

Article

# Carbon and Nitrogen Isotopic Signatures of Zooplankton Taxa in Five Small Subalpine Lakes along a Trophic Gradient

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**Abstract:** Interest in Stable Isotopes Analyses (SIA) is increasing in freshwater ecology to better clarify ecosystems' functioning. By measuring carbon and nitrogen isotopic signatures, food sources and organism trophic levels can be tracked, providing quantitative estimates of bi-dimensional niches. In order to describe some general patterns of carbon and nitrogen stable isotope signatures in lakes, we applied SIA to zooplankton community in five subalpine lakes sampled in spring and summer along a trophic gradient (from oligotrophy to hypereutrophy). Within zooplankton taxa, temporal variation in food sources and trophic levels were compared to find out taxon-specific patterns. Carbon and nitrogen isotopic signatures differed among the five lakes, reflecting depth, topography, and trophic status of the lakes. Carbon isotopic signatures varied more considerably in deeper and larger lakes (Mergozzo and Pusiano) than in a shallower and smaller lake (Lake Endine). Nitrogen isotopic signatures were generally more enriched in lakes Pusiano and Moro than in Lake Mergozzo, whereas in summer, they were depleted in all lakes. These observations indicate that zooplankton taxa specific trophic roles differed among lakes and in time.

**Keywords:** Stable Isotopes Analysis; trophic gradient; small lakes; zooplankton

## 1. Introduction

Stable isotopes are increasingly used in aquatic ecological studies to clarify food web functioning by quantifying carbon and nitrogen flows through water ecosystems [1]. The great advantage of this approach is that complex interactions among organisms are simultaneously captured [2–4], providing information about roles of the different taxa in the environment.

The basic idea is that the isotope ratio of a consumer depends on its diet. Stable carbon isotope ratio ( $\delta^{13}\text{C}\%$ ) reflects the input of carbon revealing the contributions of different food sources, and nitrogen isotope ( $\delta^{15}\text{N}\%$ ) indicates the trophic role because a consumer is typically enriched with respect to its diet (e.g., [5–8]).

Increasing  $\delta^{15}\text{N}$  enrichment is usually observed with the increasing lake trophic status [9–11]. Carbon contents of planktonic grazers and their food sources differ in response to trophic status in lakes, mainly because of differences in contribution of phytoplankton to the food sources [10]. On the other hand, less to more  $^{13}\text{C}$ -depleted carbon signatures from littoral to pelagic carbon sources are observed (e.g., [6,12]).

Lake ecosystem functioning is influenced by length and complexity of food webs, which in turn vary with environmental conditions. In this context, zooplankton plays a crucial role, linking

primary producers to secondary consumers (e.g., zooplankton predators and fish) in a dynamic process, affecting transfer of matter and energy through lake ecosystems.

Complexity of trophic interactions increases along with lake size: increasing area and depth allow for increasing taxa and competitors, with a higher degree of specialization of predators to prey [13].

In the present study, we compared carbon and nitrogen isotopic signatures of zooplankton taxa in five minor subalpine Italian lakes with different trophic status to outline some patterns already observed in other natural and artificial lakes. We tested if the baseline and the role of different zooplankton taxa vary depending on lake trophic status and size and along seasons.

## 2. Materials and Methods

### 2.1. Study Sites

Five Southern subalpine lakes located within the River Po catchment basin (Northern Italy) at an altitude between 194 and 389 m a.s.l. (Figure 1 and Table 1) were selected as representative of different trophic status (from the hypereutrophic lakes Pusiano and Comabbio to the oligotrophic Lake Mergozzo) and size (from the small and shallow Lake Comabbio to the deepest and relatively large Lake Mergozzo).

All lakes, except Lake Moro, are located in anthropized areas, and the vegetation of all catchment basin is composed by permanent meadows, softwood, and hardwood [14,15].

