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Carbon transfer from dissolved organic carbon to the cladoceran *Bosmina*: a mesocosm study

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Abstract – A mesocosm study illuminated possible transfer pathways for dissolved organic carbon from the water column to zooplankton. Organic carbon was added as ^{13}C enriched glucose to 15 mesocosms filled with natural lake water. Stable isotope analysis and phospholipid fatty acids-based stable isotope probing were used to trace the incorporation of ^{13}C into the cladoceran *Bosmina* and its potential food items. Glucose-C was shown to be assimilated into phytoplankton (including fungi and heterotrophic protists), bacteria and *Bosmina*, all of which became enriched with ^{13}C during the experiment. The study suggests that bacteria play an important role in the transfer of glucose-C to *Bosmina*. Furthermore, osmotic algae, fungi and heterotrophic protists might also contribute to the isotopic signature changes observed in *Bosmina*. These findings help to clarify the contribution of dissolved organic carbon to zooplankton and its potential pathways.

Keywords: t-DOC / bacteria / *Bosmina* / PLFA-SIP

Résumé – Transfert de carbone du carbone organique dissous au cladocère *Bosmina* : une étude en mésocosmes. Une étude de mésocosmes a permis d'éclairer les voies de transfert possibles pour le carbone organique dissous de la colonne d'eau au zooplancton. Le carbone organique a été ajouté sous forme de glucose enrichi en ^{13}C à 15 mésocosmes remplis d'eau naturelle du lac. Une analyse d'isotope stable et un marqueur isotopique stable à base de phospholipides ont été utilisés pour tracer l'incorporation du ^{13}C dans le cladocère *Bosmina* et ses sources alimentaires potentielles. On a montré que le glucose-C était assimilé dans le phytoplancton (y compris les champignons et les protistes hétérotrophes), les bactéries et *Bosmina*, qui ont tous été enrichis en ^{13}C pendant l'expérience. L'étude suggère que les bactéries jouent un rôle important dans le transfert du glucose-C à *Bosmina*. En outre, les algues, les champignons et les protistes hétérotrophes pourraient également contribuer aux changements de signature isotopique observés chez *Bosmina*. Ces résultats permettent de clarifier la contribution du carbone organique dissous au zooplancton et ses voies potentielles.

Mots-clés : t-DOC / bactérie / *Bosmina* / PLFA-SIP

As a constituent of organic carbon content of lakes, terrestrial dissolved organic carbon (t-DOC) has significant ecological effects on food webs by serving as a substrate for heterotrophic metabolism (Tranvik, 1998; Karlsson *et al.*, 2003; Solomon *et al.*, 2015). Despite evidence of terrestrial support for secondary production in lakes (Berggren *et al.*, 2010a,b; Tanentzap *et al.*, 2015), the transfer of t-DOC to higher trophic levels remains contentious (Ducklow *et al.*, 1986; Brett *et al.*, 2012; Kelly *et al.*, 2016).

Previous studies have used mesocosms with artificial t-DOC additions to address whether t-DOC affects zooplankton. Most published studies report zooplankton density increases after t-DOC input (Faithful *et al.*, 2011; Cooke *et al.*, 2015; Geddes, 2015). However, Kelly *et al.* (2016) suggested that it is the increase t-DOC-associated phosphorus, rather than t-DOC itself that boosts phytoplankton growth and ultimately causes zooplankton density increases. Others have confirmed zooplankton assimilation of t-DOC based on the natural abundance of stable carbon isotopes ($\delta^{13}C$) as a tracer (Karlsson *et al.*, 2007; Hitchcock *et al.*, 2016). Yet sometimes question exists when differences in $\delta^{13}C$ between terrestrial

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and aquatic primary producers are too small to distinguish by this technique.

