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Selective assimilation by deposit feeders: Experimental evidence using stable isotope ratios

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Summary

Deposit-feeding animals form important links between sediment organic matter (SOM) and carnivorous fish in aquatic ecosystem food webs. They inevitably assimilate food from different origins in a selective manner from SOM, including benthic and planktonic microalgae. Lake Katanuma has a very simple community structure due to its strong acidity. It includes chironomid larvae (*Chironomus acerbiphilus*) as the consumer, and two primary producers: a benthic diatom (*Pinnularia acidojaponica*) and a planktonic green alga (*Chlamydomonas acidophila*). We collected the deposit feeder *C. acerbiphilus*, its potential food (phytoplankton, benthic diatoms, and terrestrial plant litter), and the sediments forming its diet from Lake Katanuma, and conducted feeding experiments to determine the required parameters for the mixing model. The results of the isotope-mixing model clearly showed that *C. acerbiphilus* larvae selectively assimilated phytoplankton and benthic diatoms as fresh deposits from bulk sediments, and assimilated phytoplankton more readily than benthic diatoms. Moreover, the assimilation rate of benthic diatoms from sediments by *C. acerbiphilus* larvae tended to decrease with increasing water depth due to the decrease in benthic primary production, while the rate of phytoplankton tended to increase with depth.

Zusammenfassung

Die Sedimentfresser bilden wichtige Verbindungen zwischen dem organischen Material des Sediments (SOM) und den carnivoren Fischen in aquatischen Nahrungsnetzen. Sie nehmen unweigerlich Nahrung verschiedenen Ursprungs in selektiver Weise aus dem SOM auf, einschließlich benthischer und planktonischer Algen. Lake Katanuma hat eine sehr einfache Lebensgemeinschaftsstruktur aufgrund seiner starken Übersäuerung. Sie schließt Zuckmückenlarven (*Chironomus*

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acerbiphilus) als Konsumenten und zwei Primärproduzenten ein: eine benthische Kieselalge (*Pinnularia acidojaponica*) und eine planktonische Grünalge (*Chlamydomonas acidophila)*. Wir sammelten den Sedimentfresser *C. acerbiphilus*, seine potenzielle Nahrung (Phytoplankton, bentische Kieselalgen und Streu terrestrischer Pflanzen) sowie die Sedimente, die seine Nahrung im Lake Katanuma bilden, und führten Fütterungsexperimente durch, um die notwendigen Parameter für das Mischungsmodell zu bestimmen. Die Ergebnisse des Isotopen-Mischungsmodells zeigten deutlich, dass die *C. acerbiphilus*-Larven selektiv Phytoplankton und benthische Kieselalgen als frische Sedimente aus der Hauptsedimentmasse aufnahmen, und dass sie Phytoplankton bereitwilliger aufnahmen als benthische Kieselalgen. Darüber hinaus zeigte bei *C. acerbiphilus* die Aufnahmerate für benthische Kieselalgen aus dem Sediment aufgrund der Abnahme der benthischen Primärproduktion die Tendenz zur Abnahme mit zunehmender Wassertiefe, während die Rate für Phytoplankton die Tendenz zeigte mit zunehmender Tiefe zu steigen.

Introduction

Deposit-feeding animals form important links between sediment organic matter (SOM) and carnivorous fish in aquatic ecosystem food webs. They feed on SOM derived from several sources, including many species of benthic and planktonic microalgae and aquatic and terrestrial plants. They inevitably assimilate food sources from SOM, selectively. In the littoral zone of lakes, benthic algae serve as a food source for benthic animals (Bootsma, Hecky, Hesslein, & Turner, 1996; Vadeboncoeur et al., 2003; Yoshii, 1999). However, the contribution of benthic microalgae to benthic food webs has not been quantified in lake ecosystems, and the importance of benthic algae as an energy resource for the littoral invertebrate assemblage is poorly resolved for lakes (Lamberti, 1996; Wetzel, 2001). Therefore, it is necessary to determine the selective assimilation of deposit feeders to fully understand the benthic food web in lake ecosystems.