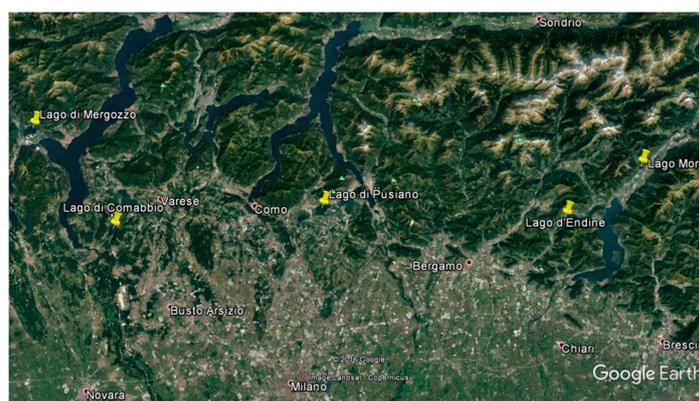


Figure 1. Satellite view of studied lakes (from Google Earth).

Table 1. Morpho-edaphic characteristics of studied lakes [16–19] ( $P_{\text{tot}}$ : total phosphorus).

Lake	Altitude (m a.s.l.)	Geographic Coordinates (Latitude Longitude)	Mixing Regime	Volume ( $10^6 \text{ m}^3$ )	Depth <sub>max</sub> (m)	$P_{\text{tot}}$ ( $\mu\text{g L}^{-1}$ )
Mergozzo	194	45°57'20" N 8°27'52" E	monomictic	83	73	1
Moro	389	45°52'47" N 10°09'25" E	meromictic	4	42	8
Endine	334	45°46'41" N 9°56'22" E	dimictic	12	9	17
Comabbio	243	45°45'55" N 8°41'37" E	polymictic	16	8	72
Pusiano	259	45°48'09" N 9°16'17" E	monomictic	69	24	74

The principal morpho-edaphic characteristic of the five lakes are reported in Table 1. All lakes, except Lake Mergozzo, may be covered with ice during very cold winters. As in most temperate lakes, the zooplankton community of these lakes is composed of cold and warm stenothermal and eurythermal taxa (Table S1).

## 2.2. Zooplankton Sampling and SIA

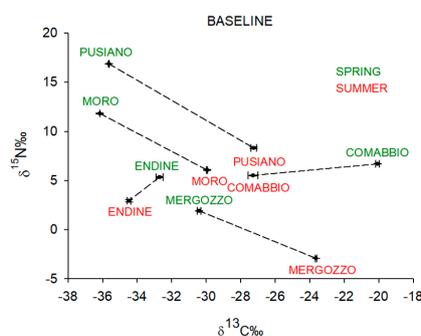
Zooplankton samples were collected at the deepest part of the lake during growing season in spring (May) and late summer (September) 2004 by vertical hauls with a 126  $\mu\text{m}$  light plankton nylon net of 20 cm-diameter opening mouth and preserved in ethanol 95%. Crustacean zooplankton were sorted by taxa in two replicates, oven-dried (60  $^{\circ}\text{C}$  for 48 h), finely ground, and transferred into tin capsules. Because of different feeding habits of copepod developmental stages, we performed SIA only on samples made up of adults and/or sub-adults of cyclopoids and diaptomids, respectively. Depending on individual body weight, between 50 and 600 specimens were necessary to get 1 mg d.w./replicate sample. Samples were sent to the G.G. Hatch Stable Isotope Laboratory of Ottawa (Ottawa, ON, Canada) for SIA analyses (Continuous Flow Isotope-Ratio Mass Spectrometry for  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ ). Data were reported in delta ( $\delta$ ) notation, the units expressed as ‰ and defined as  $\delta = ((R_x - R_{\text{std}})/R_{\text{std}}) \times 1000$ , where R is the ratio of the abundance of the heavy to the light isotope, x denotes sample and std is the abbreviation for standard. All  $\delta^{15}\text{N}$  was reported as ‰ vs. AIR and normalized to internal standards calibrated to International standards IAEA-N1 (+0.4‰), IAEA-N2 (+20.3‰), USGS-40 (−4.52‰), and USGS-41 (47.57‰). All  $\delta^{13}\text{C}$  was reported as ‰ vs. V-PDB and normalized to internal standards calibrated to International standards IAEA-CH-6 (−10.4‰), NBS-22 (−29.91‰), USGS-40 (−26.24‰), and USGS-41 (37.76‰).

