

Cyanobacteria as a carbon source for zooplankton in eutrophic Lake Taihu, China, measured by ^{13}C labeling and fatty acid biomarkers

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Abstract

Using a combined stable-isotope and fatty-acid approach, we examined carbon-transfer routes from the cyanobacterium *Microcystis* to zooplankton in eutrophic Lake Taihu, China. *Microcystis* is generally considered poor food for zooplankton, and we hypothesized that most *Microcystis* carbon flows to zooplankton via dissolved organic matter (DOM)–bacteria and detritus–bacteria pathways rather than via direct grazing. The hypothesis was tested by analyzing ^{13}C isotopes at natural abundance in field samples and in tracer experiments with ^{13}C -enriched *Microcystis*. ^{13}C -enriched *Microcystis* was added as live *Microcystis*, *Microcystis* detritus, or *Microcystis* DOM to lake-water incubations with *Bosmina* sp. and *Daphnia similis* as the dominant species. The ^{13}C isotope signatures of *Microcystis*, heterotrophic bacteria, and eukaryotic algae in seston were determined from isotope analyses of specific fatty acids, and the presence and labeling of these fatty acids were also analyzed in zooplankton consumers. *Bosmina* and *Daphnia* consumed carbon via all pathways, but the amount of carbon transfer from the *Microcystis* DOM was the highest, followed by the *Microcystis* detritus. *Bosmina* consumed relatively more live *Microcystis* than *Daphnia*. The presence and high ^{13}C enrichment of bacteria-specific fatty acids in the zooplankton consumers showed that heterotrophic bacteria were an important link between *Microcystis* and zooplankton. Microbial pathways dominate the energy flow from cyanobacteria to zooplankton in eutrophic lakes with heavy cyanobacteria blooms, such as Lake Taihu.

Eutrophication is one of the most widespread and pertinent environmental problems in aquatic environments; it often results in degradation of aquatic ecosystems, which causes shifts in primary producers, bacterial, and zooplankton communities (Carpenter et al. 1998). Severe eutrophication in freshwater ecosystems causes extensive and recurrent cyanobacteria blooms, which are expected to occur more frequently in a future warmer climate. Studies on zooplankton–cyanobacteria interactions have generally shown that cyanobacteria cause rapid decrease of large crustaceans in favor of smaller crustaceans and rotifers (Fulton and Paerl 1988). Such studies have primarily examined herbivory and proposed poor nutritional value, filtering rate interference and toxicity as important adverse factors (Haney 1987; Lampert 1987). Grazing on cyanobacteria, or herbivory, is however, only one of the energy pathways from primary producers to consumers. Zooplankton can also acquire carbon via the microbial food web by grazing on heterotrophic bacteria directly or on protists (ciliates and heterotrophic flagellates) as an intermediate trophic link. Some earlier laboratory studies indicated that cladocerans are efficient grazers on bacteria (Peterson et al. 1978; Porter et al. 1983). Field studies confirmed that zooplankton can meet a large part of their carbon requirement via the microbial food web (Hessen et al. 1990; Wylie and Currie 1991; Koshikawa et al. 1996). The importance of the microbial food web as a food source for zooplankton depends largely on lake productivity and on

plankton community structure (Pace et al. 1983). Generally, the microbial food web becomes more important when lakes move toward eutrophic systems, and this phenomenon can be explained by an increasing dominance of cyanobacteria and a co-occurring shift in zooplankton community structure (Gliwicz 1969). Based on this knowledge, we hypothesize that, in eutrophic systems, cyanobacteria are an important carbon source for zooplankton, but that much more carbon flows via the microbial food web rather than via the herbivory pathway. The importance of the microbial food web as a carbon source for zooplankton during and after a cyanobacteria bloom was previously demonstrated by Christoffersen et al. (1990). Their results were based on carbon budgets combined with modeling. Here, we present an integrated stable-isotope, biomarker approach to trace the importance of *Microcystis* carbon for zooplankton nutrition.

Stable-isotope analysis (SIA) of carbon at natural abundance is a powerful tool that enables us to trace organic carbon flows and examine food-web interactions in ecosystems. Zooplankton consumers generally reflect the carbon isotope signature ($\delta^{13}\text{C}$) of their diet, so portions of resources in the diet of zooplankton can be determined when carbon sources are sufficient isotopically distinctive. A major challenge in aquatic ecology is to separate the $\delta^{13}\text{C}$ of potential carbon sources at the base of the food web from bulk particulate organic carbon (POC). Compound-specific isotope analysis of fatty-acid biomarkers is a valuable method to determine the isotope signature of certain groups of organisms (Boschker and Middelburg 2002) and was used in this study to determine $\delta^{13}\text{C}$ of eukaryotic algae,

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cyanobacteria, and heterotrophic bacteria. Although, SIA of carbon at natural abundance can be used to identify carbon origins, it is limited in its utility for resolving pathways from basal resources to consumers. It is, for example, difficult to distinguish direct grazing by zooplankton on phytoplankton from indirect grazing on phytoplankton-derived detritus or bacteria that grew on phytoplankton carbon. Furthermore, the isotope signatures of *Microcystis* and eukaryotic algae are likely to overlap, although large isotopic differences have been reported for different phytoplankton groups (Bontes et al. 2006; Vuorio et al. 2006). A valuable alternative to avoid the problem of overlapping resources is to manipulate the isotope signature of a potential carbon source by ^{13}C enrichment and subsequently trace the incorporation into consumers (Middelburg et al. 2000; Pel et al. 2003). In this study, we combine a natural abundance and tracer ^{13}C study with fatty-acid measurements to assess the importance of carbon from *Microcystis* in zooplankton nutrition. We first analyzed natural-abundance isotope composition of carbon sources and zooplankton consumers in field samples. Then, we examined the assimilation of ^{13}C -enriched carbon sources derived from *Microcystis* within the plankton food web in lake-water incubations, with the cladocerans *Bosmina* sp. and *Daphnia similis* as end-consumers. These species are representatives for small and large cladocerans, respectively, and we expected higher consumption of *Microcystis* carbon by *Bosmina* than by *Daphnia*. The different labeled substrates were (1) live *Microcystis* to test for herbivory, (2) *Microcystis*-derived (particulate) detritus, and (3) *Microcystis*-derived dissolved organic matter (DOM) to test for carbon flow via the microbial food web. Fatty acids (FA) were used to quantify biomass and isotope composition of *Microcystis*, algae, and bacteria in the seston. We also examined the presence of labeled FA in zooplankton to retrieve additional information on zooplankton grazing on labeled *Microcystis* and bacteria. Finally, FA profiles were used to retrieve dietary information. This fatty-acid trophic marker approach (FATM) is based on the conservative incorporation of FA from the food source into primary consumers, such as zooplankton (Dalsgaard et al. 2003; Brett et al. 2006). FATM has been successfully applied to show feeding of zooplankton on phytoplankton and bacteria in previous studies (Taipale et al. 2009).