Selective assimilation is difficult to determine using only indirect methods, such as gut content and feces analyses. In contrast, stable isotope ratios are a powerful tool for investigating the food sources assimilated by deposit feeders. Carbon and nitrogen stable isotope ratios (δ^{13}C and δ^{15}N) are used to analyze food sources. The assimilation rates of each source can be calculated using isotope mixing models, which require the isotope fractionation value (Δ^{13}C and Δ^{15}N) of the animal as well as the δ^{13}C and δ^{15}N values (units, ‰) of each food source and the animals that feed on it. However, it is not always easy to measure and determine these values, especially the isotope fractionation values. Consequently, mixing models commonly use general Δ^{13}C and Δ^{15}N values for consumers and their food of 0.8 ± 1.1‰ and 3.4 ± 1.1‰, respectively, although Δ^{13}C and Δ^{15}N vary over the range -0.6–2.7‰ and 1.3–5.3‰, respectively (DeNiro & Epstein, 1978; Minagawa & Wada, 1984). Moreover, in the recent studies, the Δ^{15}N values of all studied taxa and the consumers in freshwater are 2.54 ± 0.11‰ and 2.98‰, respectively (Vanderklift & Ponsard, 2003), and the Δ^{13}C and Δ^{15}N values of species are 0.4 ± 0.12‰ (from -3‰ to 3‰) and 2.0 ± 0.20‰ (from 0‰ to 4‰) (McCutchan, Lewis, Kendall, & McGrath, 2003). Therefore, it could be misleading to infer average isotope fractionations that do not take into account species differences. Species-specific isotope fractionation is needed to calculate the precise assimilation of each food source. However, few studies have used species-specific fractionation in mixing model calculations (but Grey, Jones, & Sleep, 2001).

In this study, we examined the simple ecosystem of Lake Katanuma, an acidic volcanic lake (mean pH 2.2). The community structure is very simple, due to the lake's strong acidity. There is only one consumer, a deposit-feeding chironomid larva (*Chironomus acerbiphilus* Tokunaga). The primary producers are limited to two dominant species of microalgae: the benthic diatom *Pinnularia acidojaponica* (Idei and Mayama) and the planktonic green alga *Chlamydomonas acidophila* (Negoro) (Doi et al., 2003a; Shikano, Kikuchi, Takagi, & Doi, 2004).

For the detailed food source analysis using stable isotopes, it is required that the food sources (such as primary producers and consumers) can be collected separately and their isotope ratios can be determined. However, it is generally very difficult to separate microalgal species, which are the main producers in lake ecosystems, from sediment and phytoplankton community.

Therefore, the isotope ratios of microalgae are generally measured for an entire algal community as particulate organic matter (POM, mainly phytoplankton.) or benthic algal mat (e.g., Bootsma et al., 1996; Yoshii, 1999). In Lake Katanuma, the microalgal community is very simple, with only one phytoplankton species, *C. acidophila*, and one benthic diatom species, *P. acidojaponica*. In addition, *C. acidophila* often accumulates at the water surface, producing dark-green scum close to the lakeshore. During these periods, almost pure samples of *C. acidophila* can be obtained for use in experiments. Furthermore the benthic diatom *P. acidojaponica* cells can be separated from the sediment using their phototactic movement (Coach, 1989; Doi et al., 2003a, b). Thus, almost pure samples of *C. acidophila* and *P. acidojaponica* are obtained, which can be used for feeding experiments.

We conducted feeding experiments using several diets and *C. acerbiphilus* as the consumer. The diets consisted of SOM including planktonic, benthic microalgae, and terrestrial plant litter collected from Lake Katanuma as the food sources. Our experimental procedure was as follows: (1) we determined the specific isotope fractionation of *C. acerbiphilus* larvae in feeding experiments; (2) we determined the isotope values of the chironomids after feeding on Lake Katanuma diets; (3) we calculated the assimilation rates of each food source as the *C. acerbiphilus* larva biomass using isotope mixing models; and (4) we examined the changes in the food sources of the chironomids using sediments from different depths as diets.

Materials and methods

Diets for the feeding experiment

Six types of diet were collected directly from Lake Katanuma for the feeding experiments: benthic diatoms (*P. acidojaponica*), phytoplankton (*C. acidophila*), litter from terrestrial higher plants, and surface sediments from depths of 0.5, 2, and 10 m. In addition to these diets, a mixture of benthic diatoms and phytoplankton was examined to investigate the selective assimilation of *C. acerbiphilus* larvae in each diet experimentally.

