages of the Balma Lakes.
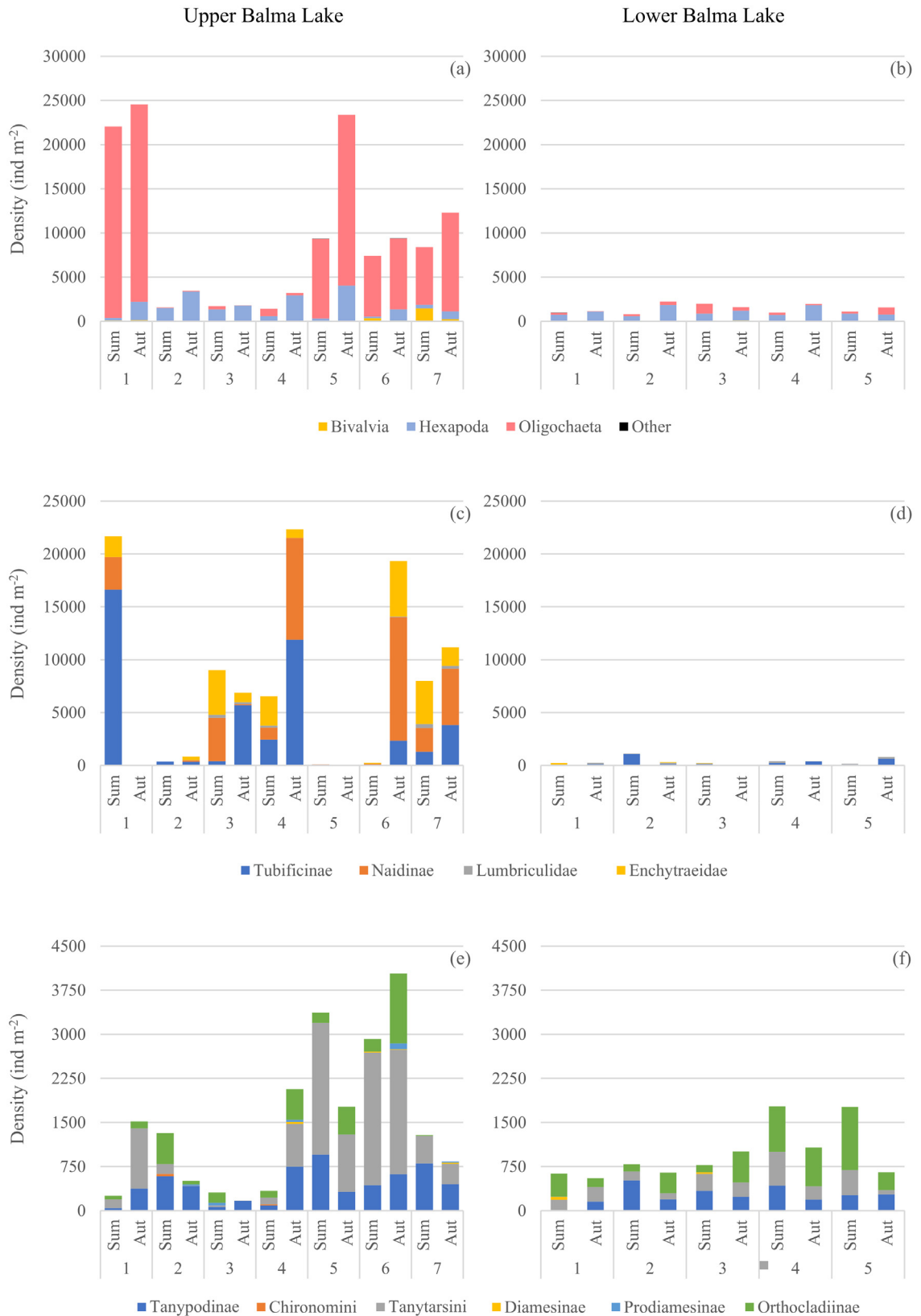
Fig. 4. Densities of the main macrobenthic invertebrate taxa (a, b), Oligochaeta (c, d) and Diptera Chironomidae (e, f) observed in the littoral zone of the Balma Lakes (Sum = summer; Aut = autumn).

Table 2
Results of PERMANOVA tests based on macrobenthic invertebrate assemblages observed in the Balma Lakes littoral area. Significance is reported in bold.