On each date, *Daphnia* signatures identified lake-specific pelagic baseline, representing time-specific signature of seston particles (in a range 1.2–50  $\mu\text{m}$ ), fueling the pelagic food web [5,6,8,12,20–22]. Taxa-specific signatures were referred to time-specific baseline signatures, allowing for tracing seasonal changes of deeper vs. more surficial carbon sources and relative position in the food web.

Carbon isotopic distance with respect to baseline ( $\text{ID} = \delta^{13}\text{C}_{\text{consumer}} - \delta^{13}\text{C}_{\text{baseline}}$ ) was used to identify pelagic carbon sources ( $\text{ID} \leq 0.8 \pm 1.1$ ) [2]. We calculated taxa-specific enrichment ( $\text{E} = \delta^{15}\text{N}_{\text{consumer}} - \delta^{15}\text{N}_{\text{baseline}}$ ) to infer trophic position of each taxa with respect to the baseline.

## 3. Results

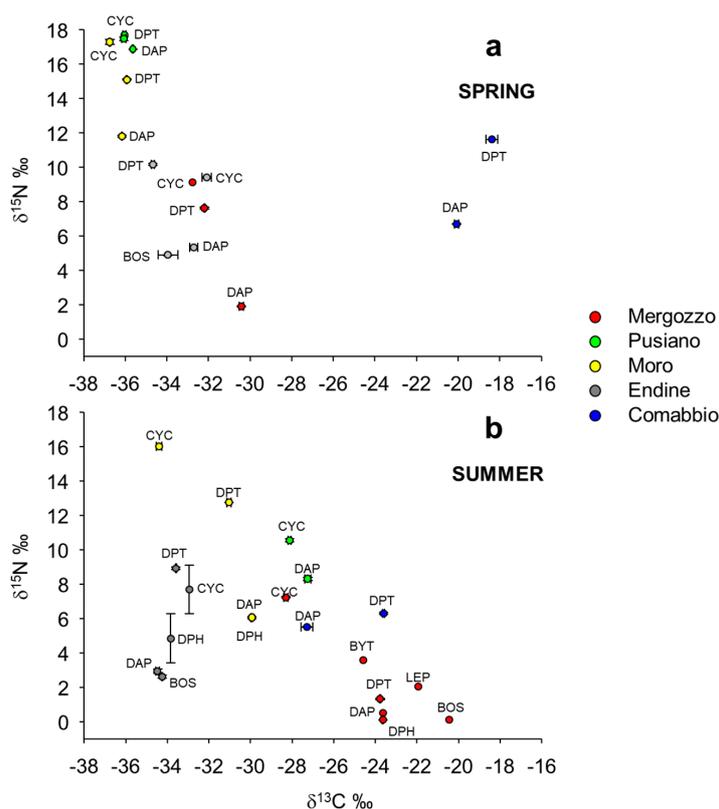
Carbon and nitrogen isotopic signatures of baseline were lake and season-specific (Figure 2). In spring,  $\delta^{13}\text{C}$  ranged between high depleted values (ca.  $-36 \delta^{13}\text{C}\text{‰}$  in lakes Pusiano and Moro) and the least depleted value of Lake Comabbio ( $-20 \delta^{13}\text{C}\text{‰}$ ). In summer,  $\delta^{13}\text{C}$  signatures were less  $^{13}\text{C}$  depleted than in spring in three out of five lakes, namely, Mergozzo, Pusiano, and Moro. An opposite trend characterized the shallowest Lake Comabbio, with a more  $^{13}\text{C}$  depleted carbon signature in summer than in spring. The lowest seasonal range of variation (of ca.  $2 \delta^{13}\text{C}\text{‰}$ ) was measured in Lake Endine. In spring, more enriched baseline  $\delta^{15}\text{N}$  signatures characterized lakes Pusiano and Moro, while Lake Mergozzo was the least enriched. In summer, nitrogen isotopic signatures were less enriched than in spring in all lakes but Comabbio, in which no seasonal variation was measured. Despite of the clear difference in the isotopic fingerprint of the baselines among lakes, we did not find statistically significant correlations between them and morpho-edaphic characteristics.