The diets were collected from Lake Katanuma in June 2001. Phytoplankton (*C. acidophila*) often accumulates at the water surface near the shore and turns the water surface to a dark green color. On such occasions, surface water containing extremely high concentrations of *C. acidophila* was collected and freeze dried to obtain pure *C. acidophila* samples. Benthic diatoms, *P. acidojaponica*, were separated from sediments (depth of 0–1 cm), which were collected at 1 m water depth, by making use of the phototactic movement of *P. acidojaponica* (Coach, 1989; Doi et al., 2003a, b). Terrestrial plant litter (predominantly oak litter; C_3 plants) was collected using an Ekman–Birge grab from a depth of 2 m and washed with deionized water. Surface sediment samples (depth of 0–1 cm) were collected from the lake with an Ekman–Birge grab at depths of 0.5, 2, and 10 m. All the materials were preserved at −20 °C until used in the feeding experiments. A mixed diet was prepared by combining phytoplankton and benthic diatoms in a ratio of 1:2.8. In this ratio, the same amounts of carbon were derived from phytoplankton and benthic diatoms, because the mean carbon contents of the phytoplankton and benthic diatoms were 27.6% and 9.7%, respectively.

Feeding experiment and isotope measurement

Eggs of *C. acerbiphilus* were collected from the lakeshore, and allowed to hatch in Petri dishes containing filtered lake water. We reared the larvae in Petri dishes (2.3 cm diameter) containing 2 cm of filtered lake water at 20 °C. About 10 first instar larvae were put in each dish. During the feeding experiments, the chironomids were transferred to new Petri dishes and provided fresh diet at 3-day intervals. Dead larvae were removed. The amount of food provided each day was arbitrary, approximately 5–20 mg per individual, but had the same carbon contents and was increased gradually with larval growth. After reaching the third instar, individual larvae were put in dishes establishing five replicates. Feeding experiments were conducted for 25 days until all larvae in each dish had reached the fourth instar. Fourth instar larvae were collected and put in filtered lake water for 24 h to eliminate their gut contents before freeze-drying.

All the samples were freeze dried and preserved in a freezer at −20 °C until the isotope ratio and elemental analyses were determined. The carbon and nitrogen contents of the diets were measured using a CN analyzer (NA-2500; CE Instruments) with four replicates. The carbon and nitrogen isotope ratios of the samples were measured with a mass spectrometer (DELTA plus; Finnigan Mat) with four replicates. The results are reported using delta notation obtained as follows:

$$\delta^{13}\mathrm{C}\ \text{or}\ \delta^{15}\mathrm{N} = (R_{\mathrm{sample}}/R_{\mathrm{standard}} - 1) \cdot 1000(‰),$$

where R is the $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratio for $\delta^{13}C$ or $\delta^{15}N$, respectively. As standards, PDB was used for $\delta^{13}C$ and air nitrogen was used for $\delta^{15}N$. The analysis errors were within $\pm 0.2‰$ for both $\delta^{13}C$ and $\delta^{15}N$.

Isotope fractionation and mixing model of *C. acerbiphilus* larvae

We calculated the isotope fractionation values ($\Delta^{13}C$ and $\Delta^{15}N$) of the chironomid larvae from their diets. The $\Delta^{13}C$ and $\Delta^{15}N$ values are defined as

$$\Delta^{13}C \text{ or } \Delta^{15}N(‰) = \delta X_{consumer} - \delta X_{diet} (\text{with } X = {}^{13}C \text{ or } {}^{15}N). \quad (1)$$

To determine which of the two food sources (phytoplankton and benthic diatoms) and three food sources (phytoplankton, benthic diatoms, and terrestrial plant litter) were assimilated more readily by *C. acerbiphilus*, larvae were fed each diet. We calculated the contribution of each source to the biomass of the chironomids using two- and three-source mixing models, with reference to Phillips (2001), respectively. The models are defined as follows:

Two-source mixing model:

$$\delta^{13}C_c = f_p(\delta^{13}C_p + \Delta^{13}C) + f_b(\delta^{13}C_b + \Delta^{13}C), \quad (2)$$

$$f_p + f_b = 1. \quad (3)$$

Three-source mixing model:

$$\delta^{13}C_c = f_p(\delta^{13}C_p + \Delta^{13}C) + f_b(\delta^{13}C_b + \Delta^{13}C) + f_t(\delta^{13}C_t + \Delta^{13}C), \quad (4)$$

$$\delta^{15}N_c = f_p(\delta^{15}N_p + \Delta^{15}N) + f_b(\delta^{15}N_b + \Delta^{15}N) + f_t(\delta^{15}N_t + \Delta^{15}N), \quad (5)$$

$$f_p + f_b + f_t = 1, \quad (6)$$

where the subscripts c, p, b, and t refer to the chironomid larvae, phytoplankton, benthic diatoms, and terrestrial plant litter, respectively; f_p, f_b, and f_t are the contributions of phytoplankton, benthic diatoms, and terrestrial plant litter as food sources to the chironomid biomass, respectively; and $\Delta^{13}C$ and $\Delta^{15}N$ are the carbon and nitrogen isotope fractionations of the chironomid larvae obtained in our feeding experiment.

Results

Isotope ratios and organic contents of the diets

Table 1 shows $\delta^{13}C$, $\delta^{15}N$, and the carbon and nitrogen contents of the diets used in the feeding experiment. The $\delta^{13}C$ values of benthic diatoms, phytoplankton, and terrestrial plant litter differed significantly (multiple comparison Holm test, $p<0.01$, $n=4$). The $\delta^{13}C$ of benthic diatoms was the highest and that of litter was the lowest. In contrast, the $\delta^{15}N$ of phytoplankton was significantly lower than those of benthic diatoms and plant litter (Holm test, $p<0.01$, $n=4$). The three food sources used in our feeding experiments had distinct isotope signatures and could consequently be used to evaluate the contribution of each diet to the chironomids using isotope mixing models. Moreover, the $\delta^{13}C$ of phytoplankton were not significantly different from those of phytoplankton, which were not blooming at 1 m depth ($-25.7\pm0.7‰$, Doi et al., 2003b) (t-test, $p>0.05$, $n=4$ (this study) and 3 (Doi et al., 2003b)). The $\delta^{13}C$ and $\delta^{15}N$ of mixed diets combining phytoplankton and benthic diatoms were significantly different from those of phytoplankton and benthic diatoms (Holm test, $p<0.01$, $n=4$). The $\delta^{15}N$ of mixed diets is closer to the phytoplankton, since the nitrogen contents were different between benthic diatoms (1.7%) and phytoplankton (3.9%).

The $\delta^{13}C$ of surface sediments differed significantly with depth (Holm test, $p<0.05$, $n=4$). The $\delta^{13}C$ and $\delta^{15}N$ of sediments from 0.5 m were similar to those of pure benthic diatoms (Fig. 1), and the SOM is composed mainly of fresh benthic diatoms. In contrast, the $\delta^{13}C$ of sediments from 10 m was similar to that of phytoplankton (Table 1), and the SOM is derived mainly from phytoplankton (see Doi et al., 2003a). Both the $\delta^{13}C$ and $\delta^{15}N$ of sediments from 2 m were intermediate between benthic diatoms and phytoplankton, and the SOM is

Table 1. $\delta^{13}C$, $\delta^{15}N$, and carbon and nitrogen contents of the diets used in the feeding experiments The values are given as means $\pm$ 1 s.d. ($n=4$)

Diets	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	C (%)	N (%)
Benthic diatoms	-22.1 ± 0.5	-0.1 ± 1.0	9.7 ± 0.3	1.7 ± 0.2
Phytoplankton	-24.3 ± 0.7	-5.4 ± 1.4	27.6 ± 1.9	3.9 ± 0.5
Mixed diet	-22.8 ± 0.2	-4.3 ± 0.8	20.6 ± 1.7	2.8 ± 0.5
Plant litter	-28.2 ± 0.1	-0.3 ± 0.6	46.6 ± 5.3	0.6 ± 0.2
Sediment (0.5 m)	-22.4 ± 0.5	-0.1 ± 0.8	12.5 ± 1.0	0.8 ± 0.1
Sediment (2 m)	-22.8 ± 0.5	-1.5 ± 0.8	5.7 ± 1.1	0.4 ± 0.1
Sediment (10 m)	-24.2 ± 0.7	-1.8 ± 0.8	1.9 ± 1.2	0.1 ± 0.2