Methods

Site description—The study was conducted in Lake Taihu, the third-largest freshwater lake in China, with a total area of 2338 km². The lake is located in the Yangtze Delta in eastern China (30°55′–31°32′N, 119°52′–126°36′E) and has economically and socially important values in the region. The lake is shallow, with an average depth of ~ 2 m. Increased nutrient inputs into the lake during the past decades caused eutrophication of Lake Taihu, which led to massive, toxic, and recurring blooms of *Microcystis* (cyanobacteria) in summers. Meiliang Bay, located in the northern part of the lake, is perhaps the most eutrophic part of the lake; it is characterized by high densities of *Microcystis* sp., comprising up to 98% of total phytoplankton bio-volume in summer (Chen et al. 2003).

The study was conducted in May 2009 in Meiliang Bay. At the time of sampling, the phytoplankton community in Meiliang Bay was dominated by the cryptophyte *Cryptomonas erosa* ($3.94 \pm 2.24 \text{ mm}^3 \text{ L}^{-1}$), and *Microcystis* sp. ($1.63 \pm 2.24 \text{ mm}^3 \text{ L}^{-1}$). Cyanobacteria bloom or surface scum was absent during our sampling. The zooplankton community was dominated by *Bosmina* sp. (152 ± 10 individuals [ind.] L^{-1}). Other zooplankton genera included *Ceriodaphnia* ($34 \pm 10 \text{ ind. L}^{-1}$), rotifers ($24 \pm 4 \text{ ind. L}^{-1}$), and *Cyclopoid* copepods ($22 \pm 9 \text{ ind. L}^{-1}$).

^{13}C -labeled substrate production—Floating *Microcystis* was collected using a 200- μm net from the surface of Lake Taihu and concentrated by subsequent collection of the upper layer. The concentrate was transferred to GF/C (Whatman)-filtered lake water. *Microcystis* was labeled in two different cultures; the first culture was used to produce the detritus and DOM substrates, and the second culture was labeled closely before the experiment and used to produce live *Microcystis* substrate. The final *Microcystis* concentration was 21.3 g fresh weight L^{-1} and 10.6 g fresh weight L^{-1} in the first and second culture, respectively. The cultures were incubated outside in air-tight and magnetically stirred bottles for 24 h with 20% ^{13}C -bicarbonate (> 98% pure ^{13}C) and 30% ^{13}C -bicarbonate in the first and second culture, respectively. To check for physical label uptake and nonphotosynthetic or dark uptake, a small part of the culture was filtered immediately after label addition and the other part was incubated in the dark. There was no physical or dark uptake of label. After incubation, *Microcystis* was rinsed at least three times using a 30- μm sieve to remove the label adhering to the surface of the cells. Size-distribution analysis of *Microcystis* collected with the same method revealed that *Microcystis* consisted of 55% of colonies smaller than 25 μm and of 39% of colonies in the range of 25–60 μm (Wang 2008).

Labeled *Microcystis* for DOM and detritus production (first culture) was concentrated by centrifugation (10 min, 4500 $\times g$) and freeze-dried. The freeze-dried culture was resuspended in demi water to induce osmosis-induced lysis of the cells (Kim et al. 2009). The particulate and dissolved fractions were separated by centrifugation (15 min, 4500 $\times g$). The pellet was again resuspended in demi water, split into dissolved and particulate fractions, and this was repeated a couple of times. The pooled dissolved fraction was glass-fiber-filtered (GF/F), and the particulate fraction was additionally rinsed and both fractions were freeze-dried again and stored frozen until used in the experiments. Live *Microcystis* (second culture) was rinsed and concentrated and could be used directly in the experiments. A subsample of each substrate was kept for carbon content and isotope-labeling analyses, and a subsample from live *Microcystis* was kept for composition and isotope labeling of FA.

Incubation experiments—The incubation experiments were carried out at the Taihu research station, Wuxi, China between 28 and 31 May 2009. *Daphnia similis* was added to natural lake water of Meiliang Bay to a final concentration of 30 individuals L^{-1} . The water was divided

over 12 buckets with 6 liters in each bucket. The buckets were floating in a pond to prevent large temperature changes, bound tight with ropes, and covered with nets. Triplicate buckets were used for the three treatments and control. Live *Microcystis* (treatment 1) was added to a final concentration of 2 g L⁻¹ fresh weight (i.e., 86 mg L⁻¹ dry weight), which corresponded to a bloom situation (Qin et al. 2010). *Microcystis* detritus (treatment 2) and *Microcystis* DOM (treatment 3) were both added to a final concentration of 8.6 mg L⁻¹ dry weight. This corresponded to a DOM concentration increase of ~ 25%. In the control treatment (treatment 4), no substrate was added. The incubations were run for 3 d and water in the buckets was mixed ~ 4 times d⁻¹. As a result of differences in substrate additions, the final POC concentrations were 8.6 times higher in treatment 1 (30.6 ± 3.6 mg C L⁻¹) compared with treatments 2 (3.8 ± 0.8 mg C L⁻¹) and 3 (3.3 ± 0.5 mg C L⁻¹).

