

## Seasonal changes in the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures of the Lago Maggiore pelagic food web

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### ABSTRACT

Seasonal variation in the relative contributions of littoral and pelagic food sources to the diets of open-water zooplankton and subsequent changes in their trophic positions were investigated with carbon and nitrogen Stable Isotope Analysis (SIA). We selected three open water stations as truly pelagic, but also influenced by littoral and riverine carbon sources. During each of the four seasons, integrated pelagic zooplankton samples were collected over 0-50 m depth intervals at each site along with seston in the size range 1.2-76  $\mu\text{m}$ . In addition, vertical temperature profiles were measured. Littoral benthos from three sites along the main longitudinal axis of the lake was sampled to serve as a reference for tracing Lago Maggiore's littoral carbon isotopic signature. Among stations differences in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  signatures of the different components of the pelagic food web, from seston to predatory zooplankton, were statistically non significant, thus confirming that allochthonous input may become important only after exceptional rainfall events. Changes in the  $\delta^{13}\text{C}$  pelagic baseline mirrored mean water temperature (0-50 m) seasonal changes. Similarly to Lake Geneva, they were likely driven by changes in carbon sources for phytoplankton growth during stratification and vertical water mixing. Differently from what observed for the other taxa, the role of littoral food sources was far from negligible (>50%) for diaptomids during winter and spring. We do not know however, whether such a result could be at least partially attributed to the heavy infestation by algal epibionts, or was consequent to the fact that these zooplankters may carry littoral carbon to the pelagial via horizontal migration. In winter, *Bythotrephes longimanus* was able to prey on *Cyclops*, thus occupying a trophic position comparable to that of planktivorous fish. Such a result confirms an ability of this visual, invertebrate predator to compete with young zooplanktivorous fish for food resources, similarly to what observed in lakes it recently invaded.

Key words: carbon, nitrogen, stable isotopes, zooplankton

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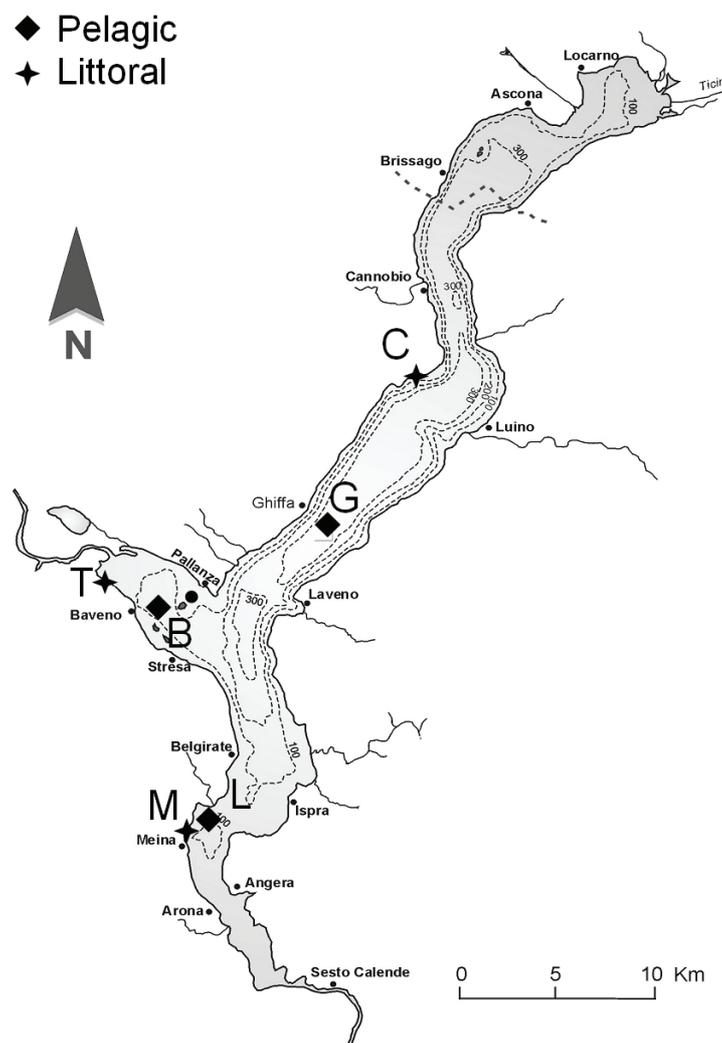
### 1. INTRODUCTION

Ecological studies are increasingly devoted to quantifying the functional roles of species through direct approaches, allowing for a better definition of food webs (Grey & Jones 1999; Leavitt *et al.* 2009; Moss *et al.* 2009). These studies are particularly important for aquatic environments, which are characterized by large temporal and spatial variability in biological as well as physico-chemical structure (de Bernardi *et al.* 1988; Bertoni *et al.* 2004). Such variability may be accompanied by important seasonal changes in the relative positions of zooplankton taxa in the food web, and in the role of primary and secondary consumers in the transfer of matter and energy along food web pathways. Such studies are also of great importance for defining the operationally based diversity and redundancy of ecosystems, which is a pre-requisite for environmentally sound ecosystem management (Moss *et al.* 2009).

