Phytoplankton functional traits and seston stable isotopes signature: a functional-based approach in a deep, subalpine lake, Lake Maggiore (N. Italy)

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ABSTRACT

The seasonal variation of seston stable isotopes signature of carbon ($\delta^{13}C$) in Lake Maggiore during 2008 was related to seasonal variation of the lake phytoplankton community, investigated in terms of phytoplankton taxonomic groups, morpho-functional groups (MBFG), cell size classes and cell shape classes. Three open water stations were selected to reflect truly pelagic, influenced by littoral and riverine carbon sources; phytoplankton samples were collected from two water depths, 0-20 m and 25-50 m. Among stations differences in $\delta^{13}C$ signatures of seston were statistically non significant, confirming that allochthonous input may become important only after exceptional rainfall events. Nonparametric multiplicative regression (NPMR) was utilized to identify among the phytoplankton parameters (taxonomic groups, MBFG, cell shapes, cell sizes) which were the best predictors of the $\delta^{13}C$ variation. Bacillariophyceae and two morpho-functional groups, Group 6 (non-flagellated organisms with siliceous exoskeletons) and Group 3 (large filamentous algae with aerotopes) were statistically significant. Non-metric multidimensional scaling (NMS) ordination was used to investigate differences in phytoplankton samples and their relationship with $\delta^{13}C$ variation. When morphological and functional phytoplankton traits were superimposed, the NMS ordination showed that cylinder cells (for cell shape classes), class 500-2000 μm^3 (for cell size classes) and Group 6 (for MBFG) were the most related to $\delta^{13}C$ variation. Our study confirms that the variation in the pelagic $\delta^{13}C$ seston most probably reflects changes in phytoplankton carbon isotopic signature, consequent to changes in availability of carbon sources depending on the season and due to different isotopic fractionation of phytoplankton taxonomic groups. Statistical investigations have allowed us to investigate the potential role of phytoplankton morphological and functional traits in the seasonal variation of $\delta^{13}C$ and to suggest their importance in the interpretation of natural stable isotopes studies in lake ecosystems.

Key words: stable isotopes, seston, carbon, phytoplankton, functional analysis.

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INTRODUCTION

The use of natural stable isotopes signatures of carbon ($\delta^{13}C$) has become an important tool to study food web relationships and potential sources of organic matter in aquatic ecosystems. Carbon stable isotope signatures in animals are known to reflect quite closely those of their diet, whereas nitrogen stable isotopes signatures in animals often show considerable enrichment compared to their diet (Peterson, and Fry 1987; Michener, and Schell 1994). These basic principles can provide much information about feeding relationships and carbon sources and have been used extensively in lake food web studies.

Phytoplankton represents the autotrophic, primary producer at the base of the aquatic food web, it is therefore fundamental to identify its isotopic signal when performing a study of the whole-lake food web through stable isotopes.

Several variables can influence phytoplankton $\delta^{13}C$ in a lake ecosystem. Primary determinants are the isotopic signatures of assimilated inorganic carbon and phytoplankton isotopic fractionation during assimilation. Phy-

toplankton $\delta^{13}C$ can be affected by the taxonomic composition of the phytoplankton communities, because different species fractionate $\delta^{13}C$ differently (Falkowski 1991, Vuorio et al. 2006).

Seasonal changes in phytoplankton $\delta^{13}C$ generally occur in lakes (Zohary et al. 1994, Gu, and Schelske 1996, Lehmann et al. 2004). These variations have been related to changes in external loadings, phytoplankton species composition, primary productivity and concentration of dissolved inorganic C and N (DIC and DIN) (Rau et al. 1989, Grey et al. 2001). Differentiated utilization of bicarbonate (HCO_3^-) by some phytoplankton species may also contribute to the seasonal $\delta^{13}C$ variation (Takahashi et al. 1990). Physical variables, such as temperature, are also important determinants of $\delta^{13}C$ seasonal changes (Rau et al. 1989). Temperature controls the solubility of CO_2 and in turn influences the fractionation of carbon isotopes during phytoplankton uptake (Rau et al. 1989, 1992). Water column thermal stratification influences cell activity, nutrients assimilation and competition, light com-

petition, rate of CO_2 and HCO_3^- diffusion and ultimately phytoplankton species succession, abundance and community structure. Thus water stratification has, in the end, a strong effect on the phytoplankton $\delta^{13}C$ (Zohary et al. 1994).

While it is established that phytoplankton taxonomic composition can influence phytoplankton $\delta^{13}C$, less recognized and documented is the potential role of cell size, shape and functional traits for phytoplankton $\delta^{13}C$ in natural communities. Several models for marine microalgae have demonstrated that species-specific parameters such as CO_2 permeability, enzymatic fractionation and cell surface area to volume ratio play a significant role in the variation of plankton $\delta^{13}C$ (Rau et al. 1996; Popp et al. 1998; Burkhardt 1999). Studies on marine phytoplankton cultures suggested that cell size and cell geometry can significantly influence phytoplankton carbon isotopic fractionation (Goericke et al. 1994; Popp et al. 1998). Phytoplankton cell size is a key feature in the ecological relationships, being related to the efficiency of many ecophysiological processes (nutrient assimilation, photosynthetic efficiency, respiration, buoyancy), most of which are affected by trophic changes (Litchman et al. 2010).