Sampling—Field sampling was done in the center of Meiliang Bay, where triplicate water samples were taken with a 5-liter Plexiglas water-sampler from the upper 0.5 m of the water column and triplicate zooplankton samples were taken with a 64- μ m mesh-size net from the upper 1 m of the water column. Triplicate water samples for t_0 or control measurements of the experiment were taken before the water was divided over the different buckets. Water samples of the incubations were only taken at the end of the experiment (t_{end}). Start and end samples of the incubations for zooplankton were collected using a 64- μ m mesh-size net. Both field and experiment water samples were subdivided for POC, dissolved organic carbon (DOC), polar lipid FA in seston, and dissolved inorganic carbon (DIC) analyses. Start and end samples from the experiment were also taken for purposes of counting algae, bacteria, and zooplankton numbers (data not shown) and for recording temperature, pH, alkalinity, chlorophyll *a*, and inorganic nutrients (data not shown).

Analyses—For POC analyses, ~ 400 mL of water was filtered over precombusted and preweighted GF/F filters, which were dried at 60°C. The carbon concentration and isotopic composition of POC were analyzed on a Thermo Electron Flash EA 1112 elemental analyzer (EA) coupled to a Delta V isotope ratio mass spectrometer (IRMS). Headspace vials (10 mL) were filled with GF/F-filtered water, preserved with mercury chloride, and stored at room temperature for DIC analyses. In the lab, a helium headspace was created and samples were acidified with H₃PO₄ solution. The CO₂ concentration and isotope ratio in the headspace were measured on the same EA-IRMS. GF/F-filtered water was stored frozen in clean vials for DOC analysis. In the laboratory, the samples were acidified and flushed with helium to remove DIC, and subsequently the DOC was measured with liquid chromatography–isotoplink–IRMS (Boschker et al. 2008). Individual zooplankters were sampled for measurements of carbon content and isotope ratios and for FA concentrations and isotope ratios. For the incubation experiments, the zooplankters were collected on a 112- μ m sieve and transferred to clean,

demi water, and made to stay for a minimum of 4 h to remove label adhering onto the animals and to clear their gut contents. About 15 *Daphnia* and 50 *Bosmina* were handpicked for each analysis. About 15 cyclopoids and 50 *Bosmina* were handpicked from the field samples. The zooplankton samples were dried at 60°C for total carbon analyses and those for FA analyses were stored frozen. The carbon content and isotopic composition of zooplankton was measured in the same way as for POC. Zooplankton and seston lipids were extracted by a modified Bligh and Dyer method (Middelburg et al. 2000). For seston, the lipids were fractionated in different polarity classes by column separation on a heat-activated silic-acid column and subsequent elution with chloroform, acetone, and methanol. The methanol fractions, containing the polar lipid FA, were collected and derivatized to fatty-acid methyl esters. For zooplankton, total FA were analyzed. 12:0 and 19:0 FA were used as internal standards. (PL)FA were separated on the nonpolar HP5 column (60 m × 0.32 mm × 0.25 μ m) and the $\delta^{13}\text{C}$ of individual (PL)FA were measured using gas chromatography-combustion isotope ratio mass spectrometry (GC-c-IRMS) (Middelburg et al. 2000). *Bosmina* FA concentrations were low, so the extracts were analyzed using large-volume (20 μ L) injection with a Cis4 PTV injector (Gerstel, Germany). The GC-c-IRMS settings used were the following: start temperature 35°C for 0.5 min, warm up by 16°C s⁻¹ to 150°C for 0 min, and finally warm up by 12°C s⁻¹ to 300°C for 3 min. The injection speed was 2.5 μ L s⁻¹ with the solvent vent mode of injection. In this way, sample injections could be increased to 100 μ L.

Data analyses—Stable-isotope ratios are expressed in the delta notation ($\delta^{13}\text{C}$), which is the isotope ratio of ¹³C: ¹²C relative to Vienna-PeeDee Belemnite standard. The weighted isotope ratio of specific FA was used to determine the isotope signature of the carbon source. *Microcystis*, in this study, contained high amounts of 16:0, followed by relatively high concentrations of C18 mono-unsaturated FA (e.g., 18:1 ω 7c, 18:1 ω 9c) and C18 poly-unsaturated FA (PUFA; e.g., 18:4 ω 3, 18:3 ω 3), in agreement with other studies on FA composition of *Microcystis* (Ahlgren et al. 1992; Gugger et al. 2002). Some of the abundant *Microcystis* FA are also present in bacteria (e.g., 18:1 ω 7c) or cryptophytes (e.g., 18:4 ω 3), the most important algae in our samples, and these FA were not used as markers. The weighted $\delta^{13}\text{C}$ of FA 18:3 ω 3, 18:3 ω 6, 18:2 ω 6c, and 18:1 ω 9c, were used to determine *Microcystis* isotope ratios. Long-chain (C20, C22) PUFA are generally absent in cyanobacteria (Ahlgren et al. 1992), so the weighted ratio of PUFA 20:5 ω 3, 20:4 ω 6, and 20:3 ω 6 were used to determine (eukaryotic) algae. Branched FA are characteristic for heterotrophic (gram-positive) bacteria, so the weighted $\delta^{13}\text{C}$ of the branched FA i14:0, ai15:0, and i15:0 were used to determine bacteria isotope ratios. Because FA are generally depleted relative to other structural components, a fractionation factor of 3‰ was applied to obtain $\delta^{13}\text{C}$ values for whole cells in the natural-abundance analyses (Hayes 2001).

Label uptake is reflected in enrichment in $\delta^{13}\text{C}$ and is calculated as $\Delta\delta^{13}\text{C}$ (‰) = $\delta^{13}\text{C}_{\text{sample}} - \delta^{13}\text{C}_{\text{background}}$.

Table 1. Average natural-abundance $\delta^{13}\text{C}$ values (\pm SD; $n = 3$) of carbon pools in Lake Taihu during spring 2009.