Among the different approaches that can be used for addressing these goals, studies using carbon and nitrogen stable isotope analysis (SIA) have been important. Originally applied in marine environments, and usually with regard to fish, SIA is increasingly being used in lacustrine systems and to assess the ecological roles of zooplankton taxa. SIA provides signatures or "finger-

prints" that like their human analogues are unique and provide a means of tracing recent activities. In this case, the recent feeding histories of different zooplankton taxa can be traced and quantified through comparison of their carbon and nitrogen stable isotope signatures with those of potential food sources (Wada 2009).

Large, deep lakes are of great importance in this respect due to their relatively large stability and level of homeostasis, and the presence of both horizontal and vertical gradients that facilitate empirical study of ecological relationships. While seasonal changes in the carbon and nitrogen isotopic signatures of zooplankton have been documented, the mechanism for these changes is poorly understood. It may be related to seasonal change in the relative contributions of littoral and pelagic food sources (Matthews & Mazumder 2003). So far, SIA of zooplankton taxa in European subalpine lakes has been limited to a handful of cases (e.g., Lake Geneva; Perga & Gredeaux 2006) but the results obtained encourage extension of the approach to other systems. A comparison of lakes with the same typology and age, but with different primary productivity levels may in fact contribute to a better definition of the factors that drive changes in the flux of energy and matter along aquatic food web pathways (Karlsson *et al.* 2003). While carbon isotopic signatures are mainly related to lake typology, nitrogen isotopic signatures are strongly



**Fig. 1.** Sampling stations from which zooplankton taxa and seston were collected for carbon and nitrogen SIA in Lake Maggiore (0–50 m) in 2008. Sites from which littoral organisms were collected as a reference for carbon littoral signature are also indicated. For further explanation see text.

influenced by trophic position (Cabana & Rasmussen 1996; Grey *et al.* 2000; Cattaneo *et al.* 2004).

With the intent of quantifying seasonal changes in trophic position and sources for secondary production, we applied carbon and nitrogen SIA to Lago Maggiore's open water zooplankton. Lago Maggiore offered a unique occasion for investigating the trophic role of different predatory zooplankton, including cyclopoid copepods and two predatory cladocerans native of the lake, *Leptodora kindtii* and *Bythotrephes longimanus*. Moreover, we sought to determine whether littoral food sources are represented in the stable isotopic signatures of pelagic organisms throughout the year.

## 2. MATERIALS AND METHODS

### 2.1. Study site and SIA method

Lago Maggiore (45°57'N; 8°32'E) lying at an altitude of 194 m a.s.l., is the second deepest (mean depth, 177 m; maximum, 370 m) and largest (area, 212.5 km<sup>2</sup>;

volume, 37.5 km<sup>3</sup>) subalpine lake in Italy. Phosphorus-limited, the lake was brought back to oligotrophy by the middle 1980s and 1990s (total phosphorus concentration at winter mixing *ca* 10 µg L<sup>-1</sup>), after eutrophication in the 1960s and late 1970s. The impact of multiple stressors on Lago Maggiore is discussed in several papers (e.g., de Bernardi *et al.* 1990; Manca *et al.* 1992; Guilizzoni *et al.* 2011).

For almost 30 years, zooplankton have been monitored at an open-water station at the lake's maximum depth (Ghiffa, G: 45°58'30"N; 8°39'09"E) (Fig. 1) with at least monthly frequency. Accordingly, in 2008, integrated 0–50 m quantitative samples were collected with a Clarke-Bumpus plankton sampler (126 µm nylon net), along a sinusoidal trajectory from a boat moving at a low speed (0.6 m s<sup>-1</sup>). Two additional sites were sampled, one (Baveno, B: 45°54'28"N; 8°31'44"E) because it is influenced by the inflow of the River Toce (Ambrosetti *et al.* 1980); the other at the southern end of the lake (Lesa, L: 45°49'70"N; 8°34'70"E) because it is

likely to be more influenced by littoral inputs, (Fig. 1). Samples were preserved in pure ethanol (99% by volume) and counted to estimate abundance of the different taxa. Live zooplankton samples for SIA analysis were collected at the same sites from *ca* 13 m<sup>3</sup> of filtered lake water with a large (opening mouth diameter, 58 cm) 126- $\mu$ m mesh plankton net. Zooplankters were kept in filtered water for gut clearance overnight before sorting to the species level. Among the Cladocera, *Daphnia galeata* (Daphniidae), *Bythotrephes longimanus* (Leydig; Onychopoda) and *Leptodora kindtii* (Focke; Haplopoda) were analyzed. Calanoid (*Eudiaptomus padanus*, Burckhardt + *E. gracilis*, Sars) and cyclopoid (*Mesocyclops leuckarti* Klaus + *Cyclops abyssorum* Sars 1863) copepods were also selected. Live organisms in the samples mainly consisted of adult particle-feeding Cladocera, and the last copepodite stage of copepods. Depending on individual mass, between 50 and 600 individuals per taxon were used to reach the dry weight necessary for reliable SIA. Three replicates of each taxon were prepared on each date as described in Manca *et al.* (1994).