Major concern of t-DOC transfer to zooplankton centers on food quality (Brett *et al.*, 2012; Taipale *et al.*, 2014). Bacteria grown on t-DOC lack some biochemicals essential for the growth and reproduction of consumers (*e.g.* highly-unsaturated fatty acids, HUFA), and have thus been deemed unsuitable as a sole food source (Martin-Creuzburg *et al.*, 2011). However, mixed algae-bacteria diet can support the growth and reproduction of zooplankton (Wenzel *et al.*, 2012). Besides, microbial food for zooplankton consists of additional heterotrophic microbes in addition to bacteria, *e.g.* fungi and bactivorous protists (Sherr and Sherr, 1994; Rösel *et al.*, 2012). Their ability of HUFA synthesis was previously reported (Hauvermale *et al.*, 2006; Chu *et al.*, 2008). So, we hypothesized that heterotrophic microbes may play important roles in transferring t-DOC to zooplankton.

To test our hypothesis, we traced the fate of ^{13}C enriched glucose added to 15 aquatic mesocosms during June to July, 2013. Similar-sized microbes like algae and bacteria being difficult separated for sampling could be distinguished by PLFA (phospholipid fatty acids), for which specific microbial groups produce signature PLFA profiles (Boschker and Middelburg, 2002). Moreover, microbial PLFA and whole-cell share similar isotopic signatures (De Kluijver *et al.*, 2015). Hence carbon stable isotopic analysis of their signature PLFA can be used to study the source of carbon they assimilated (PLFA-based stable isotope probing, PLFA-SIP), and is applied in our experiment.

Each mesocosm contained 100 L surface water from the shore of Fuxian Lake in China (24°21′28″–24°38′00″N, 102°49′12″–102°57′26″E). Fuxian Lake with an area of 211 km^2, is a deep oligotrophic lake with low phosphorus content, while with macrophyte in shores. After setting up, 3 mesocosms were sampled to provide reference values (T0). 30 mg ^{13}C-glucose was then added to rest 12 mesocosms, and each three mesocosms were sampled on days 1, 3, 6 and 9 during the experiment (T1, T3, T6 and T9 respectively). Each mesocosm was sampled only once. Total nitrogen (TN), total phosphorus (TP) and Chlorophyll *a* (Chl *a*) concentrations of mesocosm water were measured as previously described (Zhang *et al.*, 2016). DOM (dissolve organic matter) concentrations were analyzed using a TOC analyzer (Shimadzu TOC-L CPH CN200, Japan).

Both particulate organic matter (POM) and microbial samples were gathered by filtering 2–5 L water through Whatman (GF-F) glass-fiber filters (pore size 0.7 μm). Water after filtration was dried 48 °C to form DOM samples. Zooplankton was collected from the mesocosms using a 64 μm mesh-size net. The dominant species in all mesocosms, *Bosmina* sp., was picked out. POM and *Bosmina* samples were dried also at 48 °C and subjected to carbon isotopic analysis by carrying on an EA 1112 elemental analyzer coupled to an isotope ratio mass spectrometer (Thermo Finnigan Delta V) together with DOM samples. Phospholipid fatty acids of microbial samples were extracted according to Guckert *et al.* (1985). $\delta^{13}C$ of single PLFA was detected by a GC-c-IRMS (Thermo Finnigan, Germany). Isotopic analysis was conducted in Key Laboratory of Global Change and Marine-Atmospheric Chemistry, State Oceanic Administration, China. Based on sample replicates, the analytical precision was 0.3‰ for $\delta^{13}C$.