Carbon pool	Isotope ratio $\delta^{13}\text{C}$ (‰)
POC	-26.5 ± 0.5
Bacteria in seston	-26.1 ± 0.5
Algae in seston	-29.2 ± 0.6
<i>Microcystis</i> in seston	-30.6 ± 0.5
<i>Microcystis</i> of surface bloom	-20.9 ± 0.4
<i>Bosmina</i>	-26.9 ± 0.2
Copepods	-29.0 ± 3.4
Bacteria in <i>Bosmina</i>	-24.6 ± 0.1
Algae in <i>Bosmina</i>	-28.9 ± 0.6
<i>Microcystis</i> in <i>Bosmina</i>	-29.5 ± 0.6

Label uptake in consumers reflects relative consumption: uptake of the enriched carbon source relative to consumer carbon biomass. To compare labeling in the different treatments, the data were normalized for the amount of ^{13}C that was added to each of the incubations.

All results are presented as average \pm SD ($n = 3$). The contribution of potential carbon sources to zooplankton was examined using natural-abundance isotope ratios with the Isosource computer program, with 1% increment and 0.01% tolerance (Phillips and Gregg 2003). Bacteria, algae, and *Microcystis* were used as potential carbon sources for both cyclopoid copepods and *Bosmina* sp. To test for statistically significant ($p < 0.05$) differences between zooplankton species and treatments in the labeling experiment, (factorial) analyses of variance (ANOVAs) and Bonferroni post hoc tests were applied to the data.

Results

Natural-abundance isotope ratios—The average $\delta^{13}\text{C}$ of the different carbon pools in Meiliang bay are summarized in Table 1. POC and bacteria had similar $\delta^{13}\text{C}$ values of $-26.5\text{‰} \pm 0.48\text{‰}$ and $-26.1\text{‰} \pm 0.53\text{‰}$. Both algae and *Microcystis* were more depleted with isotope values of $-29.2\text{‰} \pm 0.64\text{‰}$ and $-30.6\text{‰} \pm 0.48\text{‰}$, respectively. This implies the presence of ^{13}C -enriched detritus in POC. The carbon sources of ^{13}C -enriched detritus were unknown, but scum-forming *Microcystis* could be a possible source. The floating, scum-forming *Microcystis* we collected for substrate production had much more enriched ^{13}C values than *Microcystis* in the field samples. The isotope signature of scum *Microcystis* was $-20.9\text{‰} \pm 0.36\text{‰}$ when analyzed in total or -22.6‰ based on FA. The isotope value of *Bosmina* ($-26.9\text{‰} \pm 0.20\text{‰}$) was similar to those of POC and bacteria, while $\delta^{13}\text{C}$ of cyclopoid copepods ($-29.0\text{‰} \pm 3.4\text{‰}$) reflects a more mixed diet. Overall, the isotope range of carbon sources was quite narrow, from -26.1‰ to -30.6‰ , which makes it difficult to precisely allocate the contribution of each source to zooplankton consumers. Isotope mixing models using isosource indicate that *Bosmina* received $77\% \pm 2.5\%$ of their carbon via bacteria, $14\% \pm 8.3\%$ from algae, and $9.2\% \pm 5.7\%$ from *Microcystis*. Copepods received $21\% \pm 8\%$ of their carbon from bacteria, $31\% \pm 19\%$ from *Microcystis*, and $48\% \pm$

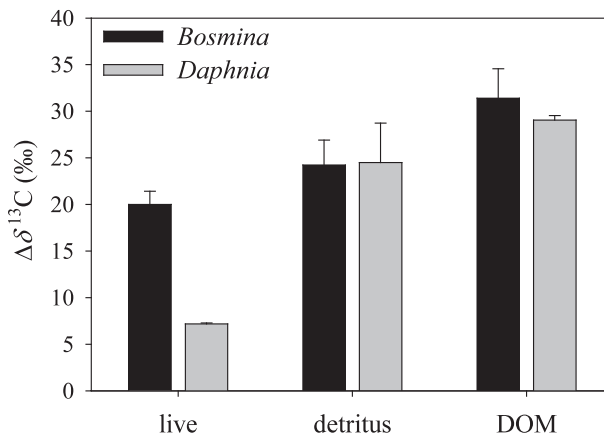


Fig. 1. ^{13}C enrichment ($\Delta\delta^{13}\text{C}$) of *Bosmina* and *Daphnia* for the three different labeled *Microcystis* substrates added. Data are presented as average with SD error bars ($n = 3$). Note that *Bosmina* has significantly higher labeling than *Daphnia* when fed live *Microcystis* (first treatment).

27% from algae. *Bosmina* contained bacteria, *Microcystis*, and algae-specific FA, and they had similar $\delta^{13}\text{C}$ values to the corresponding FA in the seston (Table 1). The similarity of $\delta^{13}\text{C}$ in seston and zooplankton FA indicates a coupling between zooplankton consumers and the measured carbon sources.

Tracer assimilation—After 3 d of lake-water incubation with ^{13}C -enriched *Microcystis* substrates, ^{13}C enrichment ($\Delta\delta^{13}\text{C}$) could be detected in all major organic carbon pools. Both *Bosmina* and *Daphnia* incorporated ^{13}C of the *Microcystis* substrates, but the uptake differed by substrate (Fig. 1). Zooplankton showed the highest incorporation of *Microcystis* when it was added in the form of DOM, with $\Delta\delta^{13}\text{C}$ values of $31.4\text{‰} \pm 3.2\text{‰}$ for *Bosmina* and $29.1\text{‰} \pm 0.5\text{‰}$ for *Daphnia*, followed by *Microcystis* added as detritus with $24.2\text{‰} \pm 2.7\text{‰}$ and $24.5\text{‰} \pm 4.2\text{‰}$ for *Bosmina* and *Daphnia*, respectively (Fig. 1). Tracer assimilation was lowest for both species in the live *Microcystis* incubations, and *Daphnia* consumed significantly fewer live *Microcystis* ($7.17\text{‰} \pm 0.11\text{‰}$) than *Bosmina* ($20.0\text{‰} \pm 1.4\text{‰}$; ANOVA, $F_{3,4} = 243$, $p < 0.0005$) relative to their carbon biomass.