Source	Sum of squares	d.f.	Mean square	F	p
Lake	3.670	1	3.670	14.289	**0.000**
Season	0.879	1	0.879	3.423	**0.004**
Interaction	0.524	1	0.524	2.039	**0.049**
Residual	17.464	68	0.257		
Total	22.536	71			

Table 3
Results of SIMPER tests based on macrobenthic invertebrate assemblages observed in the Balma Lakes littoral area. Taxa which showed a contribution lower than 2% are not shown.

Taxon	Av. dissim	Contrib. %	Cumulative %
T. tubifex	5.66	9.73	9.73
Nais	4.98	8.55	18.28
Psectrocladius	4.71	8.09	26.37
Cognettia	4.56	7.84	34.20
S. ferox	4.48	7.70	41.90
L. variegatus	4.05	6.96	48.86
Paratanytarsus	3.62	6.21	55.07
Pisidium	3.30	5.67	60.74
Cricotopus/Orthocladius	3.24	5.56	66.30
Macropelopia	2.96	5.10	71.40
Zavrelimyia	2.07	3.56	74.96
H. memnonius	2.04	3.50	78.46
Hydrobaenus	1.79	3.07	81.53
Mesenchytraeus	1.72	2.95	84.48
Cernosvitoviella	1.47	2.52	87.00
Sialis	1.36	2.33	89.33
Micropsectra	1.24	2.12	91.45

1116.7 ind m^{-2}), (Fig. 4b, d). Tubificinae was the most abundant worm subfamily in both seasons (species *S. ferox* and *T. tubifex* and genus *Potamothrix*) while Enchytreidae (*Cognettia* and *Henlea*) were observed only in summer and Naididae were absent. *Lumbriculus variegatus* was the only species belonging to the family Lumbriculidae. The Lower Lake community was mainly constituted by Chironomidae (556.7- 1830.0 ind m^{-2}) with the same subfamilies observed in the Upper basin: *P. austriacus* was one of the most abundant, while Orthocladiinae were mainly represented by *Psectrocladius, Hydrobaenus* and *Cricotopus/Orthocladius* group. As for the Upper Lake, the Tanypodinae *Zavrelimyia* and *Macropelopia* were observed and, among the other taxa, *Pisidium* was collected at all sampling sites. Finally, some taxa were characteristic exclusively of the site 1 (inlet zone), such as *Ecdyonurus, Nemoura cinerea* and *Limnephilus coenosus* (Table S1).

The application of the PERMANOVA allowed for the detection of significant differences between the investigated lakes (Table 2), and the SIMPER test highlighted that the observed dissimilarity between littoral communities was mainly related to Oligochaeta taxa (i.e., *T. tubifex, Nais, Cognettia, S. ferox, L. variegatus*), and to the chironomids *Psectrocladius, P. austriacus, Macropelopia* and *Zavrelimyia*. Considering also the Bivalvia *Pisidium*, together these organisms contributed to the 74.96% of the observed dissimilarity. The contribution due to other taxa was less than 3% (Table 3).

3.3. Macrobenthic invertebrates-environment relationships

The results of the RDA application are shown in Fig. 5a. The RDA 1 and RDA 2 axes were both significant and explain respectively 25.9 and 6.7% of the observed variability (Fig. 5a; Table 4). The main gradient is represented by the RDA 1 and is characterized by nutrients and substrates. Lower Lake sites take place in the right side of the figure, corresponding to low P concentration, high levels of NO_3^- and NH_4^+ and coarser substrates. These sites are mostly characterized by presence of chironomid genera (*Zavrelimyia, Psectrocladius* and *Paratanytarsus austriacus*). Upper Lake sites take place in the left and bottom side of the figure and are mostly characterized by high presence of annelids (*Cognettia, T. tubifex, S. ferox, Nais*). Water temperature is mostly related to the RDA 2, which represents a weak seasonal gradient, as most of the summer sites take place in the upper side of the figure. All the variable groups taken into consideration to explain the variation of the macrobenthic invertebrate assemblages of the Balma Lakes were significant (Table 4) and explained 41.1% of the total variance (Fig. 4a, c). Among individual fractions, substrates explain most of the variation (10.5%) followed by nutrients (6.8%), whereas chemical-physical features explain only the 1.6% (Fig. 5b; Table 4).