Phytoplankton trait-based approaches are being increasingly used in aquatic ecology (*e.g.*, Reynolds et al. 2002; Salmaso, and Padisak 2007; Litchman, and Klausmeier 2008; Kruk et al. 2010). Classifications based on morphological and functional proprieties generally group species with common features, indicating that algae belonging to a specific group have common morphological characteristics and are powerful predictors of optimum dynamic performance (Reynolds, and Irish 1997). In fact, species-specific morphological characteristics are related to ecological strategies of different phytoplankton species.

Phytoplankton morphological traits are relatively simple to measure and their relation to physiology is potentially well established. Specific growth rates, resource utilization, light competition, grazer resistance all depend on organism dimensions, volume and shape. A tight link between morphology and autecology of species is also suggested by the fact that distribution in time and space is linked to phytoplankton morphometry (Kruk et al. 2010).

The present study examines the temporal variation of the phytoplankton community and seston (1.2 to≤76 μm) $\delta^{13}C$ in Lake Maggiore during 2008. Previous studies report that POC and seston in this lake are mainly of autochthonous origin, and that the former is significantly correlated with chlorophyll a (r=0.651, p<0.001, d.f.=134; Bertoni, and Callieri 1993). This study was part of a wider project investigating aquatic food web relationships in Lake Maggiore through the use of stable isotopes analysis (SIA) of carbon and nitrogen. Part of the research addressed the issue of whether seasonality affected 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 (Visconti, and Manca 2011). Because phytoplankton is a key link in lacustrine trophic webs and in specific they represent a source of food for grazing zoo-plankton, we have examined the nature and thus the composition of the phytoplankton community of the lake. Furthermore, along with taxonomic species and group classification, we have explored the potential use of phytoplankton cell size, cell shape and morpho-functional groups for investigating whether they can be related to carbon isotopic variation in the lake.

MATERIALS AND METHODS

Study site and SIA methods

Lake Maggiore (45°57'30"N; 8°32'E) lying at an altitude of 193.30 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 mid eighties (total phosphorus concentration at the winter mixing *ca* 10 μg L⁻¹), after eutrophication in the 1960s and the late 1970s.

For almost 30 years, a regular monitoring of the lake has been carried out in an open-water station in the deepest part of the lake basin (station Ghiffa, latitude 45°58'30"N; longitude 8°39'09"E, depth 370 m), with at least monthly frequency. In 2008, two additional sites were sampled near the Toce River inflow (station Baveno: latitude 45°54'28"N; longitude 8°31'44"E) and in a shallow basin near the lake littoral (station Lesa: latitude 45°49'70"N; longitude 8°34'70"E, at the southern end of the lake (Fig. 1).

On each sampling date, 0-20 m (epilimnion) and 25-50 m (hypolimnion) integrated water samples were collected with a 5 L Niskin bottle, filtered through a 76 μm nylon filter. From each, a sub-sample fixed in Lugol acetic solution was taken for phytoplankton abundance and biovolume estimates. Chlorophyll-*a* was also measured on each date (Lorenzen 1967). In the lab, water samples were filtered through GF/C fiber glass filters, to obtain seston material (1.2-76 μm).

The water temperature vertical profile and the Secchi depth water transparency were also measured on each date.

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). The $\delta^{13}C$ and percentage of carbon was determined. 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 Ad-

vantage, manufactured by Thermo, Germany). Samples and standards were flash combusted at about 1800°C (Dumas combustion) and the resulted gas products carried by helium through columns of oxidizing/reducing chemicals optimized for CO_2. The gases were separated by a "purge and trap" adsorption column and sent to IRMS interface, then to IRMS.

Internal standards were ($\delta^{13}C$ in ‰): C-51 Nicotinamide (0.07, 22.95), C-52 mix of 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 (‰) and defined as $\delta=[(R_x R_{std})/R_{std}]\times1000$ 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 $\delta^{13}C$ 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 deviation for $\delta^{13}C$ was 0.05.

Laboratory methods for phytoplankton

The phytoplankton classification and counts were performed on samples fixed by acetic Lugol's solution following the Utermöhl method (Utermöhl 1958; Lund et al. 1958; Rott 1981). The phytoplankton analysis was done under an inverted microscope; small species were enumerated under 400× magnification while bigger and colonial species under a lower 200× magnification. Cell biovolumes were calculated from recorded abundance and specific biovolumes approximated to simple geometrical solids (Rott 1981). Taxa have been classified according to phytoplankton keys edited by A. Pasher- Suβwasserflora von Mitteleuropa and by G. Huber-Pestalozzi-Das Phytoplankton des Süβwassers.

In this study the functional classification of phytoplankton species was carried out according to Kruk et al. (2010); the so-called "morphologically based functional classification" abbreviated to MBFC or morphologically based functional group (MBFG), listed in Fig. 4. Phytoplankton was classified in five size classes (minimum visible-100 μm^3, 100-500 μm^3, 500-2000 μm^3, 2000-5000 μm^3 and >5000 μm^3) according to species biovolume. Phytoplankton cell shape classification was carried out according to Hillebrand et al. (1999), listed in Fig. 4.