^{13}C incorporation into FA in the seston was used to determine bacteria, *Microcystis*, and algae tracer assimilation. Heterotrophic bacterial FA showed high ^{13}C labeling in all treatments, showing that *Microcystis* is an important carbon source for bacteria (Fig. 2). Part of the observed labeling can be explained by the presence of labeled bacterial markers in *Microcystis* substrates (Table 2). However, the concentrations of substrate bacteria FA were too low to cause the observed enrichment in bacterial FA in the incubations. *Microcystis* FA showed expected high ^{13}C labeling in the live *Microcystis* incubations followed by the detritus incubations (Fig. 2), because of their presence in the *Microcystis* substrates. Small ^{13}C enrichment was detected in algae in the live *Microcystis* and DOM treatments (Fig. 2). Part of their labeling can be explained by presence of algae biomarkers in the substrate (Table 2)

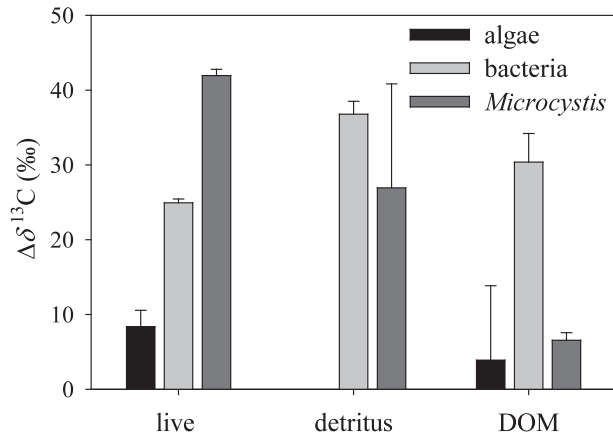


Fig. 2. ^{13}C enrichment ($\Delta\delta^{13}\text{C}$) of algae, bacteria, and *Microcystis* in the seston for the three different labeled *Microcystis* substrates added. Data are presented as average with SD error bars ($n = 3$).

and by growth on respired ^{13}C -DIC; DIC was enriched in all treatments, ranging from 5.7‰ (live treatments) to 15.2‰ (DOM treatments). DOC also showed ^{13}C enrichment in all treatments, and the enrichment was highest in the additions where labeled *Microcystis* DOM was added, with 33.7‰, as expected. POC was also enriched in all treatments, with highest enrichment in the detritus additions (19.4‰).

Fatty-acid labeling in zooplankton—The presence and isotope enrichment of the bacteria, algae, and *Microcystis* FA in zooplankton enabled us to combine *Microcystis* carbon incorporation with FA as trophic markers. Both *Bosmina* (Fig. 3A) and *Daphnia* (Fig. 3B) contained FA representative for each resource. The bacterial FA in zooplankton showed the highest labeling in the *Microcystis* DOM additions, clearly showing that *Bosmina* and *Daphnia* grazed on the bacteria that grew on *Microcystis* DOM (Fig. 3). Zooplankters in the detritus treatments also contained relatively high labeled bacterial FA, showing that zooplankton grazed on bacteria living on *Microcystis* detritus (Fig. 3). Even *Bosmina* in the live treatments contained labeled bacterial FA, demonstrating that part of the live *Microcystis* that *Bosmina* consumed was shuttled via bacteria (Fig. 3A). The bacteria might have been attached to *Microcystis*, because there were no labeled bacterial markers found in *Daphnia* in the live *Microcystis*

Table 2. Average percentages of marker fatty acids (FA) to total FA (\pm SD, $n = 3$) in zooplankton (1, 2) and seston (3) in control incubations (without substrate) and in *Microcystis* used to produce the substrates (4). PUFA = poly-unsaturated FA.

Carbon pool	% algae (PUFA)	% bacteria FA	% <i>Microcystis</i> FA
(1) <i>Bosmina</i>	5.9 \pm 1.1	0.7 \pm 0.3	8.9 \pm 1.8
(2) <i>Daphnia</i>	9.9 \pm 0.4	4.2 \pm 0.4	21.4 \pm 1.1
(3) Seston	4.6 \pm 1.6	2.8 \pm 0.2	27.8 \pm 2.0
(4) <i>Microcystis</i>	0.8	0.6	28.5

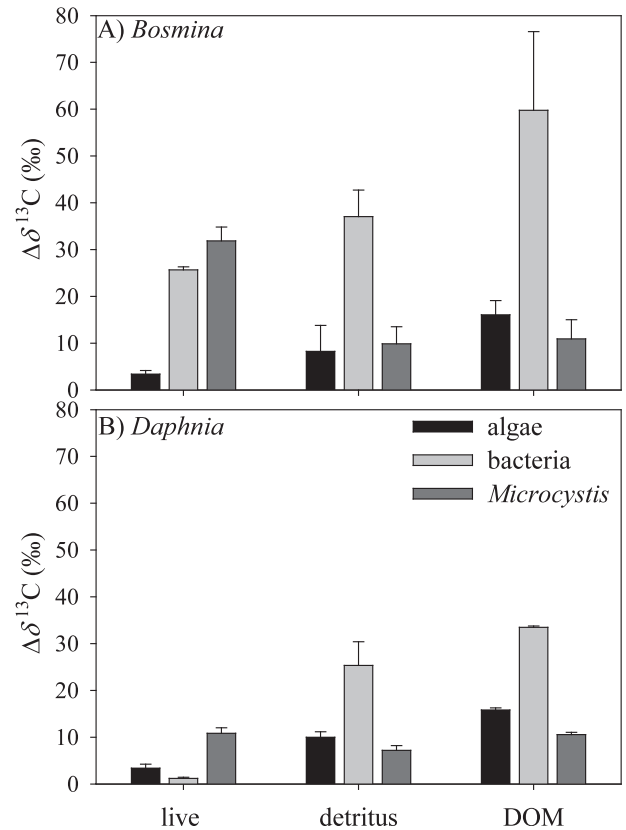


Fig. 3. ^{13}C enrichment ($\Delta\delta^{13}\text{C}$) of algae-, bacteria-, and *Microcystis*-specific FA within zooplankton for the three different labeled *Microcystis* substrates added; (A) *Bosmina*, and (B) *Daphnia*. Data are presented as average with SD error bars ($n = 3$).

treatments (Fig. 3B). Consumption of live *Microcystis* by *Bosmina* was confirmed by the presence of labeled *Microcystis* markers in the zooplankters (Fig. 3A).

