

Variations and Relationships of Stable Isotope Composition in Size-Fractionated Particulate Organic Matter

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Received: 10 August 2009

Accepted: 31 March 2010

Abstract

$\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of crustacean zooplankton and size-fractionated suspended particulate organic matter (SPOM), ranging from bacteria to $>200\ \mu\text{m}$ plankton, were studied seasonally in the large shallow eutrophic Taihu Lake in China. Stable isotopic ratios of carbon and nitrogen both showed temporal variation with heaviest values in summer accompanying the progression of cyanobacterial bloom, and tended to be low in winter and early spring. During four seasons, the $\delta^{15}\text{N}$ values of plankton ($>50\ \mu\text{m}$) were generally enriched more than those of small size classes ($<20\ \mu\text{m}$), which indicated size-dependent consumption. But the regressions between $\delta^{15}\text{N}$ and the logarithmic organism size were only significant at 3 of 7 occasions, reflecting more numerous trophic interactions per size-class. The results indicated that a size-structured approach was partially successful in the depiction of the microbial food web structure in Taihu Lake. Zooplankton was largely depleted in ^{13}C relative to the size-fractionated SPOM. There existed a continuum in the $\delta^{13}\text{C}$ differences between SPOM and zooplankton from highly productive summer to poorly productive winter. These differences probably reflected the high contribution of nonalgal allochthonous sources of organic carbon in winter and the less detritus assimilation efficiency by zooplankton.

Keywords: particulate organic matter, seasonal variation, size-fractionated trophic structure, stable isotope, Taihu Lake

Introduction

Stable isotope analysis is an excellent tool to identify sources of organic matter [1, 2], to study material transport in food-webs [3, 4], and to elucidate biogeochemical cycles in aquatic systems [5, 6]. Taihu Lake, the third largest freshwater lake (water surface area: $2,334\ \text{km}^2$) in China, has suffered from cyanobacterial blooms from May to October in recent decades [7]. The seasonal variation in production is pronounced, and causes substantial fluctuations in the

isotopic composition of plankton organisms. Studies of seasonality of isotopic signatures in suspended particulate organic matter (SPOM) were commonly reported, but only a few focused on that of different size-fractionated SPOM. Rau et al. [1] found the lowest values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in size fractions $<8\ \mu\text{m}$ and higher values in larger particles over a 10-month period. Wainright and Fry [8] reported a summer and autumn maximum enrichment of $\delta^{13}\text{C}$ and a summer maximum enrichment of $\delta^{15}\text{N}$ during 2-year cycles. Rolff [3] presented a bi- ^{13}C and tri- ^{15}N annual cycle of size-fractionated plankton from $<0.7\ \mu\text{m}$ to $500\ \mu\text{m}$ in the northern Baltic.

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Table 1. Codes of sample types and dominant organisms observed in each class during the sampling period.

Code	Size-class	Dominating group	Method of isolation
PL ₁	GF/F-GF/D filter	Bacteria	GF/F filtration
PL ₂	GF/D-20 µm	Phytoplankton, cyanobacteria	GF/D filtration
PL ₃	20-50 µm	Phytoplankton, cyanobacteria, ciliates	Nylon sieve
PL ₄	50-200 µm	Colony cyanobacteria, diatoms, rotifers, phytoplankton	Nylon sieve
PL ₅	>200 µm	Copepodites, cladocerans, rotifers	Nylon sieve
BPOM	GF/F-500 µm	Particulate organic matter	GF/F filtration
Zoop	>64 µm	Cladocerans, copepodites	Plankton net/hand-pick
CB	Colonies	Cyanobacteria	Flotation

BPOM – bulk particulate organic matter, Zoop – zooplankton, CB – cyanobacteria.