4. Discussion

Despite the short distance between the two investigated water bodies, different characteristics were observed in relation to physicochemical features and bottom characteristics. Consequently, the macrobenthic invertebrate assemblages were significantly different.

4.1. Physicochemical water parameters

As expected, water temperatures differ seasonally in both catchments and are in line with ranges found in literature for high-altitude lakes. Füreder et al. (2006) reported that water temperature usually remains below 12°C in consequence of the short irradiation period related to the ice-cover months. Rarely, temperature may be higher than 15°C. However, this value was exceeded during summer in the Balma Lower Lake. Despite the lakes were investigated in different years, differences in water temperatures between Upper and Lower Balma Lake should reflect their elevation, as altitude is strictly related to a climatic temperature gradient (Lotter et al., 1997; Čiamporová-Zaťovičová et al., 2010). Temperature plays a significant role in shaping littoral macrobenthic assemblages of both mountain and

Fig. 5. Redundancy analyses (RDA) showing the relationships between macrobenthic invertebrate taxa and abiotic features of the Balma Lakes (a). The Venn diagrams shows the variance partitioning (VPA) results for the variable groups associated with chemical-physical features (water temperature, dissolved oxygen concentration, pH and conductivity), nutrients (NH_4^+, NO_3^- and P), and substrate characteristics in relation to the RDA (b). Unexplained variance and explanations <1% are not shown.

boreal lakes (De Mendoza and Catalan, 2010), especially for Diptera Chironomidae (Boggero et al., 2006).

Dissolved oxygen concentrations show generally good levels in Balma Lakes, especially in the Lower one, despite values were not high. However, increasing elevation comes with decreasing atmospheric pressure, leading to decreasing dissolved oxygen concentrations, whereas lower water temperature (related to increasing elevation) leads to higher oxygen saturation (Luoto and Nevalainen, 2013; Bartels et al., 2021). Oxygenation levels observed in the Balma Lakes agree with those reported by Tiberti et al. (2010) for 12 alpine lakes in the Gran Paradiso National Park (Western Alps, Italy). Values record for pH and conductivity recorded in both lakes during the present study are also in line with those commonly reported for alpine lakes located in granitic

Table 4

Results of the application of the Redundancy analysis (RDA) and variance partitioning (VPA) performed on macrobenthic invertebrate assemblages observed in the Upper and Lower Balma Lakes littoral areas. VPA groups of variables were associated with physicochemical water parameters (temperature, dissolved oxygen, pH, conductivity), nutrients (NO_3^-, NH_4^+ e P) and substrates. Significance is reported in bold.

RDA			
	RDA1	RDA2	RDA3
Eigenvalue	0.141	0.036	0.028
Proportion Explained	0.259	0.067	0.051
Cumulative Proportion	0.259	0.326	0.377
Significance (999 permutations)	**p<0.001**	**p<0.001**	**p<0.001**

VPA					
Variable	**r^2**	**Adjusted r^2**	**d.f.**	**F**	**p-level**
Chemico-physical + Nutrients + Substrate	0.411	0.336	8	5.7611	**p<0.001**
Chemico-physical	0.228	0.182	4	4.9378	**p<0.001**
Nutrient	0.198	0.163	3	5.6011	**p<0.002**
Substrate	0.213	0.202	1	18.995	**p<0.003**

bedrocks (Kownacki et al., 2000; Boggero et al., 2006; Füreder et al. 2006; Fjellheim et al., 2009; Tiberti et al., 2014; Hamerlík et al., 2017).