Statistical methods

Non-parametric multiplicative regression (NPMR) was used to model $\delta^{13}C$ using taxonomic groups, cell size, cell shape and morpho-functional groups. NPMR was ap-
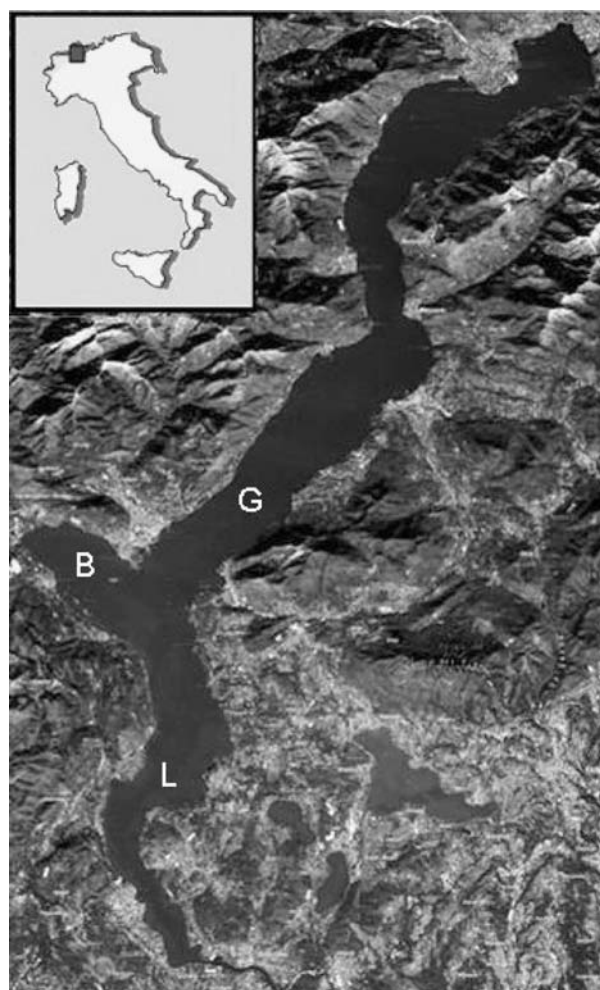

Fig. 1. Sampling stations from which phytoplankton samples were collected in Lake Maggiore in 2008 (G=Ghiffa, B=Baveno, L=Lesa).

plied using the software HyperNiche version 1.46 (McCune, and Mefford 2004). Non-metric multidimensional scaling (NMS) ordination was used to investigate differences among phytoplankton samples. NMS was applied using the software PC-ORD (McCune, and Mefford 2006).

RESULTS

Differences in seston $\delta^{13}C$ among the three monitored stations (Ghiffa, Baveno, Lesa) were not statistically significant (nonparametric Kruskal-Wallis test, n=35; P=0.075). Density and biovolume of phytoplankton in the three monitored stations were similar (Fig. 2), with density ranging between 550 and 9000 cells mL^{-1}, excluding the maximum value at Ghiffa in July with 21,900 cells mL^{-1}. Biovolume ranged between 77 (in autumn) and 1480 (in spring) mm^3 m^{-3}.

Differences of seston $\delta^{13}C$ in the two layers (depth of

0-20 m and depth of 25-50 m), in all three stations pooled together, were borderline significant (Wilcoxon test; P=0.059; n=24). The difference of phytoplankton total biovolume in the two layers (Fig. 2), in all three stations pooled together, was significant (P=0.0022; n=24). Phytoplankton biovolume differences were confirmed by chlorophyll a data, with results statistically different in the two depth layers (Wilcoxon test; P=0.008; n=18).

The seston δ^{13}C followed a seasonal trend (Fig. 3) with depleted values in winter and springtime and enriched values during the summer. Seasonal changes in δ^{13}C (pooled data from the epilimnion and hypolimnion) were statistically significant (n=23; F=5.67; P=0.006), with higher (less negative) δ^{13}C signatures in summer (-28.13; P=0.01) than in spring (-31.21; P=0.012) and winter (-30.74; P=0.038) (Scheffè *post-hoc* test).

The phytoplankton assemblage's structure changed seasonally (Fig. 2). In general, the phytoplankton community of the lake was dominated by Bacillariophyceae during late winter and spring, especially with large pennate species like *Asterionella formosa*, *Fragilaria crotonensis* and *Tabellaria flocculosa*, along with smaller

species like *Cyclotella* sp. During the summer months there was coexistence of Bacillariophyceae and Cyanobacteria, the latter group mainly represented by colonial species like *Aphanotheche clathrata*, *Snowella lacustris* and filamentous species like *Planktothrix agardii/rubescens* and *Geitlerinema* sp.

During the summer also the Crysophyceae were an important group with species like *Uroglena* sp. and *Chrisochromulina parva*. In the autumn the assemblage was dominated once again by Bacillariophyceae, mostly with *Asterionella formosa* and *Cyclotella* species. A complete taxonomic list and a detailed description are given in Manca et al. (2009).

In terms of density and biovolume there was, above all in the summer, a decrease of Bacillariophyceae and an increase of Cyanobacteria (Fig. 2). This was particularly evident in the epilimnion (0-20 m), and at the Ghiffa station where a small bloom of the cyanobacteria species *Aphanotheche clathrata* occurred in July.