Most of these studies focused on SPOM, which was considered the primary food source for zooplankton, although the degree of food selection from within the SPOM may vary between species and lake type [9]. In fact, SPOM is a mixture of phytoplankton, organic aggregates, bacteria, detritus, and suspended sediment [10]. The zooplankton are always non-specific suspension-feeders or raptorial feeders [11], depending on size-related feeding instead of food items. Size-based descriptions of trophic structure have been used increasingly over the last 20 years to provide generalizations regarding food-web properties [12,13]. Especially for the microbial food-web characterized by smaller planktonic organisms, a size-dependent approach provides an alternative method to evaluate the trophic level. Size fractions of SPOM, composed of nano-, pico-, and microplankton, are therefore likely to be representative of trophic groups in the microplankton community [1].

The trophic enrichment of ¹³C between zooplankton and POM ranged from 0.2‰ to 1.1‰ for different ecosystems [14]. Therefore, zooplankton would be expected to be enriched in ¹³C relative to SPOM, forming the base of the pelagic food web if they are utilizing this food source. In most of the studies, however, the ¹³C of bulk zooplankton was typically lower than that of SPOM. Lipid accumulation, habitat selection, and selective feeding by zooplankton can all contribute to the discrepancies between zooplankton and SPOM ¹³C signatures [14-16].

This study addressed the seasonal variations in ¹³C and ¹⁵N of POM, zooplankton, and cyanobacteria in a shallow eutrophic lake in China. The main purpose of this study was to demonstrate whether size-fractionated SPOM existed in size-structured trophic relationships. In addition, seasonal food availability to zooplankton was also discussed.

Material and Methods

Samples were collected from August 2006 to August 2007 at three random sites around Lake Center (31°20'N, 120°10'E) in Taihu Lake. Only the Lake center was

chosen with respect to the relatively small allochthonous influence from the catchment. Hu et al. [17] previously described the lake characteristics. Ten liters of water, obtained from 0.2 m depth, were gently pre-filtered through 500 µm nylon mesh into a clean polypropylene container. The samples were successively gently sieved through 200, 50, and 20 µm nylon sieves and Whatman GF/D glass-fiber filters (precombusted 450°C, 4 h) giving size-classes PL₂ to PL₅ (Table 1). Samples for bulk particulate organic matter (BPOM) were obtained by directly filtering 1 L water through precombusted Whatman GF/F filters. The PL₃ to PL₅ samples were scraped from the sieves into 100 mL centrifuge tubes to remove CaCO₃ by adding 0.5 molL⁻¹ HCl drop-by-drop until no more CO₂ was released. Samples remaining on GF/D and GF/F filters were exposed to concentrated HCl vapour in a glass desiccator for 4 h at room temperature to remove inorganic carbon.

To separate bacteria for isotope analyses, bacteria were cultured according to a modified version of the method described by Coffin et al. [18] and Kritzberg et al. [19]. In brief, 120 ml Whatman GF/D-filtered and 1,080 ml 0.2 µm-filtered lake water was incubated in triplicate glass bottles in darkness at 28°C for 48 h. The bacteria were then harvested by filtering the contents of each bottle through a 25-mm GF/F filter (PL₁) for later analysis. The PL₁ samples were not acidified.

Pure cyanobacteria (mainly dominated by *Microcystis* sp.) were obtained through flotation and washed with distilled water. Crustacean zooplankton was collected by towing a 64 µm zooplankton net from 0-30 cm surface lake water. Zooplankton was later sorted manually using a fine pipette, with cladoceran being separated when numbers permitted, although a mixed zooplankton sample was also retained on each occasion. Cyanobacteria and zooplankton samples were not acidified to remove inorganic carbonates.

All samples were freeze-dried for 24 h and ground to a fine powder using mortar and pestle. For analysis of carbon and nitrogen isotope ratios, all organic samples were combusted in a Flash EA1112 elemental analyzer coupled with a Thermo FinniganMAT Deltaplus dual-inlet continuous

Table 2. Means (SD) of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ as a function of sampling occasion and sample type. Range: max.-min. average for each sample type.