Fatty-acid concentrations—The results on FA composition are only presented for control incubations, where substrate addition did not influence FA composition of the seston. Although the FA found in zooplankton matched the FA in seston, the relative abundance of FA differed (Table 2). The percentage of long-chain PUFA or algae markers was higher in zooplankton than in seston (ANOVA, $F_{2,6} = 19$, $p < 0.05$), which indicated a preferential uptake or incorporation of those markers (Table 2). *Microcystis* markers, however, were more abundant in seston than in zooplankton (ANOVA, $F_{2,6} = 99$, $p < 0.05$; Table 2). *Daphnia* contained relatively more algae and *Microcystis* markers than *Bosmina*. Bacterial markers were most abundant in *Daphnia*, followed by seston, and they were least abundant in *Bosmina* (ANOVA, $F_{2,6} = 103$, $p < 0.05$; Table 2). Overall, *Bosmina* had lower percentages of marker FA than *Daphnia*, because *Bosmina* contained relatively more unsaturated FA, such as 18:0, than did *Daphnia*, and had an overall higher diversity of FA. The percentages of bacteria and algae markers in *Bosmina* and *Daphnia* were similar between the different treatments, but the percentage of *Microcystis* FA differed

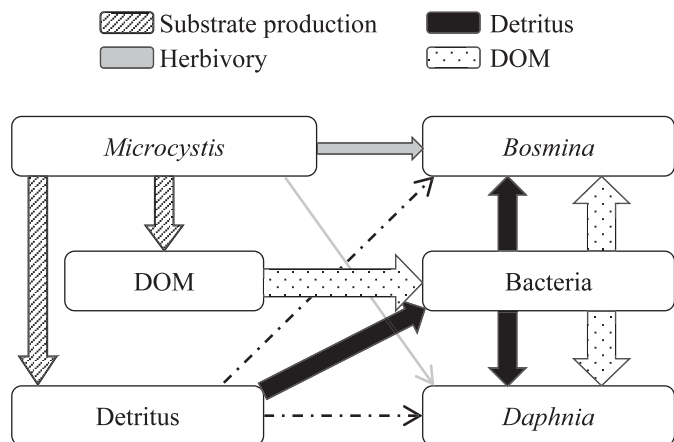


Fig. 4. A scheme of carbon flows from *Microcystis* and *Microcystis*-derived substrates to zooplankton based on our study in Meiliang Bay, Lake Taihu, China during spring 2009. Thickness of the arrow indicates the relative importance. The dashed arrows could not be confirmed.

between treatments. *Microcystis* FA were lower in *Daphnia* in the DOM treatment compared with the other treatments (ANOVA, $F_{3,8} = 13$, $p < 0.05$) and higher in *Bosmina* in the *Microcystis* additions compared with the other treatments (ANOVA, $F_{3,8} = 8.3$, $p < 0.01$).

Discussion

Carbon can flow from *Microcystis* to zooplankton via various pathways (Fig. 4), and it is not simple to identify and disentangle these routes under natural field conditions. Here, we combined stable-carbon isotope at natural abundance, FA as trophic transfer markers, and ^{13}C as a deliberately added tracer as complementary tools. Each approach has its strengths and weaknesses, but together they provide clear evidence for multiple pathways from *Microcystis* to zooplankton, and specifically for the dominance of microbial pathways.

Natural-abundance stable-isotope analyses—Carbon SIA is a powerful tool to study food-web interactions and has been successfully applied to allocate carbon sources of zooplankton. The natural values of carbon isotope ratios have been used to calculate the contribution of *Microcystis* carbon to zooplankton diets with bacteria and eukaryotic algae as the other potential carbon sources. Although stable-isotope signatures of metazoan consumers can be easily determined by handpicking the organisms, determination of the isotope signatures of the potential food sources remains challenging. By using compound-specific isotope analyses, we could resolve the isotope signatures of bacteria, *Microcystis*, and eukaryotic algae. Another pitfall is the natural variability of carbon isotope signatures within sources and the overlap between sources. Phytoplankton cells are known to have variable isotope signatures, which are partly taxa-dependent but also influenced by physiology (e.g., growth) and environmental characteristics, such as temperature and CO_2 availability (Laws et al 1995;

Vuorio et al. 2006). Cyanobacteria display especially large variability in isotope signatures, which can be partly explained by colony-cell density and, subsequently, CO_2 availability. Furthermore, cyanobacteria are known to be able to fix aqueous CO_2 , atmospheric CO_2 , and bicarbonate, which have distinct isotope signatures and thus contribute to variability. In this study, the isotopic difference within *Microcystis* was larger (8‰) than that between *Microcystis* and other algae (< 1‰; Table 1), which complicates resolution of eukaryotic algae vs. cyanobacteria contributions to zooplankton diets. A very large range in isotope signatures of cyanobacteria, from -32‰ to -5.9‰ , was also found in Finnish lakes (Vuorio et al. 2006). Similar to our study, Bontes et al. (2006) observed higher $\delta^{13}\text{C}$ in scum-forming *Microcystis* than in water-column *Microcystis* in a shallow Dutch eutrophic lake. Enrichment in the scum-forming *Microcystis* can be partly explained by CO_2 limitation due to higher density and fixation of atmospheric CO_2 or bicarbonate.