The phytoplankton community was analyzed according to different parameters (as described in the Methods), namely cell shapes, cell sizes (biovolume classes) and
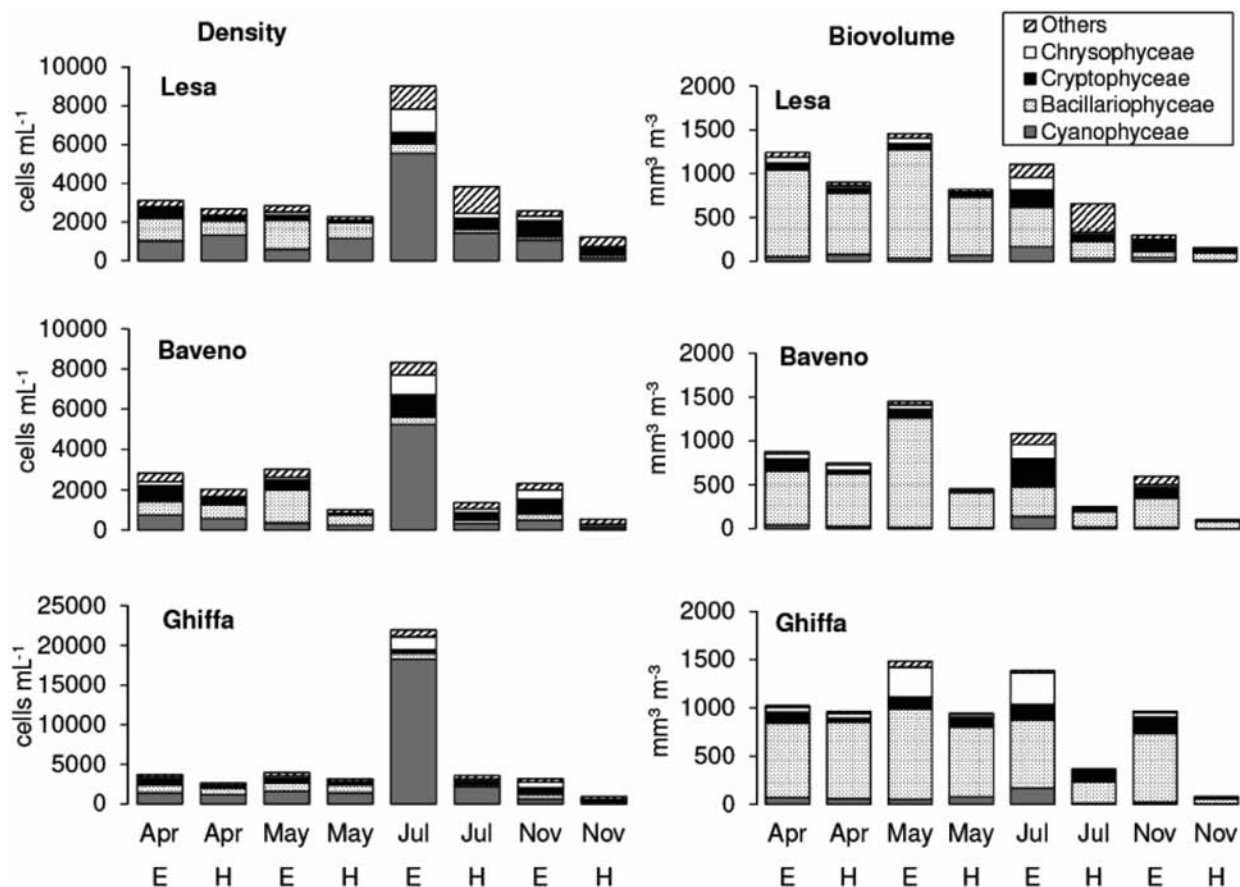

Fig. 2. Seasonal trend of phytoplankton δ^{13}C in the three stations (GHI: Ghiffa, BAV: Baveno, LES: Lesa). Data from the two layers were pooled.

morpho-functional or MBFG groups (Fig. 4).

According to cell shapes, the community was quite diversified in springtime; during the summer there was a relative increase in density and biovolume of phytoplankton species with prolate spheroid shape particularly in the epilimnion (0-20 m) and quite evident at the Ghiffa station, together with a general decrease of cylinder and box shaped cells in all the three stations.

According to sizes, the community was quite diversified in terms of biovolume with the 500-2000 μm^3 generally as the main size class (Fig. 4). In contrast, the dominant class in term of density in both epilimnion and hypolimnion (0-20 m and 25-50 m) was the smallest 0.1-100 μm^3, during the whole period and in all the three stations. In the summer there was a marked increase of such size class (0.1-100 μm^3) of small organisms, particularly in the epilimnion (data not shown).

According to morpho-functional groups the phytoplankton community, in terms of biovolume, in both layers was generally dominated by Group 6 (non-flagellated organisms with siliceous exoskeletons). In the summer the relative importance of this group to some extent decreased

(Fig. 4). In contrast, in terms of density, the most important MBFG groups were Group 6, Group 3 (large filamentous algae with aerotopes) and Group 1 (small organisms with high surface/volume ratio). However, in the summer Group 7 (large mucilaginous colonies) was the most numerous morpho-functional group in both layers (0-20 m
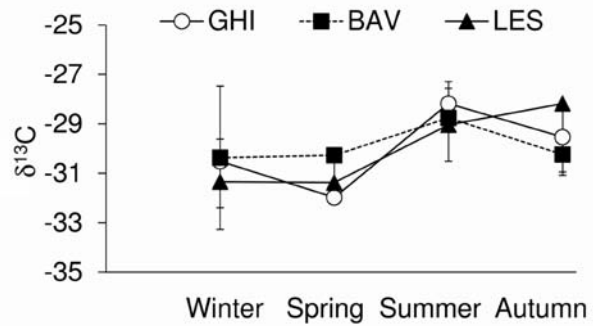

Fig. 3. Seasonal trend of phytoplankton $\delta^{13}C$ in the three stations (GHI: Ghiffa, BAV: Baveno, LES: Lesa). Data from the two layers were pooled.