	2006-08-05	2006-08-26	2006-11-08	2007-01-07	2007-03-12	2007-04-19	2007-06-12	2007-08-16	Range
$\delta^{13}\text{C}$ (‰)									
PL ₅	-22.9 (2.00)	-22.1 (2.10)	-26.1 (1.75)	-30.6 (3.53)	-32.3 (3.54)	-27.6 (1.40)	-26.1 (2.44)	-25.4 (2.38)	10.2
PL ₄	-23.7 (1.59)	-22.9 (2.23)	-25.8 (2.09)	-30.6 (1.84)	-30.4 (1.23)	-25.2 (1.17)	-23.1 (1.41)	-22.1 (1.66)	8.5
PL ₃	-25.7 (1.70)	-23.0 (0.57)	-25.4 (1.71)	-31.2 (2.71)	-30.6 (1.52)	-25.9 (2.97)	-22.8 (1.69)	-23.6 (1.94)	8.4
PL ₂	-25.0 (1.35)	-23.7 (1.30)	-25.3 (1.74)	-30.9 (3.19)	-33.7 (3.07)	-25.9 (1.81)	-22.7 (2.28)	-21.9 (1.12)	11.8
PL ₁	-33.0 (1.58)	-23.8 (0.34)	-24.4 (0.57)	-26.0 (1.21)	-24.9 (0.90)	-37.8 (9.71)	-33.7 (11.3)	-26.7 (2.09)	14.0
BPOM	-24.4 (1.21)	-23.8 (1.38)	-25.7 (1.85)	-29.1 (3.31)	-28.1 (1.20)	-25.8 (1.21)	-22.7 (3.29)	-24.6 (1.77)	6.4
Zoop	-25.0 (0.71)	-22.8 (1.47)	-28.0 (2.84)	-33.9 (1.78)	-31.7 (0.48)	-26.9 (0.70)	-26.2 (2.94)	-24.5 (2.93)	11.1
CB	-23.3 (1.03)	-21.6 (1.52)				-23.7 (0.60)	-20.8 (1.77)	-21.5 (1.11)	2.9
$\delta^{15}\text{N}$ (‰)									
PL ₅	13.5 (1.36)	13.8 (0.90)	10.7 (7.14)	7.4 (7.72)	5.8 (1.65)	17.2 (3.19)	15.0 (0.93)	16.8 (0.55)	11.4
PL ₄	12.8 (1.25)	10.9 (3.22)	10.4 (7.99)	6.9 (7.61)	4.7 (3.47)	16.7 (3.77)	14.7 (1.22)	15.8 (1.25)	12.0
PL ₃	10.8 (1.06)	9.9 (3.04)	10.3 (7.36)	7.6 (7.50)	5.4 (2.64)	16.9 (3.53)	14.2 (1.13)	15.4 (0.57)	11.5
PL ₂	9.4 (2.13)	8.0 (2.78)	10.8 (6.94)	6.8 (7.47)	5.8 (2.84)	16.7 (4.10)	14.7 (0.92)	14.7 (2.03)	10.9
PL ₁	10.2 (6.43)	5.5 (2.87)	8.1 (1.83)	5.9 (4.35)	5.4 (6.94)	3.7 (2.29)	8.5 (4.0)	9.6 (1.46)	6.5
BPOM	10.7 (0.66)	9.9 (3.18)	10.5 (7.49)	4.8 (5.37)	3.9 (5.06)	12.5 (2.05)	13.0 (0.50)	14.6 (0.12)	10.7
Zoop	13.1 (1.52)	15.4 (0.84)	15.1 (4.70)	4.1 (6.41)	5.6 (1.62)	17.0 (3.02)	15.2 (1.57)	16.3 (2.13)	12.9
CB	13.7 (1.17)	14.0 (0.09)				18.1 (2.28)	14.4 (0.26)	15.1 (1.65)	4.4

flow isotope ratio mass spectrometer. The results of isotopic analysis are presented in conventional delta (δ) notation, defined as:

$$\delta X = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000$$

...where X is ^{13}C or ^{15}N and R is the ratio of $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$. Isotopic ratios are expressed relative to Vienna Pee Dee Belemnite (VPDB) for $\delta^{13}\text{C}$, and to atmospheric nitrogen for $\delta^{15}\text{N}$. Precision for the analyses was better than $\pm 0.1\%$ for both carbon and nitrogen.