To trace seasonal changes in the sestonic pelagic baseline (1.2-76  $\mu$ m size fraction; hereafter defined as "seston"), 0-20 m (epilimnion) and 25-50 m (hypolimnion) integrated water samples were collected on each sampling date with a 5l Niskin bottle. Subsamples were fixed with Lugol acetic solution for later phytoplankton microscopic analyses, and the remainder of the samples filtered through GF/C glass fibre filters, coupled with 12- $\mu$ m nucleopore membranes. The filters were used for SIA and measurement of chlorophyll-*a* concentration using the methodology of Lorenzen (1967). We also measured the water temperature vertical profile with a thermistore, and Secchi depth water transparency. In addition, a detailed study on phytoplankton taxa composition, biovolume and results of SIA is now in press (Caroni *et al.* 2011).

To characterize the isotopic signatures of Lago Maggiore's littoral zone we collected aquatic invertebrates (chironomids and amphipods) from three different stations (Canero: C; Meina: M; Toce: T; Fig. 1), located along the main longitudinal axis of the lake-shore. Signatures of three zooplanktivorous fish (*Rutilus rutilus* L.; *Coregonus lavaretus* L. and *Alosa fallax lacustris* Fatio), collected monthly in the open water with benthic gillnets were also analyzed (dorsal muscle, 5 individuals/species with three replicates each), and individual size (total length and total weight) was measured (Manca *et al.* 2009). Detailed and specific analyses on littoral organisms and on fish carbon and nitrogen isotopic signatures will be discussed in two separate manuscripts under preparation.

All the collected samples were dried at 60 °C for one day. Once dry, the samples were ground to a fine powder and 1-mg subsamples weighted into 5×9 mm tin capsules. The samples were analyzed by continuous

flow isotope-ratio mass spectrometry (CF-IRMS) at the G.G. Hatch Stable Isotope Laboratory (University of Ottawa, Canada) for  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , percentage of carbon and nitrogen, and C:N ratio. The Elemental Analyzer (Vario EL III manufactured by Elementar, Germany) was interfaced with a Conflo II (manufactured by Thermo, Germany), to an Isotope Ratio Mass Spectrometer (IRMS; Delta XP Plus Advantage, manufactured by Thermo, Germany). Samples and standards were flash-combusted at about 1800 °C (Dumas combustion) and the resulting gaseous products carried by helium through columns of oxidizing/reducing chemicals optimized for CO<sub>2</sub> and N<sub>2</sub>. The gases were separated by a "purge and trap" adsorption column and sent to an IRMS interface, then to the IRMS.

Internal standards for  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  were: C-51 Nicotinamide (0.07-22.95), C-52 ammonium sulphate + sucrose (16.58-11.94), C-54 caffeine (-16.61 -34.46), blind standard C-55: glutamic acid (-3.98 -28.53). Data were reported in Delta notation ( $\delta$ ), the units being per mil (‰) 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 an abbreviation for standard. All  $\delta^{15}\text{N}$  values were 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  $\delta^{13}\text{C}$  were reported as ‰ vs V-PDB (Pee Dee Belemnite) 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‰). PDB and V-PDB are identical and interchangeable. Analytical precision based on the laboratory internal standard (C-55, not used for calibration) was better than 0.2 ‰. Standard deviations were 0.05 and 0.12, 0.12 and 0.06, 0.12 and 0.06, 0.06 and 0.16 for <sup>13</sup>C and <sup>15</sup>N of seston, zooplankton, fish and littoral samples, respectively.

We calculated  $\delta^{13}\text{C}$  fractionation ( $F = \delta^{13}\text{C}_{\text{consumers}} - \delta^{13}\text{C}_{\text{baseline}}$ ) of different organisms with respect to both seston and *Daphnia* isotopic signature, taking 0.8 (SD  $\pm$  1.1‰, according to de Niro & Epstein 1978) as a threshold value for non-pelagic carbon sources. We also calculated  $\delta^{15}\text{N}$  enrichment ( $E = \delta^{15}\text{N}_{\text{consumers}} - \delta^{15}\text{N}_{\text{baseline}}$ ) along the food chain.

An organism's trophic level (T) at each sampling was calculated as:

$$T_{\text{seston}} = \lambda + [(\delta^{15}\text{N}_{\text{consumer}} - \delta^{15}\text{N}_{\text{baseline}})/2.3] \quad (1)$$

Where:  $\lambda$  is the trophic position of the organism used as a baseline (with  $\lambda = 1$  for primary producers;  $\lambda = 2$  for primary consumers (Vander Zanden *et al.* 1997)), and 2.3 represents the minimum nitrogen enrichment value calculated for Lago Maggiore with respect to the pelagic baseline. When *Daphnia* ( $\lambda = 2$ ) was the reference organism however, the equation (1) was modified into:

$$T_{\text{consumer}} = 2 + [(\delta^{15}\text{N}_{\text{secon. consumer}} - \delta^{15}\text{N}_{\text{prim. consumer}})/2.3] \quad (2)$$