The carbon isotope signature of bacteria resembled more that of POM than that of phytoplankton, which indicated that carbon of the total POM pool was utilized by bacteria rather than by phytoplankton only (Table 1). The enrichment of POM relative to phytoplankton can be explained by the presence of detritus. In lakes, detritus is often found to be enriched in ^{13}C due to preferential mineralization of ^{12}C or presence of enriched allochthonous material (Del Giorgio and France 1996). In Taihu, enriched scum-forming *Microcystis* probably contributed to enriched detritus.

In spite of overlapping resources, it was still possible to differentiate between bacteria-POM vs. phytoplankton (algae and *Microcystis*) contributions to zooplankton diets. In our study, *Bosmina* received a high (average 77%) carbon contribution from POM-bacteria, while copepods were feeding more on algae (average 48%). This is in agreement with the general consensus that cladocerans can graze effectively on bacteria, while copepods generally feed selectively on larger particles (Wylie and Currie 1991). Due to overlap in isotope signatures, feeding of the cladoceran *Bosmina* on bacteria could not be distinguished from consumption of bulk POM. However, the presence of bacterial FA in *Bosmina* indicated that they were grazing on bacteria. The $\delta^{13}\text{C}$ of FA in *Bosmina* matched the $\delta^{13}\text{C}$ of seston FA, providing additional evidence that the zooplankton feeds on bacteria.

Fatty-acid biomarkers—The presence of FA in zooplankton consumers can be used to infer dietary information, specifically feeding on different types of algae and bacteria. FA present in the food are often directly incorporated into consumers and the zooplankton FA profile therefore mimics the diet (Dalsgaard et al. 2003; Taipale et al. 2009). However, zooplankton can selectively assimilate certain FA, making it difficult to apply them in a quantitative way. The PUFA are physiologically essential and cannot be synthesized by zooplankton; therefore, they are considered essential FA. The long-chain PUFA 20:5 ω 3, 20:4 ω 6, and 22:6 ω 3 are high-quality FA for zooplankton, and their absence in the diet can limit growth and

reproduction of cladocerans (Von Elert 2002). These FA will be preferentially assimilated by zooplankton and stored. However, when these FA are short in supply, zooplankton can convert $18\omega3$ PUFA, present in *Microcystis*, into the necessary long-chain PUFAs, although this would be at higher energetic costs. In agreement with previous studies on PUFA concentrations in zooplankton (Brett et al. 2006), zooplankton PUFA content was higher than seston PUFA concentrations in this study, which indicated a preferential assimilation of PUFA (Table 2). Less attention has been paid to the trophic transfer of bacterial FA within the plankton food web (Taipale et al. 2009; Kürten et al. 2011). Bacterial FA are not essential for zooplankton, and whether they semiquantitatively reflect the bacterial contribution to zooplankton diets remains to be tested because these FA are likely metabolized rather than stored or assimilated.

Fatty-acid labeling—In addition to higher concentrations in zooplankton, the algae markers in zooplankton also showed higher labeling than did algae markers in the seston. There are at least three possible explanations for accumulation of labeled algae markers in zooplankton. (1) Zooplankton could selectively assimilate FA of newly produced and, thus, labeled algae. During the experiment, ^{13}C present in the substrates got respired, resulting in ^{13}C increase in DIC, which was subsequently assimilated by primary producers. This can also explain the labeling of algae in the seston (Fig. 2). (2) The algae markers could have been produced by the zooplankton itself, by transformation of ^{13}C -PUFA ($18:3\omega3$, $18:4\omega3$, $18:2\omega6$, and $18:3\omega6$), which were present in *Microcystis* and *Microcystis*-derived substrates. (3) They could have been produced by protists (ciliates and heterotrophic nanoflagellates) as part of the microbial food web, which were subsequently consumed by zooplankton. Protists feeding on bacteria are known to produce PUFA and other essential molecules, causing trophic upgrading of food quality along the food chain (Zhukova and Kharlamenko 1999). In this study, we cannot distinguish transfer of carbon via protists from direct uptake of bacteria by zooplankton, and we considered them all to be part of the microbial food web. The high labeling of bacterial markers in the seston showed that *Microcystis* and *Microcystis*-derived substrates were an important carbon source for bacteria (Fig. 2). The high labeling of bacterial FA in zooplankton suggests consumption of bacteria or bacterivorous protists by zooplankton (Fig. 3). Comparison of FA labeling between the zooplankton species is difficult, because of their different FA profiles (Table 2). For example, higher labeling of bacterial markers in *Bosmina* compared with *Daphnia* could result from higher bacterial consumption but also from lower concentrations in *Bosmina* compared with *Daphnia*. It should also be noted that the produced substrates were not from axenic *Microcystis* cultures, but from field concentrates, where other organisms were present as well. Trace amounts of labeled algae PUFA were present in our labeled *Microcystis* cultures and can potentially explain the relative high labeling of algae in the seston in the live treatments (Fig. 2). *Microcystis* has been shown to be a hot-spot for bacterial activity (Worm and

Søndergaard 1998), and bacterial markers were labeled in the same way as *Microcystis* markers during substrate production. A small part of the measured bacterial labeling can thus be due to substrate labeling.