**Figure 2.** Carbon and nitrogen isotopic fingerprint of the pelagic baselines (*Daphnia*) in spring (green) and summer (red) in all lakes.

In spring, cyclopoids and diaptomids were the most enriched with respect to the baseline (Figures 3a and 4a,b). Marked differences were found among lakes  $\delta^{15}\text{N}$  signatures, while the range of variation in  $\delta^{13}\text{C}$  was relatively small.

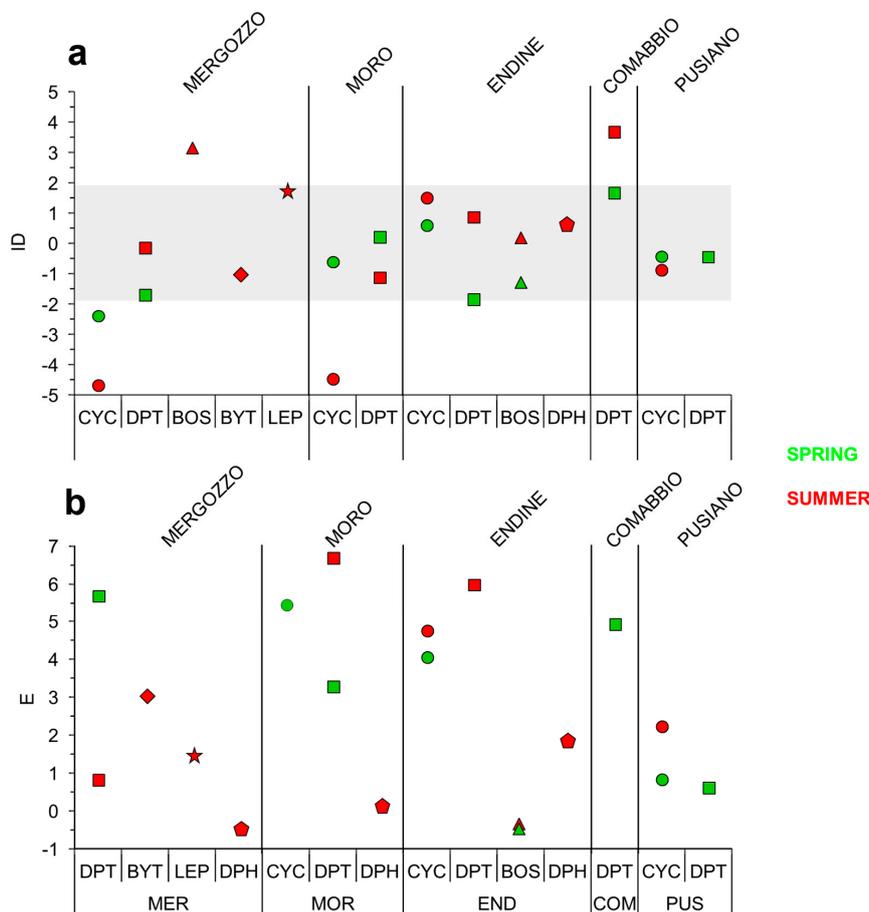
Lakes Pusiano and Moro lay on the top of the trophochemical graph, the former also characterized by an almost complete overlapping of isotopic signatures of cyclopoids and diaptomids; in the latter, cyclopoids were more enriched than diaptomids with respect to the baseline (respectively  $5.47 \delta^{15}\text{N}\%$  and  $3.28 \delta^{15}\text{N}\%$ ). In both lakes Pusiano and Moro, copepods were tightly linked with pelagic carbon sources. In Lake Mergozzo, carbon sources of cyclopoids were clearly separated from the pelagic isotopic signature, being more  $\delta^{13}\text{C}$  depleted than the baseline. In this lake, as in all others, cyclopoids had the highest  $\delta^{15}\text{N}\%$  in spring. Diaptomids were closely related to the cyclopoids for nitrogen isotopic signature, and the enrichment with respect to the pelagic baseline was high ( $5.70 \delta^{15}\text{N}\%$ ). In Lake Endine, diaptomids and cyclopoids shared similar  $\delta^{15}\text{N}$  signatures, while being well separated for  $\delta^{13}\text{C}$  signature: cyclopoids relying upon pelagic carbon sources, and diaptomids being more  $\delta^{13}\text{C}$  depleted than pelagic baseline carbon signature. In Lake Endine, diaptomids appeared tightly related to *Bosmina*, with respect to which they were  $\delta^{15}\text{N}\%$  enriched of ca. 5. Enrichment of the cyclopoids with respect to *Daphnia* was also high (4.06). Carbon fractionation between diaptomids and the pelagic baseline is indicative of deeper food sources than those integrated by *Daphnia* and better represented by *Bosmina*. The most distinct isotopic signatures were those of Lake Comabbio, in which carbon signatures of diaptomids and of the pelagic baseline were the least depleted, while being  $\delta^{15}\text{N}$  enrichment substantial (4.93), although lower than in Lake Mergozzo (5.70).