Fig. 4. Seasonal trends of the phytoplankton for Shapes, Sizes and MBFG groups for the three sites (Ghiffa, Baveno and Lesa). Each bar in the matrix represents percentage biovolume in the sample and was calculated separately for Shapes, Sizes and MBFG.

and 25-50 m) in all the three stations, but particularly at Ghiffa; a correspondent decrease in the relative importance of Group 6 occurred. NPMR was utilized to search all possible phytoplankton descriptors to determine the best predictors of $\delta^{13}C$ variation. Through an iterative process NPMR searches all possible multiplicative combinations of variables to produce a model using the best predictors. Four statistically significant models were found ($P \leq 0.05$) through NPMR (Tab. 1). For the different taxonomic groups, Bacillariophyceae (BACILL) had the most important relation with $\delta^{13}C$. For MBFG groups, Group 6 (non-flagellated organisms with siliceous exoskeletons) and Group 3 (large filamentous algae with aerotopes) seemed to be the most related to $\delta^{13}C$.

Analyzing the data through multiple linear regression models between phytoplankton parameters (taxa groups, MBFG, shapes, sizes) and $\delta^{13}C$ also revealed significant (p <0.02) relationships (Tab. 2). There were similarities to the NPMR results with Bacillariophyceae and MBFG

6 being significant predictors of $\delta^{13}C$. NMS analysis carried out using taxonomic composition revealed that samples were mainly separated according to depth (axis 1), with most of the epilimnetic samples (0-20 m) found on the left of the ordination and the hypolimnetic samples (25-50 m) on the right (Fig. 5). Shape, size and morphofunctional classification classes were then superimposed as secondary variables in joint plots, together with $\delta^{13}C$ values, in order to observe patterns as indicated by the direction and length of overlain lines (Figs 5A, 5B and 5C).

In the NMS ordination of phytoplankton with cell shapes, the $\delta^{13}C$ gradient stretched from the center of the plot towards the bottom (Fig. 5A). Cylinder shapes (a category which mainly included the diatom *Cyclotella* sp.) were positioned at the top of the plot, directly opposite to $\delta^{13}C$, probably indicating the light-est (more negative) $\delta^{13}C$ signal among the cell shapes classes. *Cyclotella* sp. density and biovolume in all the three lake stations decreased considerably during the summer, a pattern that

Tab. 1. NPMR results for prediction of phytoplankton $\delta^{13}C$. Data were expressed as eight types.

| Data type	Density or biovolume	Eval xR^2	P	Average size neighbourhood	Variable 1	Tolerance	Variable 2	Tolerance	Variable 3	Tolerance
MBFG	density	0.164	0.02	11.9	MBFG3	534				
MBFG	biovolume	0.131	0.03	12.8	MBFG6	359718				
Taxa Groups	density	0.129	0.02	13.5	BACILL	453				
Taxa Groups	biovolume	0.131	0.04	12.8	BACILL	359718				
Shapes	density	0.207	0.18	12.2	2 half e	1.3	box + 2	162	cylinder	658
Shapes	biovolume	0.135	0.21	13.1	box	219332	box + 2	66874		
Size	density	0.005	0.29	14.8	500-2000	322				
Size	biovolume	0.007	0.41	13.4	0.1-100	175688	500-2000	360906		
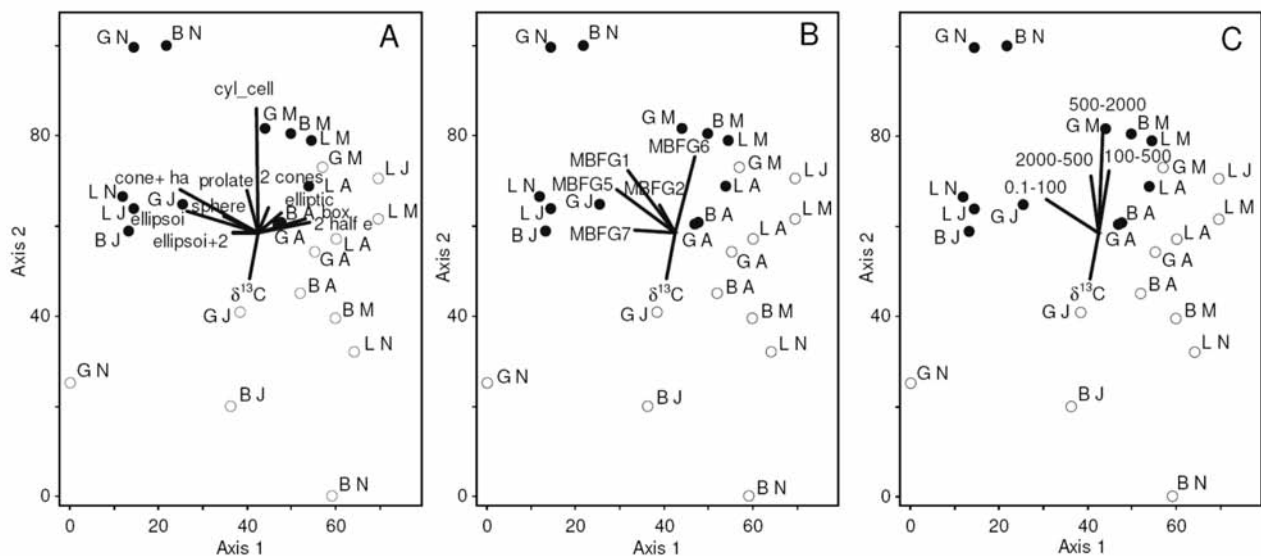
Fig. 5. NMS ordination of phytoplankton (square root transformed quantitative data). Parameters that had a correlation with an axis ≥0.1 were overlain for cell shapes (A), MBFG (B), Sizes (C), and $\delta^{13}C$. Stress=8.80936. Samples are coded by station initial (G: Ghiffa, B: Baveno and L: Lesa) and month initial (A: April, M: May, J: July, N: November). Depth is indicated by closed circles (epilimnion) and open circles (hypolimnion).