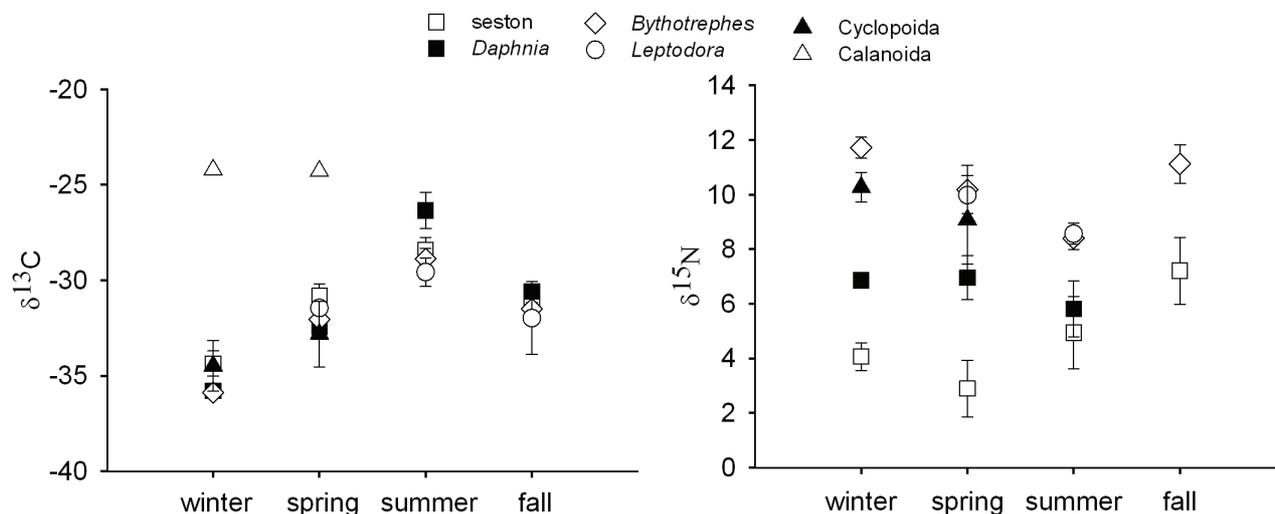


Fig. 2. Seasonal changes in carbon and nitrogen isotopic signatures of pelagic zooplankton taxa, and seston, in Lake Maggiore, 2008. For further explanation see text.

## 2.2. Statistical analyses

Statistical analyses, performed on Statistica for Windows (StatSoft 1994), included examination of potential correlations between seston and *Daphnia*  $\delta^{13}\text{C}$  signature, between the latter and water temperature (mean of 0–50 m) and between various zooplankton taxa and the pelagic baseline signatures. Pearson's product-moment  $r$  or  $R$ -Spearman Rank correlation coefficients were determined, depending on the results of the Levine test for homogeneity of variance. Slope coefficient values were compared to 1 by a  $t$ -test. Residual variability values, obtained from the corresponding ANOVA model, were compared to the variability predicted from the trophic fractionation of  $^{13}\text{C}$  and  $^{15}\text{N}$  (Standard Deviation = 1.3 and 1.0‰) by  $F$  tests (Matthews & Mazumder 2003). Friedman ANOVA was used to test the statistical significance of differences among stations, water depths and seasons of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  signatures and of C:N ratio.

## 3. RESULTS

A total of 23 samples of seston and 54 of zooplankton were analyzed for their  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  isotopic signatures. Among-stations differences in seston and zooplankton taxa signatures were not statistically significant ( $p < 0.05$ ) (Friedman ANOVA test). Similarly, the three stations did not differ with regard to the C:N ratios of their different components. Data from the different stations regarding both seston and the different zooplankton taxa therefore were pooled together by date. Also the differences between epilimnetic and hypolimnetic seston signatures were not statistically significant at  $p < 0.05$  (Friedman ANOVA test;  $\chi^2$  ( $N = 11$ ,  $df = 1$ ) = 0.82;  $p < 0.37$ ). In the epilimnion, seasonal changes of  $\delta^{13}\text{C}$  sestonic signatures were significant ( $\chi^2$  ( $N = 5$ ,  $df = 4$ ) = 12.73;  $p < 0.013$ ), as a consequence of significant

differences between winter and summer signatures ( $p = 0.028$ ; multiple comparisons  $p$  values (2-tailed)). They were non-significant at  $p < 0.05$  however for hypolimnetic  $\delta^{13}\text{C}$  seston.

Overall differences between epilimnion and hypolimnion  $\delta^{15}\text{N}$  signatures did not differ statistically (Friedman ANOVA test;  $\chi^2$  ( $N = 4$ ,  $df = 5$ ) = 10.68;  $p < 0.058$ ). Changes with the season of pooled data also were non-significant at  $p < 0.05$  ( $\chi^2$  ( $N = 4$ ,  $df = 3$ ) = 5.1;  $p < 0.16$ ). Multiple comparisons'  $p$  values (2-tailed), however, revealed that differences were statistically significant ( $p = 0.04$ ) for spring *versus* autumn values.