Interestingly, both lakes did not show seasonal differences in concentrations of P, NH_4^+ and NO_3^-. NH_4^+ and NO_3^- are higher in the Lower Lake than in the Upper, while P levels follow an opposite trend. Increased nitrate levels could be related to inputs due to cattle or sheep (Bartels et al., 2021). Cattle excrements have been observed near both lakes during each sampling campaign, and grazing livestock was present in the Balma Lakes area. It was demonstrated that the Lower Balma Lake was subject to an increase in nutrients over time, especially nitrates (Cantonati et al., 2021), which could be related to cattle and fish introduction. On the other hand, P concentrations are higher than expected in the Upper Lake, which shows levels typical of calcareous catchment, not expected in granitic bedrocks (Boggero et al., 2006; Fjellheim et al., 2009). Total phosphorous is a limiting factor for primary production (Junmei et al., 2022) and inputs could increase algal productivity in remote lakes (Reche et al., 2009; Tiberti et al., 2010). In the Lower Balma Lake water transparency was higher than in the Upper Lake. This result suggests that algal productivity should likely be higher in the Upper Lake than in the Lower. In fact, presence of algal biomass was detected during the sampling operations in the Upper Lake, despite values of total phosphorous seem to be not typical for blooming. However, observed values are higher than those reported by Tiberti et al., 2010 for alpine lakes in the Gran Paradiso National Park. Filamentous algae can be early warning indicators of environmental change, including eutrophication, acidification, or food web alterations (Lambert et al., 2008), despite the cause of increase is not always clear (Naranjo et al., 2019) and further analyses are required.

4.2. Macrobenthic invertebrates of the Balma Lakes

Biodiversity observed in the Balma Lakes system agrees with data found in literature. Čiamporova-Zaťovičová et al. (2010) found a number of genera ranging between

5 and 18 for three lakes placed between 1724 and 2157 m a.s.l. in the Tatra mountains (Slovakia). Values of the Shannon-Wiener Index and Evenness are also comparable to those observed by Füreder et al. (2006), in a study which analyzes littoral macrobenthic invertebrates of 55 alpine lakes in Europe. Evenness values observed in the Balma system allow to state that there is a good organism distribution among the observed taxa, despite sometimes some of them become dominant, especially in the Upper Lake.

As expected, both Balma Lake littoral communities are dominated by Diptera Chironomidae and Oligochaeta, as usually reported for alpine lakes (Füreder et al., 2006; Kownacki et al., 2006; Oertli et al., 2008; Fjellheim et al., 2009; Čiamporová-Zaťovičová et al., 2010; Dumnicka et al., 2015; Novikmec et al., 2015; Bartels et al., 2021). However, dominant taxa are different between Upper and Lower Lake, due to the bottom characteristics of the analyzed water bodies listed above. As highlighted by the application of the PERMANOVA and SIMPER tests, The Upper Lake is dominated by Oligochaeta taxa, while the Lower is mostly characterized by Diptera Chironomidae. First, the high worm densities observed in the Upper Lake are likely related to the substrate composition, as fine sandy/muddy sediment was observed especially in site 1, at the inlet mouth, and sites 5, 6 and 7, placed near small shores at the foot of the slopes surrounding the western side of the lake (Fig. 1b, c). These sites showed high invertebrate densities, which were almost Oligochaeta. The other Upper Lake sampling sites showed lower densities and were dominated by the presence of chironomids, in line with sites of the Lower Lake (Fig. 4a). The application of the RDA confirms this result, as sites 2, 3 and 4 take places in the right side of the figure, near the Lower Lake sites, where samples were mostly characterized by chironomids.

In alpine lakes, substrate of the littoral areas is generally dominated by larger grain size from gravel to stones and boulders, as observed in the Lower Balma Lake. Fine substrates (such as silt, mud and sand) were prevalent only in rare cases, but they have been found to be among the most influential environmental drivers of macroinver-

tebrate community composition and distribution in high altitude lakes (Catalan et al., 2009; De Mendoza and Catalan, 2010; Bartels et al., 2021). Habitat types play a pivotal role for community composition, as gravel, cobbles and rocky littorals have been shown to be preferred by different species of Chironomidae, while Oligochaeta prefers sites characterized by sandy habitats (Catalan et al., 2009; Reuss et al., 2014; Bartels et al., 2021). The bivalve *Pisidium* could be also related to fine sediments, and it was found with higher densities in the Upper Lake than in the Lower. However, *Pisidium* was observed also in the Lower basin. This presence is in contrast with Nardi (2014) which reports the absence of this bivalve with pH values lower than 7, as recorded in our work during the summer campaign. On the other hand, Boggero et al. (2005) found *Pisidium* in alpine lakes with pH ranging between 6.21 and 6.74, in line with our findings. It is possible that densities of *Pisidium* in Balma system are affected by both factors, substrate, and pH, which can limit the presence in the Lower Lake.