further supports the hypothesis of their light $\delta^{13}C$ fractionation. Also prolate shape cells were positioned in the upper part of the plot, although with a less extreme position than cylinder shapes. Cell shapes indicated with box (a shape category which included the diatom *Tabellaria* sp.) were located in the right part of the plot, where deep samples were usually found, and slightly opposite to increasing $\delta^{13}C$.

When cell sizes were overlain (Fig. 5C) the class of 500-2000 μm^3 appeared in the upper part of the ordination, likely representing the lightest $\delta^{13}C$ signal size class. The class with the smallest cells, or size class 0.1-100 μm^3, was positioned in the upper part of the plot indicating a relatively light $\delta^{13}C$ signal, and toward the left of the plot which was dominated by epilimnion samples.

In the ordination with the MBFG groups, Group 6 (mainly Bacillariophyceae) was positioned in the upper part of the plot, likely representing the lightest $\delta^{13}C$ signal of the function-morphological groups (Fig. 5B). Also Group 1 (small organisms with high S/V ratio) was located in the upper part of the ordination and toward the left, or towards epilimnion samples. Group 7 (large mucilaginous colonies) appeared in the left part of the plot, being more related with axes 1 and thus found mainly in epilimnion samples; its vector appeared the closest to $\delta^{13}C$, potentially indicating a MBFG group with enriched $\delta^{13}C$.

DISCUSSION

The use of $\delta^{13}C$ POM in ecological and environmental studies in lakes is based on an assumption that POM is a surrogate of phytoplankton. However, this assumption is not always valid because POM samples also include non-living matter derived from *in situ* production, external inputs and from flagellates and ciliates. To date a major problem in studies of plankton isotopes has been the separation of phytoplankton from other microbes and detritus (Jones et al. 1998), an operation that might be very difficult or impossible to finally obtain pure phytoplankton materials without contamination from other coexistent particulates (Gu et al. 2011). As indicated by Gu et al. (2011), several reasons warrant/justify the continued use of stable isotopes of POM in research on biogeochemical processes in lakes. First, biological activities likely dictate the responses of POM to environmental changes although POM is not exclusively composed of phytoplankton. Second, microbial organisms and grazers that obtain nutrients or energy from phytoplankton may share similar isotope compositions with their sources because isotopic fractionation between consumers and their prey is typically small and predictable (Post 2002). Detritus derived from phytoplankton may also resemble, if it is not identical, to its source.

Furthermore, for Lake Maggiore, a highly significant

Tab. 2. Multiple linear regression results for relationship between $\delta^{13}C$ and phytoplankton parameters (biovolume).

Data type	Variables	R^2	P
MBFG	MBFG 6, MBFG 4	43.6	<0.02
Taxa groups	BACILL	24.8	0.01
Shapes	Cylinder (cells), sphere	42.1	<0.001

correlation between chlorophyll-*a* and sestonic carbon concentration was found over the long term (Bertoni, and Callieri 1993), suggesting that POM in the lake is mainly of autoctonous origin and likely made up of algal cells and that $\delta^{13}C$ signatures of seston in this lake are mainly related to phytoplankton. As in other deep lakes such as Lake Geneva, phytoplankton largely contributes to seston in Lake Maggiore epilimnion (Morabito et al. 2009).

In this study, overall differences among the three stations in sestonic $\delta^{13}C$ signature were statistically non-significant, suggesting that the impact of allochthonous material from the River Toce, which was expected in one of the stations (station Baveno; de Bernardi et al. 1988) may only exceptionally become important (*i.e.*, after heavy rainfall events). Phytoplankton density and biomass values were also similar in the three monitored stations. The among-stations close similarity of carbon isotopic signatures may reflect a substantial homogeneity in phytoplankton abundance and community composition. Although a recent study (Morabito et al. 2009) found a gradient in the horizontal distribution of summer phytoplankton in the lake from north to south, such variability was not evident in our study likely due to differences in the position and number of the stations monitored.