The concentration-weighted $\delta^{13}C$ of branched fatty acids i15:0 and ai15:0 were used to symbolize bacterial $\delta^{13}C$ ($\delta^{13}C_{bacteria}$) according to Boschker and Middelburg (2002). $\delta^{13}C$ of phytoplankton ($\delta^{13}C_{phytoplankton}$) was calculated as concentration-weighted $\delta^{13}C$ of polyunsaturated fatty acids 18:2ω6, 18:3ω6, 20:5ω3 and 22:6ω3 (including cyanobacteria and eukaryotic algae), as recommended by De Kluijver *et al.* (2015). However, since 18:2ω6 and 18:3ω6 are also found in fungi (Wurzbacher *et al.*, 2010), fungi must be considered as potential contributors to the observed phytoplankton isotopic signatures. One-way ANOVA and repeated measures ANOVA were conducted to compare stable isotopic signatures in different mesocosms and between different organisms respectively in SPSS 18.0. Correlation analysis used Pearson Correlation also in SPSS. Key physiochemical information of mesocosms is given in Table 1. All stable isotopic signatures are shown in Figure 1.

$^{13}C_{bacteria}$ values started at averaged −23.1‰. Subsequently, a significant increase in $\delta^{13}C_{bacteria}$ was observed on day 1 samples ($P < 0.05$), consistent with massive t-DOC support for bacterial production (Cole *et al.*, 2006). In following days, however, $\delta^{13}C_{bacteria}$ fell rapidly. Quickly use up of labile glucose and following more incorporation of depleted DOM into bacterial biomass is a potential factor in decline of $^{13}C_{bacteria}$ values. Besides, a bias towards allochthonous carbon in bacterial respiration reported (Karlsson and Jonsson, 2007) probably contributes to this rapid decrease of $\delta^{13}C_{bacteria}$.

Particulate organic matter comprises a mixture of bacteria, phytoplankton, detrital aggregates and other material, with an initial average $\delta^{13}C_{POM}$ of −14‰. $\delta^{13}C_{POM}$ was closely correlated to $\delta^{13}C_{bacteria}$ ($r = 0.984$, $P < 0.001$) and changes in $\delta^{13}C_{POM}$ recorded in the mesocosms were consistent with $\delta^{13}C_{bacteria}$. In experiments designed to record microbial uptake of ^{3}H-labeled glucose and acetate, Paerl (1974) concluded that radioactive isotopic signature could be passed to POM by bacterial uptake and their help in the formation of detrital aggregates.

$\delta^{13}C_{DOM}$ of −14.8‰ in T0 mesocosm was observed to increase by a relatively small amount on the first day and increased gradually in following days, indicating that ^{13}C-glucose supplement was exhausted in the very first day. $^{13}C_{DOM}$ values that persist later in the experiment are likely due to excretion by bacteria and zooplankton.

$\delta^{13}C_{phytoplantkon}$ was also affected by ^{13}C-glucose addition, increasing from −26.3‰ on average to a maximum of 222.6‰. There are three plausible explanations for this observed increase. Firstly, the inclusion of heterotrophic fungi, which can be supported by extra organic carbon, *e.g.* t-DOC (Rösel *et al.*, 2012). Second, the presence of mixotrophic and heterotrophic protists by grazing on bacteria or osmosis as well as osmotrophic algae, which are able to assimilate DOC and synthesize HUFA (Jones, 2000; Tittel *et al.*, 2009). A third possibility is that dissolved inorganic carbon (DIC) could be derived from respiration of non-autochthonous carbon (Karlsson and Jonsson, 2007), possibly supporting the growth of autotrophic phytoplankton. Since all these fungi, mixotrophic and heterotrophic protists, as well as algae are available as potential food for zooplankton, they offer a variety of trophic transfer pathway for glucose-C into zooplankton.

A significant increase in $\delta^{13}C_{zooplankton}$ was also observed after glucose addition ($P < 0.05$), and $\delta^{13}C_{zooplankton}$ values