Statistical analyses were conducted using SPSS for Windows software (version 13.0). Relationships between $\delta^{15}\text{N}$ and logarithmic size, ^{13}C values of all size fractions and zooplankton were characterized using linear regression analysis. Pearson's correlation coefficients were used to measure relationships between ^{13}C value of different size-classes, BPOM, and zooplankton.

Results

A general pattern of seasonal isotopic fluctuation was found in the size-fractionated SPOM and zooplankton, with the isotope ratios of both C and N exhibiting strikingly similar patterns (Table 2). The seasonal range of $\delta^{13}\text{C}$ in the size-classes PL₁ to PL₅ is considerable (6.4 to 14.0‰).

In the plankton samples (PL₂₋₅), the seasonality pattern was characterized by a strong enrichment of ^{13}C accompanying the progression of cyanobacterial bloom. There was no clear seasonal $\delta^{13}\text{C}$ cycle in PL₁, compared with other SPOM samples. The nitrogen isotope ratios of plankton tended to be low in January and March, high from April to August. In the size-classes PL₁ to PL₅, the seasonal range of $\delta^{15}\text{N}$ was between 6.5 and 12.0‰. The nitrogen isotopic composition of pure cyanobacteria collected from the bloom ranged from 13.7 to 18.1‰, indicating uptake of ^{15}N -enriched inorganic nitrogen due to eutrophication pollution rather than gaseous nitrogen depletion.

The $\delta^{15}\text{N}$ values of $>50 \mu\text{m}$ SPOM were generally greater than those of GF/F and GF/D. There was a trend toward enrichment in $\delta^{15}\text{N}$ with increasing SPOM size, indicating size-dependent consumption. But the regressions between $\delta^{15}\text{N}$ and the logarithmic organism size were significant for 3 of 8 occasions at $p < 0.05$ and one date at the limit of significance ($p = 0.051$) (Table 3).

Zooplankton $\delta^{13}\text{C}$ ranged from -22.8 to -33.9‰, and there was a significant positive relationship between the $\delta^{13}\text{C}$ of zooplankton and SPOM (PL₂ to PL₅) ($r^2 = 0.627$, $p < 0.001$). The $\delta^{13}\text{C}$ of zooplankton was mostly depleted in ^{13}C relative to the smaller planktonic size fractions on which they presumably fed (Fig. 1). When size-fractionated SPOM and zooplankton $\delta^{13}\text{C}$ data were grouped under different sampling months, the values tended to converge

Table 3. Regression parameters and significance of relationships between $\delta^{15}\text{N}$ and $\log_{10}\text{POM}$ size for each sampling date.

Date	Samples included in regression	a	b	r^2	p
2006-08-05	PL ₂ -PL ₃ -PL ₄ -PL ₅	8.273	2.342	0.937	0.032
2006-08-26	PL ₁ -PL ₂ -PL ₃ -PL ₄ -PL ₅	6.107	3.153	0.974	0.002
2006-11-08	PL ₁ , PL ₃ -PL ₄ -PL ₅	8.431	1.129	0.936	0.032
2007-01-07	PL ₁ -PL ₂ , PL ₄ -PL ₅	6.191	0.497	0.797	0.107 n.s.
2007-03-12	PL ₁ -PL ₃ -PL ₅	5.374	0.164	0.712	0.361 n.s.
2007-04-19	PL ₁ -PL ₂ , PL ₄ -PL ₅	8.936	4.338	0.550	0.258 n.s.
2007-06-12	PL ₁ -PL ₃ -PL ₅	9.389	2.740	0.910	0.194 n.s.
2007-08-16	PL ₁ -PL ₂ -PL ₃ -PL ₄ -PL ₅	11.644	2.523	0.770	0.051

n.s. – not significant

with increasing lake productivity from winter to summer (Fig. 2). No overlap of SPOM and zooplankton $\delta^{13}\text{C}$ values was seen in November and January, whereas considerable overlap was evident in June and August.