A vertical gradient of phytoplankton density and biomass, in the three stations, was observed with generally higher values in the epilimnion than in the hypolimnion, particularly manifest in the summer during thermal stratification. This gradient was confirmed by the difference in chlorophyll a concentrations in the two layers.

In this study the pelagic seston $\delta^{13}C$ followed a seasonal trend (Fig. 3) with depleted values in winter and springtime, less negative values during the summer and again more depleted values in the autumn. These $\delta^{13}C$ variations corresponded to the water temperature seasonal trend in the Lake Maggiore during 2008 (Manca et al. 2009; Visconti, and Manca 2011). Similar $\delta^{13}C$ seasonal trends have been observed in other deep lakes, such as Lake Geneva (Perga, and Gerdeaux 2006). Also in smaller lakes such as Lake Wauberg (Gu et al. 2006), enriched phytoplankton $\delta^{13}C$ values typically occurred in warm months and were generally accompanied by high water temperature, high pH and low DIC concentration.

The variation in the pelagic $\delta^{13}C$ seston baseline most probably reflects changes in phytoplankton carbon isotopic signature (Zohary et al. 1994), consequent to

changes in availability of carbon sources depending on the season, and the degree of fractionation during the uptake of dissolved inorganic carbon (DIC) for the photosynthetic activity. Plankton collected during periods of high biomass and primary productivity tends to exhibit $\delta^{13}C$ enrichment (Degens et al. 1968; Fry, and Wainright 1991; Zohary et al. 1994; France et al. 1997) and heaviest (*i.e.*, less negative) $\delta^{13}C$ signatures, due to reduced isotopic fractionation at high cell densities or growth rates, or a switch to utilizing HCO_3^- as CO_2 concentration is depleted at high cell densities or growth rates (France et al. 1997; Leggett et al. 1999, 2000). Lighter (*i.e.*, more negative) $\delta^{13}C$ signatures are again typically observed in the autumn with the gradual thermal destratification and increasing contribution of dissolved carbon from the hypolimnion, leading to a seasonal minimum during winter mixing (Zohary et al. 1994).

In this study, the phytoplankton community underwent some structural and taxonomic changes during the year. Diatoms represented the main group in the winter and springtime (when $\delta^{13}C$ was more negative), and they decreased both in density and biovolume in the summer (when $\delta^{13}C$ was less negative). This observed trend is typical of Lake Maggiore in recent years and has been confirmed for 2008 by a specific monitoring of phytoplankton for the whole year (Morabito et al. 2009). Diverse studies on phytoplankton carbon fractionation (*e.g.*, Hollander, and McKenzie 1991; Zohary et al. 1994; Jones et al. 1998) found that diatoms have lighter isotopic compositions than other algal groups. For instance, values of 32‰ of $\delta^{13}C$ for *Melosira granulata* in winter in Lake Kinnereth and values of 31.2‰ for large diatoms in Loch Ness were observed.

While $\delta^{13}C$ depletion in diatoms, compared to small phytoplankton, in lakes has been reported [*e.g.*, Lake Kinnereth by Zohary et al. (1994), Lake Greifen by Hollander, and McKenzie (1991) and Loch Ness by Jones et al. (1998)], several studies reported that marine diatoms have more positive $\delta^{13}C$ signatures than that of small phytoplankton (Gearing et al. 1984; Fry, and Wainright 1991, Gu, and Schelske 1996). One possible explanation for the $\delta^{13}C$ depletion in lacustrine diatoms is the lipid stored within the cells which is a ^{13}C-poor bio-chemical compound (Parker 1964; Gu, and Schelske 1996).

In this study, Cyanobacteria, both in density and biovolume, increased in the lake in the summer (when $\delta^{13}C$ was less negative), particularly in the epilimnion. Cyanobacteria are expected to have a high $\delta^{13}C$ due to their use of HCO_3^- and their efficient utilization of CO_2 generated in the carboxysome (Goericke et al. 1994). During the summer months, with thermal stratification conditions, pH typically increases in the epilimnion due to the high rate of CO_2 uptake for increasing primary production and algal blooms. In these conditions the carbon

balance might switch from CO_2 to HCO_3^- dominance, and groups like Cyanobacteria, capable of using HCO_3^-, may have competition advantages and dominate the phytoplankton community. Because $\delta^{13}C$ of HCO_3^- is on average 8‰ higher than that of CO_2 (Mook et al. 1974), phytoplankton are enriched with $\delta^{13}C$ when using HCO_3^- as their carbon source (Gu et al. 2006).

Detailed analysis of the phytoplankton community in this study revealed that the increase of Cyanobacteria in the summer at the surface of Ghiffa Station (the pelagic station, maximum depth) was mainly due to the colonial *Aphanotheche clathrata*. Our findings are in agreement with the ecophysiology of this species and with other studies of deep subalpine lakes (Salmaso 2000; Morabito 2002), where this species may occur at significant abundance in the summer. According to Kruk et al. (2010) *Aphanotheche clathrata* belongs to the morpho-functional Group 7 (components with low surface/volume ratio), which was also the predominant group in our study during the summer. Such relatively low ratio of phytoplankton surface/volume might be related to the general enrichment of $\delta^{13}C$ observed in the lake during the summer, being in agreement with the finding of Popp et al. (1998) on cell shape and surface/volume ratio. This was particularly evident in the Ghiffa Station, where colonies of *A. clathrata* formed a little bloom and where we found the more enriched values of $\delta^{13}C$ (27‰) of the three lake stations.