**Figure 3.** Trophochemical graph of zooplankton taxa signatures in spring (a) and summer (b). Error bars represent the Standard Error. CYC: cyclopoids; DPT: diaptomids; DAP: *Daphnia*; BOS: *Bosmina*; DPH: *Diaphanosoma*; BYT: *Bythotrephes*; LEP: *Leptodora*.

Differences among the lakes were clearer in summer, when the range of variation in carbon signatures was larger than in spring, ranging between the least depleted taxa signatures from

Lake Mergozzo to the most depleted taxa signatures of Lake Endine (Figure 3b). In the latter, *Diaphanosoma* signatures fully overlapped the pelagic baseline. Diaptomids also relied on pelagic food sources, although thanks to their nitrogen isotopic signature, they were the most enriched with respect to the baseline (6.70; Figure 4a,b).



**Figure 4.** (a) Carbon isotopic distance (ID) of zooplankton taxa with respect to the baseline (*Daphnia*) in the studied lakes. ID values ranging between  $-1.9$  and  $+1.9$  are highlighted by the grey bar; (b) Nitrogen enrichment (E) of zooplankton taxa relying upon lake pelagic baseline (*Daphnia*). CYC: cyclopoids; DPT: diaptomids; BOS: *Bosmina*; DPH: *Diaphanosoma*; BYT: *Bythotrephes*; LEP: *Leptodora*.

A full overlapping between *Daphnia* and *Bosmina* and a close similarity with *Diaphanosoma* characterized Lake Endine's summer signatures, where diaptomids were the most  $\delta^{15}\text{N}$ -enriched. In this lake, between-season variation was the least for both carbon and nitrogen signatures. Intermediate carbon signatures characterized lakes Moro and Comabbio. Lake Mergozzo's zooplankton signatures were on the right and bottom part of the trophochemical graph. In this lake, *Diaphanosoma* was clearly separated from *Bosmina* and from the *Daphnia* pelagic baseline, being characterized by the least  $\delta^{13}\text{C}$ -depleted signature. *Leptodora* was  $\delta^{15}\text{N}$  enriched with respect to *Diaphanosoma*, sharing a carbon signature intermediate between those of *Daphnia*, *Diaphanosoma*, and *Bosmina*. On the other side, isotopic signature of *Bythotrephes* was consistent with those of *Daphnia* (representing the pelagic carbon baseline), with  $\delta^{15}\text{N}$ -enrichment of 3.08. In this lake, the cyclopoids were clearly separated from all other taxa and from the pelagic baseline, being carbon signature highly depleted ( $\delta^{13}\text{C} = -28.30$ ) and nitrogen signature highly enriched ( $\delta^{15}\text{N} = 7.24$ ).