Discussion

Trophic Relationships Per Size Classes

It is difficult to assign trophic status in microbial food webs because of the complexity of such systems and the present inability to differentiate the individual components [1]. And it is also labour-intensive to isolate sufficient numbers of pure organisms for stable isotope analysis. The size-specific approach has proved to be preferable in the analysis of trophic isotope enrichment in micro- and meso-plankton food webs [3, 20, 21]. Nitrogen stable isotopes have already been used to provide evidence of links between trophic level and body size in planktonic communities [22] and to provide evidence for intraspecific relationships between body size and trophic level [23, 24]. Rolff [3] observed that $\delta^{15}\text{N}$ increased linearly with the logarithm of organism size in the interval 20 to 500 μm POM in the Baltic Sea, reflecting size-related consumption patterns of marine plankton food webs. Of course, the size-based trophic structure also has some shortcomings [25]. Since the size-fractionated material contains different taxonomic groups with different feeding ecologies, it should be careful to explain the isotopic variations observed. In this analysis, there was a trend toward enrichment in $\delta^{15}\text{N}$ with increasing plankton size, but the regression-explained variability (r^2) differed among seasons. Significant regression coefficients only occurred in August, accompanying serious cyanobacterial bloom. The relatively low amounts of dead organic matter, together with the simple structure of the pelagic community, make the size-fractionation more successful in summer than in other seasons in which all size-fractions tend to have mixed composition and yield similar isotope signals. It's been suggested that the separation of the components of microbial food web by size-fractionation was

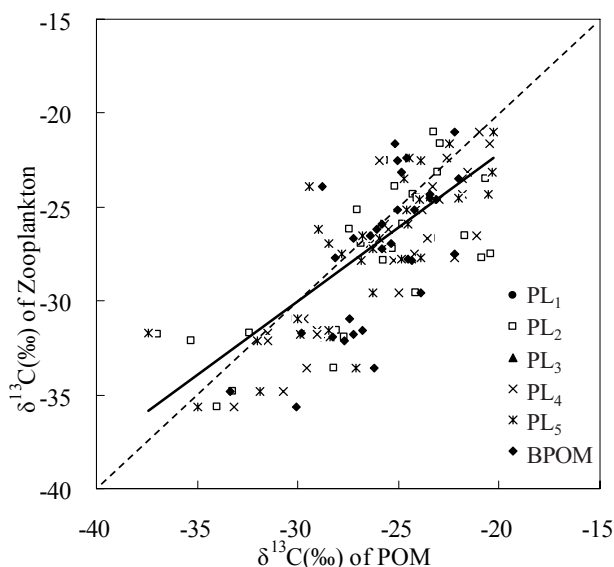


Fig. 1. Relationship between $\delta^{13}\text{C}$ of crustacean zooplankton and size-fractionated POM for all measured data. The broken line of equality is shown. The fitted regression is $Y = -5.214 + 0.856X_{\text{PL}_{2-5}}$ ($r^2 = 0.627$, $P < 0.001$).