### 3.1. Pelagic isotopic signatures

#### 3.1.1. Carbon

*Daphnia's*  $\delta^{13}\text{C}$  signature in the different seasons was closely correlated with the signature of seston ( $r = 0.86$ ;  $p < 0.01$ ;  $N = 13$ ), thus confirming that *Daphnia* is an appropriate proxy for the pelagic baseline against which the carbon isotopic signals of other zooplankton can be compared. *Daphnia*  $\delta^{13}\text{C}$  proved to be least negative in summer (-26.35‰) and most negative in winter (-35.78‰), with intermediate and similar values in spring and fall (Fig. 2).

The pelagic carbon isotopic signatures of both seston and *Daphnia* were strongly correlated with the mean temperature of the water layer from which organisms were collected (Pearson's  $r = 0.74$ ;  $p = 0.01$ ;  $N = 12$ ). Carbon signatures of the other planktonic Cladocera taxa were closely related with those of *Daphnia*, particularly in winter and spring. This relationship was less strong in summer, when *Daphnia's*  $\delta^{13}\text{C}$  was slightly less negative than those of the other taxa.

The correlation between *Daphnia* and *Bythotrephes*  $\delta^{13}\text{C}$  values was statistically highly significant (Spearman rank correlation  $r = 0.886$ ;  $p < 0.001$ ;  $N = 18$ ). Also cyclopoid copepod  $\delta^{13}\text{C}$  was tightly correlated with

**Tab. 1.**  $\delta^{15}\text{N}$  enrichment (E) and trophic position (T) with respect to seston, pooled data, and to *Daphnia* (in parenthesis) baseline of zooplankton consumers. For further explanation see text.

	Winter		Spring		Summer		Fall	
	E	T	E	T	E	T	E	T
<i>Daphnia</i>	2.8	2.2	5.04	3.2	2.7	2.2		
<i>Cyclops</i>	6.2 (3.4)	3.7 (3.5)	7.2 (2.1)	4.1 (2.9)				
<i>Bythotrephes</i>	7.7 (4.9)	4.3 (4.1)	8.3 (3.2)	4.6 (3.4)	5.2 (2.6)	3.3 (3.1)	3.9	2.7
<i>Leptodora</i>			8.1 (3.0)	4.5 (3.3)	5.4 (2.8)	3.2		

*Daphnia*'s signature, and followed the same seasonal pattern. In this case, however, data were too few to allow for testing statistical significance, as cyclopoids could be sampled only in winter and spring, because of their low abundance in summer and fall.

Different results were obtained for Calanoida whose signatures could be analyzed for the same reason only in winter and spring.  $\delta^{13}\text{C}$  values for these organisms were less negative ( $-24.22 \pm 0.05$  and  $-24.29 \pm 0.06$ ) than those of *Daphnia* ( $-35.78 \pm 0.06\text{‰}$  and  $32.42 \pm 0.5\text{‰}$ ) in winter and spring, respectively. The littoral carbon isotopic signature (obtained from periphyton and amphipods collected along the shoreline) averaged of  $-24.41 \pm 0.028\text{‰}$ . In this case, among-station variability was significant (Manca *et al.* 2009).

### 3.1.2. Nitrogen

The  $\delta^{15}\text{N}$  signature of seston was highest in winter and fall, and lowest in spring (Fig. 2). Values measured in the epilimnion were slightly lower (from 3.23 to 6.03  $\delta^{15}\text{N}\text{‰}$ ) than those measured in hypolimnion samples (ranging between 4.06 and 6.93  $\delta^{15}\text{N}\text{‰}$ ). *Daphnia* nitrogen isotopic signature was quite consistently 6.9  $\delta^{15}\text{N}\text{‰}$  in winter and spring, but declined in summer to 5.8  $\delta^{15}\text{N}\text{‰}$ . Unfortunately, we were unable to measure the fall signature for *Daphnia* due to low sample biomass. The other two zooplanktonic cladoceran taxa, namely *Leptodora* and *Bythotrephes*, however, showed a clear trend toward a decrease in nitrogen isotopic signature from a winter maximum to a summer minimum. Only *Bythotrephes* could be analyzed in fall, when the nitrogen signature appeared to approach that measured in winter. When present, i.e. in spring and summer, *Leptodora* fully overlapped with *Bythotrephes*, with regard to  $\delta^{15}\text{N}$  values; for both summer values were lower than spring  $\delta^{15}\text{N}$  values (8.6 and 9.98 $\text{‰}$ , respectively). Cyclopoid copepods (their carbon isotopic signatures measurable only in winter and spring) occupied an intermediate position in the  $\delta^{15}\text{N}\text{‰}$  plot between predatory cladocerans and *Daphnia* (Fig. 2).

### 3.1.3. $\delta^{15}\text{N}$ enrichment and trophic position of zooplankton taxa

*Daphnia*  $\delta^{15}\text{N}$  enrichment with respect to seston was close to 3 in winter and summer (E = 2.8 and 2.7 respectively; Tab. 1). Enrichment of *Bythotrephes* with respect to *Daphnia* was highest in winter (4.9), intermediate in spring (3.2) and lower in summer (2.6).