Changes in time in C isotopic values of the baseline were substantial, ranging between most  $^{13}\text{C}$ -depleted values in spring to least depleted values in summer (extremes being  $-36.74\%$  of cyclopoids

in Lake Moro in spring and  $-20.46$  of *Bosmina* in Lake Mergozzo in summer; Table S2). The least  $\delta^{13}\text{C}$ -depleted signature was measured in Lake Comabbio, in which a large variability in  $\delta^{15}\text{N}$  signature was measured in the diaptomids. Least depleted summer carbon isotopic signatures were measured in all lakes but Endine, where temporal variations of all taxa were very small. In Lake Mergozzo, within time variation in diaptomids' carbon isotopic signature was wider than that of *Daphnia*, but as in most lakes, variability in  $\delta^{15}\text{N}$  was very small.

#### 4. Discussion

Carbon and nitrogen stable isotope signatures are lake-specific. In deeper lakes, more depleted carbon signatures are expected than in shallower ones; lakes with a higher trophic status are usually more enriched in  $\delta^{15}\text{N}$  than those with a lower trophic status [9–11].

Our dataset spanned from the shallowest Lake Comabbio to the deepest Lake Mergozzo, also including the small and relatively deep Lake Moro, which is characterized by cold waters. The dataset also comprised the oligotrophic Lake Mergozzo and the hypereutrophic Lake Pusiano, thus covering a wide range of lake trophic status. Our results confirm some general patterns. In the deep subalpine Lake Mergozzo, seasonal changes in taxa-specific signatures were consistent with those observed in other deep subalpine lakes, such as Lake Maggiore [12]. With summer thermal stratification, carbon signature of the pelagic baseline tends towards less  $^{13}\text{C}$  depleted values. This observation probably reflects changes in phytoplankton isotopic signature [21]. Phytoplankton, during growth season, exhibits  $\delta^{13}\text{C}$  values less negative, because of reduced isotopic carbon fractionation at high cell densities and/or a shift on exploitation of  $\text{HCO}_3^-$  as carbon source instead of  $\text{CO}_2$  [21–31]. *Daphnia*, being an unselective filter-feeder integrates seston particle up to a size fitting the intersetular distance of filtering combs, in turn depending on *Daphnia* body size. In deep subalpine lakes, seston  $\leq 50\ \mu\text{m}$  is mainly composed by phytoplankton algal cells [32]. Carbon fractionation with respect to seston is therefore negligible, and seasonal changes in *Daphnia* carbon signature entirely overlap those of phytoplankton, thus providing a reliable pelagic baseline [6,8,33–35]. Changes in carbon baseline signature with thermal stratification in turn reflect changes in taxa composition and carbon dynamics of phytoplankton in the upper water layers [6,20,21].

According to this interpretation, also in lakes Pusiano and Moro, summer thermal stratification led to increased contribution of the upper layers to phytoplankton production in summer. In Lake Comabbio, and to a lesser extent in Lake Endine, such change is not confirmed, the summer carbon signature of both *Daphnia* pelagic baseline and the diaptomids being more  $\delta^{13}\text{C}$  depleted than in spring. Such opposite pattern is consistent with a recruitment of *Daphnia cucullata* and of *Eudiaptomus padanus* from the lake littoral, characterized by less negative carbon values (e.g., [36]), into the lake profundal during spring. Horizontal migration of zooplankton has been well documented in a number of cases, such as *Eudiaptomus padanus* in the deep nearby Lake Maggiore [36].