Bacteria as carbon source—The importance of bacteria as a food source for zooplankton has been long known, from laboratory studies with bacteria as a single food source (Peterson et al. 1978; Porter et al. 1983) or labeled bacteria in mixed assemblages (Gophen et al. 1974) and studies under more natural conditions (Pace et al. 1983). The general consensus is that cladocerans are bacterivores, while copepods are not, so the importance of the microbial loop as a carbon source for zooplankton depends on zooplankton composition (Sanders et al. 1989; Wylie and Currie 1991; Karlsson et al. 2007 [i.e., the presence of cladocerans that feed on bacteria, in addition to algae]). Our findings of $\sim 77\%$ of carbon coming from bacteria in cladocerans and $\sim 23\%$ in copepods, based on an isotope mixing model with natural-abundance data, supports these earlier works. Although copepods are not grazing directly on bacteria, cyclopoid and calanoid copepods can be effective grazers on larger bacterivores, such as ciliates (Sanders and Wickham 1993). The importance of the microbial loop as food source for zooplankton also depends on the amount of bacterial production relative to primary production (Pace et al. 1983; Sanders et al. 1989). Wylie and Currie (1991) calculated that bacterial carbon contributed 16–21% of carbon ingested by cladocerans in an oligotrophic lake based on isotope labeling combined with modeling. In a marine mesocosm experiment with labeled carbon compounds, an equal contribution of bacterial and algae carbon to zooplankton (copepods and doliolida) was found (Koshikawa et al. 1996). In humic lakes, which are largely fuelled by allochthonous carbon, detritus and bacteria can have a high carbon contribution to zooplankton (Hessen et al. 1990). In an isotope-labeling experiment in a humic lake, Hessen et al. (1990), found bacteria growing on labeled DOM to contribute 11–42% to zooplankton carbon, with an even higher contribution of (unlabeled) detritus (46–82%) that could have passed through bacteria as well. The significance of bacteria and the microbial food web as a food source for zooplankton increases with increasing eutrophication (Gliwicz 1969). This phenomenon can be largely explained by the observed phytoplankton community shift toward cyanobacteria, corresponding with lake eutrophication. Still, there are few studies that showed a direct link from cyanobacteria carbon to zooplankton via bacteria and the microbial food web. Christoffersen et al. (1990) studied carbon fluxes in a plankton community during a cyanobacteria bloom, based on carbon budgets and causal relationships. They showed that macrozooplankton (mainly *Daphnia*) were assimilating carbon when cyanobacteria dominated the phytoplankton community, and that this carbon apparently originated from phytoplankton and thus cyanobacteria. Christoffersen et al. (1990) also calculated that cyanobacteria were an important carbon source for bacteria and that macrozooplankton were more important bacterivores than were microzooplankton. Work and Havens (2003) showed that

macrozooplankton were grazing extensively on cyanobacteria and on bacteria in a eutrophic lake, but the authors did not show a link between cyanobacteria and bacteria, as is shown here with the help of isotope labeling. Decomposed *Microcystis* and other cyanobacteria were found to be a more important carbon source for zooplankton than were living ones in a laboratory study by Hanazato and Yasuno (1987) and a labeling study by Gulati et al. (2001). In our study, the pathway from *Microcystis* DOM to bacteria and, subsequently, to zooplankton was more important than the *Microcystis* detritus–bacteria–zooplankton pathway (Figs. 1, 3). It is generally not known what fraction of *Microcystis* ends up in particulate or dissolved form, but both fractions are expected to be significant. Hansen et al. (1986) showed that cyanobacteria-dominated phytoplankton communities lost up to 43% of the cellular carbon content as dissolved carbon within 24 h after cell death and that a large part of the fresh DOM (71%) was used by bacteria in 24 h.

Zooplankton competition—The effect of cyanobacteria on different types of zooplankton and their competitive relations have been extensively studied over the past decades. Traditionally, most studies have focused on food quality of bloom-forming cyanobacteria for grazing zooplankton, and thus on the herbivory pathway. Our results support that zooplankton community changes are mainly caused by differences in grazing behavior; only treatments with live *Microcystis* showed a significant difference in carbon assimilation between *Bosmina* and *Daphnia*, with 48% for *Bosmina* vs. 17% for *Daphnia* (Fig. 1). *Microcystis* is generally considered ‘poor’ food for herbivorous zooplankton; it produces toxic substances, lacks essential nutrients, and can form inedible colonies and filaments (reviewed in DeBernardi and Giussani 1990). In laboratory feeding experiments, cladocerans showed reduced survivorship, growth, and reproduction when they were fed solely with cyanobacteria (reviewed in Lampert 1987). Consistent with our results, Fulton and Paerl (1987) showed higher use efficiency of *Microcystis* carbon by *Bosmina* than by *Daphnia*. The morphology of *Microcystis* colonies has shown to be the most important intrinsic property in grazing inhibition of *Daphnia*, because it interferes with the filtering system (Fulton and Paerl 1987). In the treatment with live *Microcystis*, feeding inhibition in both *Bosmina* and *Daphnia* likely contributed partially to their low consumption due to high densities of *Microcystis* colonies, which are common in Lake Taihu (Qin et al. 2010). Field studies also showed that larger cladocerans are more susceptible to the inhibiting effects are than smaller ones (DeMott et al. 2001; Ghadouani et al. 2006), consistent with the observed lower carbon use in *Daphnia* than *Bosmina* in the experiments with live *Microcystis*. The body size of cladocerans is not a good predictor of ability to graze on bacteria, especially on free-living bacteria. DeMott (1982) studied consumption of bacteria and green algae by *Bosmina* and *Daphnia* and observed that *Bosmina* consumed fewer bacteria than did *Daphnia*, relative to green algae. This could be explained by differences in feeding strategies: *Bosmina* is a more selective

feeder than is *Daphnia* (DeMott and Kerfoot 1982). The results of our study, on the other hand, indicate that *Daphnia* and *Bosmina* are both efficient grazers on total (solitary and attached) bacteria (Figs. 1, 3).

A shift in zooplankton community from large cladocerans toward small-bodied cladocerans is generally observed in eutrophic systems with cyanobacteria blooms (Gliwicz 1969). One important cause is feeding on large zooplankters by planktivorous fish, especially in lakes that are shallow and/or lack a hypolimnetic or macrophyte-bed refuge. As described above, the cyanobacteria bloom itself can contribute to the shift in zooplankton species due to feeding inhibition of larger cladocerans (DeMott et al. 2001; Ghadouani et al. 2006).

The conceptual diagram in Fig. 4 shows the major findings of this study: (1) the cyanobacterium *Microcystis* is an important carbon source for zooplankton growth in Taihu; (2) *Microcystis* carbon flows to zooplankton mainly via DOM and detritus; and (3) heterotrophic bacteria play a key role in the carbon flow from *Microcystis* DOM and detritus to zooplankton. Moreover, *Bosmina* assimilated relatively more *Microcystis* carbon than did *Daphnia*, mainly because *Bosmina* grazed more on live *Microcystis* than did *Daphnia*.

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