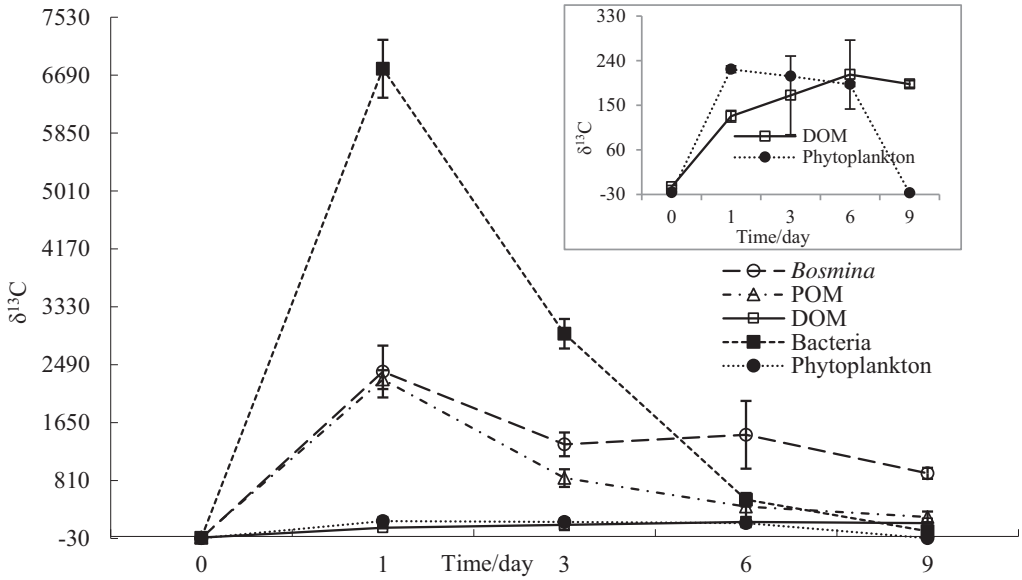

Fig. 1. Carbon stable isotope signatures of bacteria (indicated by PLFA), POM, DOM, phytoplankton (indicated by PLFA) and *Bosmina* in mesocosms after 0 d, 1 d, 3 d, 6 d, 9 d incubation with ^{13}C-glucose (mean ± SD).

Table 1. Physiochemical information of experimental groups. *Note*: Values given in average and – indicates not detected.

No	Sampling date	TN (mg/L)	TP (mg/L)	Chl *a* (μg/L)	DOM (mg/L)	*Bosmina* predator
T0	June 29	0.34	0.007	5.3	1.399	–
T1	June 30	0.34	0.015	7.2	1.360	–
T3	July 2	0.43	0.020	9.7	1.542	–
T6	July 5	0.31	0.013	3.8	1.646	–
T9	July 8	0.38	0.013	4.7	1.899	–

remained significantly higher than those of phytoplankton thereafter ($P < 0.05$). These results confirmed the rapid assimilation of glucose-C by *Bosmina*. $\delta^{13}C_{zooplankton}$ was substantially higher than $\delta^{13}C_{POM}$ after glucose addition ($P < 0.05$) indicating that *Bosmina* had utilized an isotopically heavier food source than the analyzed POM fraction. Only bacteria were isotopically heavier than POM, suggesting selective consumption of bacteria by *Bosmina*. Freese and Martin-Creuzburg (2013) found that *Daphnia magna* grew better with a mixed bacteria-algae diet than sole algae, possibly due to bacteria-derived nutrients, *e.g.* vitamins, which could also explain our results.

In summary, our mesocosm experiments confirm the rapid incorporation of glucose-C by *Bosmina*. The results provide evidence for a transfer of carbon by direct consumption of bacteria. Besides, fungi, mixotrophic and heterotrophic protists as well as algae offer further likely pathways whereby from glucose-C may be assimilated by zooplankton. To be noticed, t-DOC is a more recalcitrant carbon than labile glucose. However, Berggren *et al.* (2010a) and Attermeyer *et al.* (2014) reported t-DOC to include some low molecular weight substances labile to bacteria thus similar to glucose. Considering that labile part of t-DOC has an unproportionally large impact on aquatic secondary production compared its share of total t-DOC (Berggren *et al.*, 2010b), this experiment hints at possible transfer pathways for DOC included t-DOC into zooplankton.

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