Very close or overlapping carbon and nitrogen signatures of primary consumers, such as *Bosmina* and *Diaphanosoma*, suggest the exploitation of the same food sources in Lake Endine, particularly in summer. In Lake Mergozzo, isotopic signatures suggest that *Diaphanosoma*, *Daphnia*, and *Eudiaptomus* have a very similar trophic role, with *Bosmina* being well separated from the other three zooplankton taxa, clearly relying upon more superficial (or more littoral) carbon sources. The least  $\delta^{13}\text{C}$ -depleted signature, in fact, is indicative of warmer waters or littoral sources. Spatial and temporal variations in carbon and nitrogen isotopic signatures in different compartments of a single lake were assessed by former studies [37,38]. Previous research on Lake Maggiore demonstrated that littoral carbon isotopic signature follows the same pattern as pelagic carbon with increasingly less depleted  $^{13}\text{C}$  values in summer than in spring, the shift being responsible for a clear separation of littoral with respect to pelagic isotopic fingerprint [6,12].

Previous work on Lake Maggiore [6,12] suggested a relatively narrow range of carbon isotopic signature of cyclopoids, along with a clear separation from summer pelagic signature. The two peculiar traits are interpreted as a clear specificity of habitat and food selection [37]. Our dataset only partially

confirms these two traits: while separation with respect to summer pelagic carbon is confirmed in lakes Moro and Mergozzo, such is not the case in lakes Pusiano and Endine, where cyclopoids do not show differences with respect to pelagic carbon signature in summer. Seasonal variability of carbon signature was small in lakes Endine and Moro, while being very large, almost comparable to that of the unselective filter-feeder *Daphnia*, in the other lakes. Different taxa may contribute along the season to the cyclopoids: in Lake Pusiano, where variability was the largest, three species have been reported, namely *Cyclops abyssorum*, *Mesocyclops leuckarti*, and *Thermocyclops hyalinus*. Previous studies have suggested interspecific differences of cyclopoid copepod species in the exploitation of food sources (e.g., [38,39]) and their relative trophic position [37]. Moreover, while *Cyclops* is known to grow and reach maximum density during cold months, the other two species tend to increase during the warm season. Such a strong seasonality explains the wide range of variability in carbon signature measured in this lake. The same explanation can be recalled for Lake Mergozzo, where a transition between *C. abyssorum* and the smaller-sized *M. leuckarti* and *Mesocyclops hyalinus* is known to occur in summer [40,41].  $\Delta^{15}\text{N}$  enrichment confirms some traits found in other lakes, independently from their size, typology, and trophic position. In particular, cyclopoids were the most enriched in all but Lake Endine, in which the diatoms were more enriched than the cyclopoids [20]. The small seasonal variability of  $\delta^{15}\text{N}$  signature also suggests a small plasticity in trophic position in all lakes but Pusiano, the hypereutrophic lake. In this lake, the cyclopoids relied upon the pelagic signature also in summer.

## 5. Conclusions

Isotopic signatures can be regarded as fingerprints of lake ecosystems, integrating site-specific traits related to lake trophic position and typology. According to our results, some traits, such as the trend toward less depleted  $^{13}\text{C}$  and less enriched  $^{15}\text{N}$  signatures in summer than in spring, were confirmed, although with some exceptions. While in thermally stratified lakes, such a transition was detected, in the shallowest, Lake Comabbio, the prevalence of a horizontal gradient was likely responsible for the opposite pattern. On the other hand, while the hypereutrophic Lake Pusiano was the most  $\delta^{15}\text{N}$  enriched as expected, substantially high levels of  $\delta^{15}\text{N}$  signature were also detected in the small, oligotrophic, and deep Lake Moro, the coldest lake among those analyzed.

Our results overall confirm the importance of taxa-specific analyses within zooplankton community, which is essential for understanding trophic relationships, changes in habitat, and carbon sources fueling the pelagic food web.

**Supplementary Materials:** The following are available online at <http://www.mdpi.com/2073-4441/10/1/94/s1>.

**Author Contributions:** All authors contributed equally to the paper.

**Conflicts of Interest:** The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

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