Seasonal changes in the $\delta^{13}$C and $\delta^{15}$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 µm. 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 $\delta^{13}$C and $\delta^{15}$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 $\delta^{13}$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

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 of 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
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 km²; volume, 37.5 km³) 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⁻¹), 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⁻¹). 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 13 m³ of filtered lake water with a large (opening mouth diameter, 58 cm) 126-µm mesh plankton net. Zooplankters were kept in filtered water for gut clearance overnight before sorting.

126-µm mesh plankton net. Zooplankters were kept in water with a large (opening mouth diameter, 58 cm) Burckhardt + Eudiaptomus padanus (Focke; Haplopora) were analyzed. Calanoid (Onychopoda) and cyclopoid (Meso- cyclops 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 µm 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 51 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 microscopic analyses, and the remainder of the samples 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-µm 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 thermistor, 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 (Cannero: 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 δ¹³C, δ¹⁵N, 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 CO₂ and N₂. The gases were separated by a "purge and trap" adsorption column and sent to an IRMS interface, then to the IRMS.

Internal standards for δ¹⁵N and δ¹³C 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 (δ), the units being per mil (%o) and defined as δ = [(Rsample/Rstd)]x1000 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 δ¹⁵N values were reported as %o vs air and normalized to international standards calibrated to international standards IAEA-N1 (+0.4‰), IAEA-N2 (+20.3‰), USGS-40 (-4.52‰) and USGS-41 (47.57‰). All δ¹³C were reported as %o vs V-PDB (Pee Dee Bel- emmite) 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 ¹³C and ¹⁵N of seston, zooplankton, fish and littoral samples, respectively.

We calculated δ¹³C fractionation (F = δ¹³Cconsumers - δ¹³Cbaseline) of different organisms with respect to both seston and Daphnia isotopic signature, taking 0.8 (SD ± 1.1‰, according to de Niro & Epstein (1978) as a threshold value for non-pelagic carbon sources. We also calculated δ¹⁵N enrichment (E = δ¹⁵Nconsumers - δ¹⁵Nbaseline) along the food chain.

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

\[ T_{seston} = \lambda + \frac{[\delta^{15}N_{consumer} - \delta^{15}N_{baseline}]}{2.3} \]  

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:

\[ T_{consumer} = 2 + \frac{[\delta^{15}N_{seston\ consumer} - \delta^{15}N_{prim\ consumer}]}{2.3} \]  \( 2 \)
2.2. Statistical analyses

Statistical analyses, performed on Statistica for Windows (StatSoft 1994), included examination of potential correlations between seston and Daphnia δ¹³C 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 ¹³C and ¹⁵N (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 δ¹³C and δ¹⁵N signatures and of C:N ratio.

3. RESULTS

A total of 23 samples of seston and 54 of zooplankton were analyzed for their δ¹³C and δ¹⁵N 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 epilimnion and hypolimnion δ¹³C values 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 δ¹³C 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 δ¹³C was slightly less negative than those of the other taxa.

The correlation between Daphnia and Bythotrephes δ¹³C values was statistically highly significant (Spearman rank correlation r = 0.86; p <0.001; N = 18). Also cyclopoid copepod δ¹³C was tightly correlated with
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 Calanoidea whose signatures could be analyzed for the same reason only in winter and spring. δ^{13}C values for these organisms were less negative (-24.22 ± 0.05 and -24.29 ± 0.06) than those of Daphnia (-35.78 ± 0.06‰ and 32.42 ± 0.5‰) in winter and spring, respectively. The littoral carbon isotopic signature (obtained from periphyton and amphipods collected along the shoreline) averaged of -24.41 ± 0.02‰. In this case, among-station variability was significant (Manca et al. 2009).

3.1.2. Nitrogen

The δ^{15}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 δ^{15}N‰) than those measured in hypolimnion samples (ranging between 4.06 and 6.93 δ^{15}N‰). Daphnia nitrogen isotopic signature was quite consistently 6.9 δ^{15}N‰ in winter and spring, but declined in summer to 5.8 δ^{15}N‰. 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 δ^{15}N values; for both summer values were lower than spring δ^{15}N values (8.6 and 9.98‰, respectively). Cyclopoid copepods (their carbon isotopic signatures measurable only in winter and spring) occupied an intermediate position in the δ^{15}N‰ plot between predatory cladocerans and Daphnia (Fig. 2).

3.1.3. δ^{15}N enrichment and trophic position of zooplankton taxa

Daphnia δ^{15}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 δ^{15}N was closer to the Daphnia than the predatory cladoceran signatures, with its δ^{15}N enrichment ranging between 3.4 in winter to 2.1 in spring, relative to the baseline values of Daphnia.

We do not have δ^{15}N data for Daphnia in fall; thus, we cannot reference the δ^{15}N values of other zooplankton taxa to it to determine the degree of isotopic enrichment. During this season, Bythotrephes δ^{15}N was enriched by 3.9 relative to the value for seston; Leptodora also had δ^{15}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

### Tab. 1. δ^{15}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.

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<td>Cyclops</td>
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<td>Bythotrephes</td>
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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.

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 -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 µg L\(^{-1}\) in Geneva; Perga & Gerdeaux 2006; Rogora et al. 2009). The nitrogen signature of the pelagic baseline tends to increase with increasing trophic state (Vander Zanden & Rasmussen 1999; Grey et al. 2000; Grey & Jones 2001; Cattaneo et al. 2004).

Overall, differences among the three stations in sestonic \(\delta^{13}C\) 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. 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 \(\delta^{13}C\) 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 \(^{13}C\)-enrichment (Degens et al. 1968; Fry & Wainright 1991; Zohary et al. 1994; France et al. 1997) and thus heaviest (i.e., less negative) \(\delta^{13}C\) signatures, due to reduced isotopic fractionation at high cell densities or growth rates, or to a switch to use of HCO\(_3^-\) when CO\(_2\) is depleted (France et al. 1997; Leggett et al. 1999, 2000). Lighter (i.e., more negative) \(\delta^{13}C\) 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 δ13C signature overlapped fully the average values measured for periphyton and shoreline amphipods. Our result is in agreement with previous observations of diatomoids 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 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 zooplanktons 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 m3 with respect to 1 m3) 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 δ13C (Matthews & Mazumder 2005), and resulted from changes in both food (seston) quality and rates of population growth. Seasonal changes and enrichment in δ15N 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 δ15N 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 diatomoids fully mirrored those of littoral organisms. Unfortunately, we missed obtaining the δ15N signature of diatomids 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 δ15N 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 δ15N 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 diatomids (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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