In our statistical analysis by NPMR, we have investigated which, among all phytoplankton parameters, could be the best predictors of phytoplankton $\delta^{13}C$ variations (Tab. 1). Among the taxonomic groups, Bacillariophyceae was the most important in relation with $\delta^{13}C$ trend. For the MBFG groups, Group 3 (large filamentous algae with aerotopes) and Group 6 (non-flagellated organisms with siliceous exoskeletons) seemed to be the most related to $\delta^{13}C$ trend. As described earlier, Bacillariophyceae (included in MBFG 6) was the dominant taxonomic group in late winter and spring and its importance decreased during the summer, when Cyanobacteria (included in MBFG 3) were increasing. These two taxonomical groups usually represent the dominant groups in the phytoplankton community of Lake Maggiore (Morabito et al. 2009).

Some multiple linear regression models run with phytoplankton data and $\delta^{13}C$ variation were significant. In particular, the taxonomic group of Bacillariophyceae was related to $\delta^{13}C$ variation, so were cylinder shaped cells and MBFG 6, both categories including mainly Bacillariophyceae. The pattern of $\delta^{13}C$ seasonal variations showed that at less negative values of $\delta^{13}C$ found during the summer, the phytoplankton predictors found above have decreased, thus indicating to potentially represent phytoplankton groups with light $\delta^{13}C$.

More interconnections among the parameters were revealed by the NMS analysis, which primarily showed that

the depth factor was a strong determinant in the ordinations. The δ13C gradient seemed to be mainly related to size class 500-2000 μm3 (the most important in the lake in terms of biovolume and including most of the diatom species), cylindrical shaped cells (including all *Cyclotella* species and in agreement with the multiple linear regression model), and with the morpho-functional groups MBFG 6 (indicating a light δ13C) and MBFG 7 (indicating enriched δ13C).

CONCLUSIONS

Statistical investigation of the phytoplankton parameters that might be predictors of δ13C variation in this study seems to be mainly related to the Bacillariophyceae taxonomic group and to MBFG 6 (non-flagellated organisms with siliceous exoskeletons). More interconnection among the parameters considered emerged from the NMS analysis.

Phytoplankton shape doesn't seem to be *per se* a good indicator of δ13C variation. For instance, prolate cells, which were postulated to be a good predictor from direct observations, included diverse taxonomic groups which, from the literature have also different δ13C fractionation. It included, in fact, Cyanobacteria which are supposed to have enriched δ13C fractionation, and mainly all the Chrysophyta taxa which are supposed, instead, to have depleted δ13C fractionation. The sum of the two groups probably reduced the differences visible thereby reducing significance in the statistical tests we have applied. The same comment is valid for the group of cylinder colonies, which included both filamentous Cyanobacteria (enriched δ13C fractionation) and filamentous Bacillariophyceae (depleted δ13C fractionation). However, a better indicator shape group might be the cylinder shaped cells (including mainly *Cyclotella* sp.) since they resulted to have a significant relationship with δ13C in the multiple linear regression and in the NMS analysis.

Phytoplankton size classes were not found to be statistically significant with δ13C variation, both through the NPMR model and the multiple linear regression model. However, NMS analysis revealed that size class 500-2000 μm3 was the best related to δ13C variations. Although the classification of a phytoplankton assemblage in terms of size spectra could represent a valid instrument in describing the response to environmental gradients (Kamenir, and Morabito 2009) and in developing phytoplankton trophic indices (Phillips et al. 2011), its use in lake isotopic studies needs further insight and studies to be considered. In particular, future and already existent laboratory studies should be coupled with more investigations of carbon isotopes in natural environments.

In this study, the best predictor of δ13C variations seemed to be the phytoplankton taxonomic groups and the morpho-functional groups (MBFG). In particular, Bacyl-

lariophyceae and MBFG 6 were significantly related to δ13C variations in both NPMR model and in the multiple linear regression model and also the NMS analysis revealed their potential importance as predictor of δ13C variations. The importance of morphological and functional traits in shaping phytoplankton assemblages is well recognized and increasingly used in ecology (see the review by Litchman et al. 2010). Already thirty years ago Margalef (1978) and Reynolds (1984) suggested that morphology and functions of phytoplankton cells are determinant for the role of the organisms in the environment and that a trait-based approach can explain species distribution and auto-ecology. A simple and mechanistic classification of phytoplankton results to be strongly connected with the functioning of the community.

Our study suggests that morpho-functional grouping of phytoplankton could be a good predictor for δ13C variations in lakes, contributing to a better understanding of the factors influencing δ13C signature and variation in aquatic ecosystems. This might represent a supporting and parallel result also in the utilization of phytoplankton functional traits classification in current research for developing models and indices of water ecological quality (Phillips et al. 2011).

Our study confirms that the variation in the pelagic δ13C seston most probably reflects changes in phytoplankton carbon isotopic signature, consequent to changes in availability of carbon sources depending on the season and due to different isotopic fractionation of phytoplankton taxonomic and morpho-functional groups. Overall, this study shows the value of detailed information on phytoplankton composition and morpho-functional traits when interpreting isotopic signatures and pathways of organic matter in lake food webs.

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