Cyclopoid copepod  $\delta^{15}\text{N}$  was closer to the *Daphnia* than the predatory cladoceran signatures, with its  $\delta^{15}\text{N}$  enrichment ranging between 3.4 in winter to 2.1 in spring, relative to the baseline values of *Daphnia*.

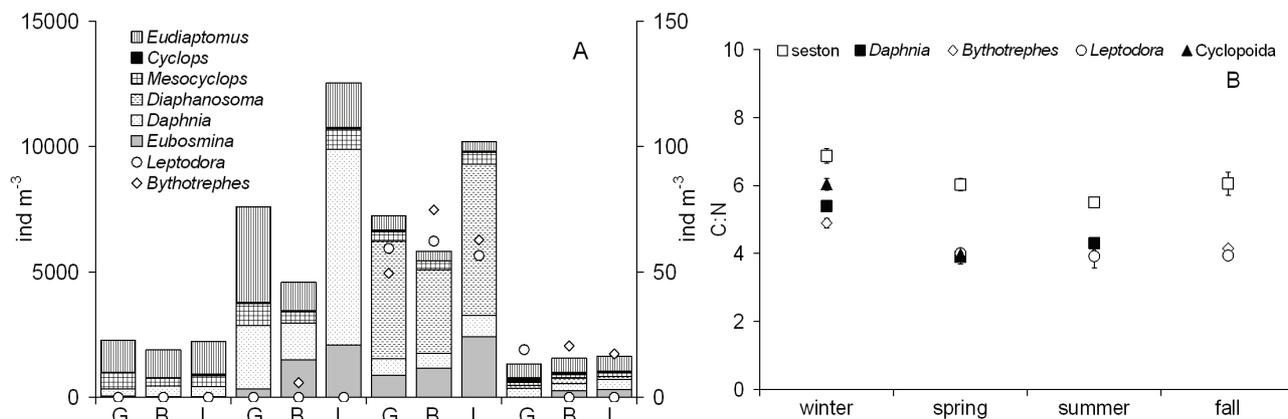
We do not have  $\delta^{15}\text{N}$  data for *Daphnia* in fall; thus, we cannot reference the  $\delta^{15}\text{N}$  values of other zooplankton taxa to it to determine the degree of isotopic enrichment. During this season, *Bythotrephes*  $\delta^{15}\text{N}$  was enriched by 3.9 relative to the value for seston; *Leptodora* also had  $\delta^{15}\text{N}$  values enriched relative to seston, the range of enrichment falling between 3.0 in spring and 2.8 in summer.

In winter, the trophic positions of the zooplanktonic taxa were in increasing order: *Daphnia*, cyclopoid copepods, and *Bythotrephes* (Tab. 1). By contrast, in spring, *Daphnia* and the cyclopoids were at the same, lower trophic level, and *Bythotrephes* and *Leptodora* in an upper one. Also in summer, *Bythotrephes* and *Leptodora* occupied the same trophic level, higher than *Daphnia*.

### 3.2. Seasonal changes in zooplankton population density and in the C:N ratio

Zooplankton population density levels were low and very similar in the different stations (G = Ghiffa, B = Baveno; L = Lesa) in winter (Fig. 3A). Diaptomids (*Eudiaptomus padanus* and *E. gracilis*) were largely dominant, followed by small cyclopoids (e.g., the species *Mesocyclops leuckarti*). The spring increase was mainly related to an increase in the population density of *Daphnia*, particularly abundant in Lesa, and to *Eudiaptomus* in Ghiffa (cfr. Fig.1). At all the sampling stations, *Diaphanosoma brachyurum* replaced *Daphnia* in summer although at generally lower densities, with slightly higher levels at Lesa than at the other two stations. In the former, *Eubosmina longispina* was also slightly more abundant than at the other two stations in spring and summer. *Bythotrephes* and *Leptodora* had similar densities at the different stations. *Leptodora* showed a modest peak in summer, while *Bythotrephes* was present in non-negligible numbers from spring into fall. *Bythotrephes* specimens collected with the large-mouth zooplankton net were abundant enough to allow for preparation of reliable SIA samples in winter as well.

Seasonal changes in C:N ratio were apparent in both epilimnetic and hypolimnetic sestonic samples; values were highest in winter, intermediate in spring and fall, and lowest in summer (Fig. 3B). Values of the two



**Fig. 3.** Seasonal changes in population density (A) and in C:N ratio (B) of seston and of zooplankton taxa from Lake Maggiore, 2008. For further explanation see text.

water layers fully overlapped in winter, but were clearly distinguished in summer, when a lower value (5.2) was found in the hypolimnion than in the epilimnion (5.8) (Manca *et al.* 2009). C:N values of the two water layers were still different in fall, when in the hypolimnion the ratio increased to approach the value recorded in spring. Zooplankton taxa showed a similar decrease between winter and summer. All had a C:N ratio of *ca* 4 from spring-fall (Fig. 3B), whereas in winter more variable ratios ranging from 6 for cyclopoids to 4.9 for *Bythotrephes* were measured. The value for *Daphnia*, 5.4, was intermediate. *Leptodora's* C:N ratio fully overlapped the range for *Bythotrephes* in summer, but was lower in winter.