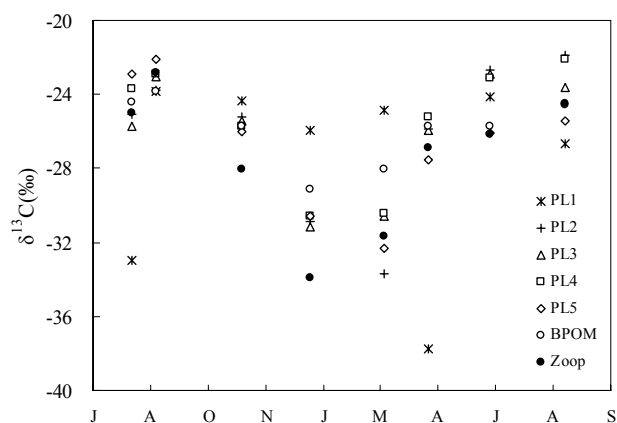


Fig. 2. Crustacean zooplankton and size-fractionated POM grouped over sampling dates.

partially successful in Taihu Lake. A similar pattern was also found by Rolff [3]. And he contributed the low slopes and r^2 s in spring and early summer to the complexity of the trophic interactions per size-class in these seasons.

Seasonal Patterns

In the study of pelagic food webs, BPOM or planktonic size classes are always used as surrogate end-points, with the implicit assumption that most of this material is algae in composition. So the changes in C and N isotopes of POM are also linked to autochthonous production over time [26]. Because of the overlap in size range between algal and detrital particles, direct measurements of algae also was not possible [27].

A pronounced seasonal pattern with a summer maximum in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were found in the size-fractionated POM from 2006 to 2007 in Taihu Lake. Seasonal changes in zooplankton lipid level and selectively feeding may account for some temporal variability in the $\delta^{13}\text{C}$ of zooplankton. The $\delta^{13}\text{C}$ value of zooplankton, BPOM and different size-classes showed significantly correlated with each other (all of the significant coefficients $p < 0.05$, data not shown). Eutrophic lakes in the temperate and subarctic regions typically show larger variations in $\delta^{13}\text{C}_{\text{POM}}$ [28]. Taihu Lake is located in a transition zone between subtropical and temperate regions and is characterized by large seasonal changes in climate. The lake water temperature ranged widely from about 5°C in winter to $>30^\circ\text{C}$ in summer (data not shown). So the range of $\delta^{13}\text{C}$ for all size classes (6.4 to 14.0‰) in Taihu Lake was correspondingly large compared to temperate lakes.

Seasonal variation in $\delta^{13}\text{C}$ POM may be affected by water temperature, productivity, and dissolved inorganic carbon (DIC) pool size. In early summer, phytoplankton (overwhelmingly dominated by *Microcystis* sp.) photosynthesis in Taihu Lake increased as water temperature increased, resulting in a low concentration of dissolved nutrients and DIC. A high rate of CO_2 uptake results in increases in pH and a switch in the C balance from CO_2 to HCO_3^- dominance, which is the major form of DIC [5]. Since $\delta^{13}\text{C}$ of HCO_3^- is on average 8‰ higher than that of dissolved free CO_2 [29], cyanobacteria are enriched with ^{13}C when using HCO_3^- as their C source. The POM $\delta^{15}\text{N}$ values markedly correlated with its $\delta^{13}\text{C}$ ($r=0.759$, $p=0.048$), with enriched isotope ratios from April to November. Mariotti et al. [30] found that seston $\delta^{15}\text{N}$ increased with temperature in the North Sea. Goering et al. [31] also observed a similar pattern in the $\delta^{15}\text{N}$ of phytoplankton in Auke Bay, Alaska. They attributed the summertime increase in $\delta^{15}\text{N}$ to decreased fractionation during rapid phytoplankton growth in warmer months. It's a pity that the fractionation factor was not measured in Taihu Lake, but the $\delta^{15}\text{N}$ of SPOM increased with the decreases in DIN concentrations on sampling dates [32]. Ferber et al. [33] reported high $\delta^{15}\text{N}$ of cyanobacteria in a small shallow lake with low N_2 fixation and high dissolved inorganic nitrogen (DIN) uptake rates. A similar process was also found in the

Childs River [10]. The huge requirement of N by cyanobacteria may draw down DIN concentrations in summer. At the same time, microbial processes such as nitrification and denitrification may also contribute to it.