In addition to substrates, nutrient concentrations may have a central role in shaping the Balma littoral communities. It was demonstrated that in alpine lakes nitrate concentrations have negative effects on oligochaetes assemblages, and that these organisms exhibit positive preferences for environments rich in phosphorous (Bartels et al., 2021), especially the genus *Nais*. These findings seem to agree with our results, as shown by the application of RDA and VPA to the Balma Lakes dataset. Finally, fluctuations in water level causing the temporary drying of the littoral and complete freezing of shorelines are factors that limit the occurrence of strictly aquatic families, such as Naidinae, Tubificinae and Lumbriculidae (Kownacki et al., 2000). In lakes with fluctuating water levels, Enchytraeidae were often abundant or the only oligochaetes to occur (Dumnicka and Boggero, 2007). It is reasonable to presume that the Balma Lakes were differently affected by water level fluctuations, as differences in the shoreline position were observed between summer and autumn sampling campaign in both lakes. However, the shift of the shoreline water level limit was more evident in the Upper Lake, especially in sites 1, 5, 6, 7, where higher densities of Enchytreidae have been observed.

Among Chironomidae, differences between the two lakes were mostly due to *Psectrocladius, Paratanytarsus austriacus, Cricotopus/Orthocladius, Macropelopia* and *Zavrelimyia*. The first taxon could be related to the nutrient levels measured in the lakes, as it is commonly associated to low conductivity and phosphate poor environments (Moller Pillot, 2009). In fact, it showed higher densities in the Lower Lake, as agreement with the RDA results. *Paratanytarsus austriacus*, and *Zavrelimyia* are two of the most widespread chironomids in alpine (Boggero et al., 2006; Füreder et al., 2006). *P. austriacus* is usually associated with oligotrophic conditions (Gandouin & Franquet). On the other hand, *Zavrelimyia* is adapted to warm temperatures and shallow habitats. Its presence could be related to an increase in the lake productivity in relation to warm summers and global warming effects, as observed by Ilyashuk et al. (2011) in an Austrian alpine Lake. These findings have been also reported by Perilli et al. (2020) for the Lower Balma lake, in a paleolimnological study. The

Upper Lake show slightly higher *Zavrelimyia* densities than the Lower Lake, where water temperatures were higher than Upper Lake, maybe because here the effect of warmer summer seasons is more incisive, due to different water temperature. On the other hand, other factors could have a role in shaping the communities. The synergic action of high temperatures, shape of the lake and catchment characteristics (such as substrates) could enhance the effect due to a well-structured brook trout population, which showed high densities during the sampling operations. Fish introduction can affect nutrient loads in alpine lakes (Tiberti et al., 2014; Sánchez-Hernádez et al., 2015; Milardi et al., 2016). Previous studies demonstrated how the Lower Balma Lake was affected by fish introduction, global warming and nutrient increase (Perilli et al., 2020; Pastorino et al., 2020b; Cantonati et al., 2021). However, the effect on the Upper Lake could be more evident due to the basin shape as this is a pivotal factor in the metabolism of the lakes, with cascading effects on the whole system. Thus, further investigations should be also performed on the Upper Lake. Furthermore, the small-scale concept should be applied to the other assemblages (diatoms, ostracods, testate amoebae) to evaluate the role of the abiotic parameters on different organisms inhabiting remote alpine lakes. Moreover, the paleolimnological history of the Upper Balma Lake need to be investigated in the near future.

5. Conclusions

The present study analyzes the variations of littoral macrobenthic invertebrate assemblages in two alpine lakes of the Western Alps in relation to local-scale environmental variability. Observed biodiversity was comparable between the Balma Lakes, but differences in community structure are related to substrate, nutrient concentrations, and water temperature, which features seem to have the major role in shaping the investigated communities. The group *Cricotopus/Orthocladius* is commonly associated to aquatic plants, algae, or submerged parts of macrophytes. Higher densities of this Orthocladiinae have been observed in the Upper Lake sites, in agreement with the presence of a large algal community. Despite the Upper Lake is more characterized by Oligochaeta than Chironomidae, the densities of the *Cricotopus/Orthocladius* group could reflect the trophic condition of the Upper Lake. These findings highlight the importance of site-specific variables that operate al small scale. These drivers shape the communities of alpine lakes and must be studied to better understand the ecological sensitivity of these environment and to predict potential changes in future.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could

have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ecohyd.2023.02.003.

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