#### 4. DISCUSSION

In deep lakes, carbon isotopic signature is strongly influenced by depth, with organisms living in deeper layers characterized by more negative values than those living closer to the surface and/or in the littoral zone (Vander Zanden & Rasmussen 1999; Grey *et al.* 2000; Cattaneo *et al.* 2004).

Lago Maggiore's carbon pelagic baseline and its changes along the season were quite close to those measured in Lake Geneva, with values ranging between *ca* -34‰ in winter and -26‰ in summer (Perga & Gerdeaux 2006). Such results indicate the importance of lake typology in determining the relative importance of allochthonous and autochthonous carbon sources (Vander Zanden *et al.* 1999; Post *et al.* 2000; Perga & Gerdeaux 2006). Nitrogen baseline signature was generally lower in Lago Maggiore than in Lake Geneva (5 and 14‰; Perga & Gerdeaux 2006), an expected outcome due to the more oligotrophic nature of Lago Maggiore (mean TP concentration 8 *versus* 35  $\mu\text{g L}^{-1}$  in Geneva; Perga & Gerdeaux 2006; Rogora *et al.* 2009). The nitrogen signature of the pelagic baseline tends to increase with increasing trophicity (Vander Zanden & Rasmussen 1999; Grey *et al.* 2000; Grey & Jones 2001; Cattaneo *et al.* 2004).

Overall, differences among the three stations in sestonic  $\delta^{13}\text{C}$  signature were statistically insignificant, suggesting that the impact of allochthonous material from the River Toce, entering near the station "Baveno" (de Bernardi *et al.* 1988) is only exceptionally important (i.e., after heavy rainfall events). Our interpretation is consistent with the fact that in the epilimnion of Lago Maggiore, chlorophyll-*a* concentration is significantly correlated with sestonic carbon concentration (Bertoni *et al.* 2004). The seasonal changes we observed are consistent with changes in carbon uptake and fractionation related to phytoplankton cell geometry, size and taxa (Popp *et al.* 1998; Caroni *et al.* 2011). The among-stations close similarity of seston carbon isotopic signatures may therefore reflect a substantial homogeneity in phytoplankton taxa composition (Caroni *et al.* 2011).

The strong correlation between pelagic carbon isotopic signature and mean water temperature (0-50 m depth) found in Lago Maggiore was observed also in Lake Geneva. In the latter lake, mean epilimnetic water temperature explained up to 80% of the measured variation in *Daphnia* carbon isotopic signature. The increase of the pelagic  $\delta^{13}\text{C}$  baseline probably reflects changes in phytoplankton carbon isotopic signature (Zohary *et al.* 1994) consequent to changes in the availability of carbon sources with season, and the degree of fractionation during the uptake of dissolved inorganic carbon (DIC) for photosynthesis. Plankton collected during periods of high biomass and primary productivity tends to exhibit  $^{13}\text{C}$ -enrichment (Degens *et al.* 1968; Fry & Wainright 1991; Zohary *et al.* 1994; France *et al.* 1997) and thus heaviest (i.e., less negative)  $\delta^{13}\text{C}$  signatures, due to reduced isotopic fractionation at high cell densities or growth rates, or to a switch to use of  $\text{HCO}_3^-$  when  $\text{CO}_2$  is depleted (France *et al.* 1997; Leggett *et al.* 1999, 2000). Lighter (i.e., more negative)  $\delta^{13}\text{C}$  signatures are again typically observed in the fall with gradual thermal de-stratification and resulting inputs of dissolved carbon from the hypolimnion, and lead to a seasonal minimum during winter mixing (Zohary *et al.* 1994). In both lakes, phytoplankton accounts for the

bulk of seston in the epilimnion, and peaks in spring (Morabito *et al.* 2009).

The good correspondence between the pelagic baseline and most zooplankton taxa indicates that the latter mainly rely upon pelagic carbon sources. As observed in Lake Geneva, the correlation between *Daphnia* and *Bythotrephes* carbon signatures was statistically significant. The only exception was for *Eudiaptomus*, which seems to rely mainly on littoral food source; its  $\delta^{13}\text{C}$  signature overlapped fully the average values measured for periphyton and shoreline amphipods. Our result is in agreement with previous observations of diaptomids feeding behaviour in other lakes (Grey & Jones 2001). Horizontal migrations from littoral to pelagic waters may be responsible for these organisms acting as carriers of littoral carbon into Lago Maggiore's pelagic zone. A littoral-like carbon isotopic signature in pelagic organisms, however, might also result from a high degree of infestation by algal epibionts (namely of the genus *Characium*; Manca *et al.* 1995), we observed in inspected animals, as commonly found in Lago Maggiore since the early 90s; Manca, *ibidem*). Although never investigated, these algal epibionts may carry a littoral-like carbon signature.