Relationship between POM and Zooplankton

The $\delta^{13}\text{C}$ for zooplankton and various size fractions of POM largely fell below the line of equality during the sampling dates, indicating that zooplankton were generally depleted in ^{13}C relative to the smaller planktonic size fraction on which they presumably fed. The zooplankton ^{13}C -depletion relative to POM might be explained by:

- (1) The accumulation in zooplankton of ^{13}C -depleted lipids, since the lipids were not removed from zooplankton samples during this study [34]. Lipid levels in zooplankton might also change seasonally and hence influence temporal variation of $\delta^{13}\text{C}$ in zooplankton [35]. This might in part explain the different set-offs between POM and zooplankton carbon isotopic signatures.
- (2) Spatial separation between where zooplankton are sampled and where they feed, with corresponding differences in carbon sources. Since three replicate samples were collected in one site in this study, this factor might be ignored.
- (3) Selective feeding by zooplankton on isotopically light carbon sources that may be masked or diluted by a large detrital contribution to POM that is enriched in ^{13}C [14].
- (4) Isotopic heterogeneity within plankton communities may confound the relationship between the $\delta^{13}\text{C}$ for zooplankton and POM [36].

When all the plankton organisms were grouped according to sampling time, the divergence between zooplankton and POM signatures did decrease from winter to summer. Crustacean zooplankton plays a pivotal role in the transfer of carbon through lake food webs, which can consume a wide range of particulate matter, including phytoplankton, bacteria, and detritus. The traditional concept considered that zooplankton predominantly grazed autochthonous primary production. In summer, the most productive season, cyanobacterial bloom occurred in Taihu Lake. Considerable overlap of different size POM classes and zooplankton $\delta^{13}\text{C}$ values was evident from April to August, indicating that zooplankton might consume nutritionally poor or even toxic cyanobacteria. Yoshioka and Wada [37] reported zooplankton may utilize *Microcystis* through a microbial loop. Bontes et al. [38] found that cyanobacteria accounted for more than 50% of the isotope signature of the zooplankton. When phytoplankton biomass was low in winter, the isotopic signature of terrestrial and littoral materials predominated in both BPOM and some size classes. Recent studies have revealed zooplankton diets supported by planktonic heterotrophs and detritus via the microbial pathway when phytoplankton production is limited. Grey et al. [16] confirmed that the diet of zooplankton in Loch Ness switched from a reliance on allochthonous carbon derived from POM during winter and early spring to heavy dependence on algal production during summer. The allochthonous carbon accounted for ~50% in December. Hessen et al.

[39] demonstrated that zooplankton can use detrital sources of organic matter directly, but with only low assimilation efficiency and that this use is probably mediated by a series of microbial links. To sum up, while zooplankton have similar carbon isotopic values to POM and BPOM classes, these values are intermediate among the major algal components of diatoms, cyanobacteria, and other microplankton, and may be the result of mixture. Alternatively, zooplankton may be consuming bacteria that are assimilating carbon from mixed algal sources or allochthonous detritus. Further work is needed, possibly using lipid biomarkers [40], to delineate the source of organic carbon more clearly.

Conclusions

A size-dependent approach is commonly considered applicable to isotopic studies of microbial food webs. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ across lacustrine SPOM size classes were investigated seasonally in this study. But the ^{15}N enrichment per unit size and the regression-explained variability (r^2) were not strong on each sampling date, suggesting a complex trophic relationship per size class in different seasons. Relatively large seasonal variations in SPOM stable isotope ratios were also found accompanied by isotope changes of zooplankton that may potentially feed on them. The variations may reflect differences in ambient environmental conditions and biological processing among systems.

Acknowledgements

We thank X.D. Wu and S.Q. Qian for help with sampling and E. L. Zhang for stable isotope analyses. This work has been supported by National Water Pollution Control and Management Technology Major Projects (No. 2008ZX07101-012), and the State Key Basic Research and Development Plan (No. 2008CB418104).

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