Although a major part of Lago Maggiore's open-water zooplankton taxa were analyzed, our initial impression of the food web led to overlooking the signatures of *Diaphanosoma brachyurum* (Liévin 1848) and *Eubosmina longispina* (Leydig 1860), which proved to be more important than anticipated. The former in fact replaced *Daphnia* during the summer of this study. The latter, although present in spring and summer at relatively low levels of abundance, may have been an important food source for invertebrate predators, as observed in other deep lakes with non-SIA techniques (Branstrator & Lehman 1991). The two zooplankters provide a good occasion for investigating the level of taxa redundancy by addressing functional (i.e. ecological) roles of the zooplankton taxa composing Lago Maggiore's pelagic zooplankton. Such an estimate is essential for a sustainable management and an assessment of the impact of alien species invasions (Manca *et al.* 2007; Riccardi & Rossetti 2007; Moss *et al.* 2009; Visconti *et al.* 2010). *Diaphanosoma* and *Eubosmina*, however, were included in a more detailed study in 2009 whose results are now in press. On the other hand, *Bythotrephes*, although present at a low density in the fixed samples, was found in sufficient numbers to allow for preparation of SIA samples in winter. Not only a larger filtered water volume (13 m<sup>3</sup> with respect to 1 m<sup>3</sup>) but also a higher capture efficiency of nets with a large opening mouth could explain the difference between the two (live and fixed) samples (de Bernardi 1984).

As expected, the C:N ratios of zooplankton taxa mirrored seasonal changes in  $\delta^{13}\text{C}$  (Matthews & Mazumder 2005), and resulted from changes in both food (seston) quality and rates of population growth. Seasonal

changes and enrichment in  $\delta^{15}\text{N}$  along the food chain allowed us to trace the trophic position (T) of the different zooplankton taxa. In winter, three levels were identified, with *Daphnia* at the bottom, cyclopoid copepods in between, and the predatory cladoceran *Bythotrephes longimanus* at the top. According to  $\delta^{15}\text{N}$  enrichment, during this season, *Bythotrephes* was able to prey on cyclopoid copepods, and thus played an ecological role much like that zooplanktivorous fish (Pangle *et al.* 2007). In spring, on the same, upper level as *Daphnia* we found the cyclopoid copepods, and *Bythotrephes* and *Leptodora*.

Unlike for the other zooplankton taxa, the carbon signature of diaptomids fully mirrored those of littoral organisms. Unfortunately, we missed obtaining the  $\delta^{15}\text{N}$  signature of diaptomids due to the low dry weight of our samples. As for other zooplankton, their responses will be assessed during description of our more detailed 2009.

In summer, according to their  $\delta^{15}\text{N}$  enrichment, the two predatory cladocerans, *Leptodora* and *Bythotrephes*, occupy the same upper trophic level relative to *Daphnia*. Unfortunately, we did not have *Daphnia* nitrogen isotopic signature in fall. However, given the very good correlation with seston signature, we calculated a seston-based  $\delta^{15}\text{N}$  enrichment, and a trophic coefficient for it. *Bythotrephes* seems to play a comparable role with some planktivorous fishes (data not shown), all of them being, during this time of the year, related to the pelagic environment. These results are in agreement also with literature information on Lago Maggiore that suggests that *Bythotrephes* may be compared to young zooplanktivorous fish regarding its predation ability, especially when large in body size (Manca *et al.* 2000; Manca *et al.* 2008; Manca 2011). Studies on *Bythotrephes* from invaded sites also suggest that this voracious visual predator can compete with young zooplanktivorous fish for prey (Pangle *et al.* 2007; Manca 2011; Dimitru *et al.* 2001; Hoffman *et al.* 2001). This result further emphasizes the similarity between the consequences of *Bythotrephes* exponential increase in Lago Maggiore and those observed in invaded lakes (Manca & Ruggiu 1998; Manca & DeMott 2009; Manca 2011).

## 5. CONCLUSIONS

The functional (ecological) roles of aquatic organisms such as zooplankton are increasingly being investigated (e.g., Obertegger & Manca 2011). Seasonal changes are of a particular interest in aquatic environments, particularly in those where predatory cladocerans may represent an important step in the food web. Most studies have been directed towards pelagic food webs of large lakes, assuming that the littoral was of little importance for open water zooplankton. We found a close similarity in the isotopic fingerprints of organisms collected from different pelagic stations, which were supposed to be differently influenced by the littoral zone

and/or allochthonous inputs of organic matter from the River Toce. Instead, we observed an unexpected contribution of littoral carbon to the open water food web and diatoms (*E. padanus* and *E. gracilis*) seemed to act as carriers of littoral carbon into Lago Maggiore's pelagia.

During winter and fall, predatory zooplankton is able to play a functional (trophic) role comparable to that of truly zooplanktivorous fish. In particular, the ability of *Bythotrephes longimanus* to prey on *Cyclops* in winter confirms that this predatory cladoceran may compete with young zooplanktivorous fish for food resources, as observed in lakes it recently invaded (Manca 2011).

Further research now in progress will allow inclusion of taxa that could not be analyzed in the present study. By identifying the level of redundancy and the degree to which various taxa are found, we will be able to define functional diversity and redundancy, information needed for an ecosystem-sounded management.

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