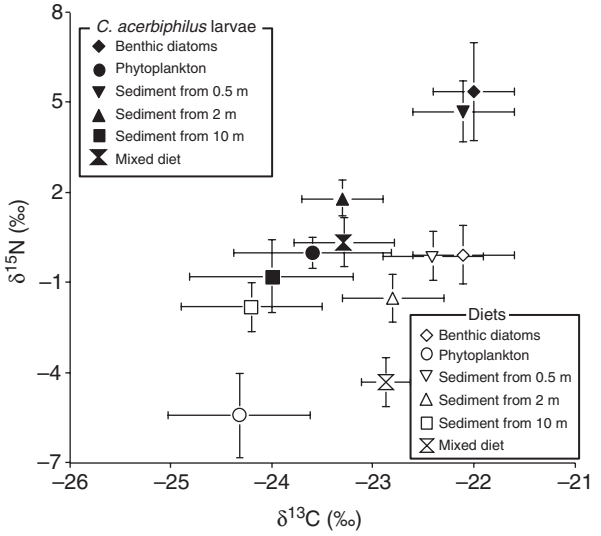

Figure 1. δ^{13}C and δ^{15}N plots of *C. acerbiphilus* larvae and the diets (except plant litter). The symbols are the mean δ^{13}C and δ^{15}N for *C. acerbiphilus* larvae and the various diets. The error bars indicate ± 1 s.d. ($n = 4$).

composed mainly of benthic diatoms and phytoplankton.

Carbon and nitrogen isotope ratios of *C. acerbiphilus* larvae

In our feeding experiments, we attempted to raise *C. acerbiphilus* larvae fed on seven diets to the fourth instar. However, *C. acerbiphilus* larvae could not grow to the third instar when fed on plant litter only. We raised chironomid larvae fed on the other six diets (benthic diatoms, phytoplankton, sediments from depths of 0.5, 2, and 10 m, and mixed diet) to the fourth instar and measured their carbon and nitrogen isotope values.

The δ^{13}C and δ^{15}N of the diets and the chironomid larvae fed on these diets are shown in Fig. 1. Both the δ^{13}C and δ^{15}N of chironomid larvae fed phytoplankton were significantly lower than those fed on benthic diatoms (*t*-test, $p < 0.001$, $n = 4$). The δ^{13}C and δ^{15}N of the chironomid larvae fed on the mixed diet were close to those of larvae fed phytoplankton only (Fig. 1). The δ^{13}C and δ^{15}N of chironomid larvae fed sediments from 0.5 m were similar to those of larvae fed on benthic diatoms (Fig. 1). In contrast, the δ^{13}C and δ^{15}N of chironomid larvae fed sediments from a depth of 10 m were similar to those of the larvae fed phytoplankton only (Fig. 1). The values for chironomid larvae fed sediments from 2 m were intermediate between those fed pure diets of phytoplankton and benthic diatoms, and closer to those of chironomid larvae fed phytoplankton.

Isotope fractionation and mixing model of *C. acerbiphilus* larvae

The Δ^{13}C and Δ^{15}N of chironomid larvae fed on phytoplankton were 0.3 ± 0.5‰ and 5.7 ± 1.1‰ (mean ± 1 s.d., $n = 4$), respectively, and the values of those fed on benthic diatoms were 0.1 ± 0.6‰ and 5.4 ± 1.9‰, respectively. These values were similar irrespective of the food source (phytoplankton and benthic diatoms). The mean Δ^{13}C and Δ^{15}N of the chironomid larvae fed on benthic diatoms and phytoplankton were 0.3 ± 0.7‰ and 5.4 ± 1.2‰, respectively. These average Δ^{13}C and Δ^{15}N values were used as the specific fractionation of *C. acerbiphilus* larvae to calculate the isotope mixing models.

The contributions of each food source to the *C. acerbiphilus* larvae biomass, as estimated by the two- or three-source mixing model (Eqs. (2)–(6)), are shown in Fig. 2. The results of the two-source mixing model indicated that *C. acerbiphilus* larvae assimilated more phytoplankton (78%) than benthic diatoms (22%) when fed the mixed diet. The contribution of each food source to chironomid larvae biomass fed sediments from a depth of 0.5 m was calculated using the three-source mixing model. The results showed that the contribution of benthic diatoms (84%) was much higher than that of phytoplankton (9%) and plant litter (7%). When fed on sediments from 10 m, the contribution of phytoplankton (86%) was much higher than that of plant litter (12%) and benthic diatoms (2%). When fed on sediments from 2 m, the contributions of phytoplankton, benthic diatoms, and plant litter were 66%, 33%, and 1%, respectively.

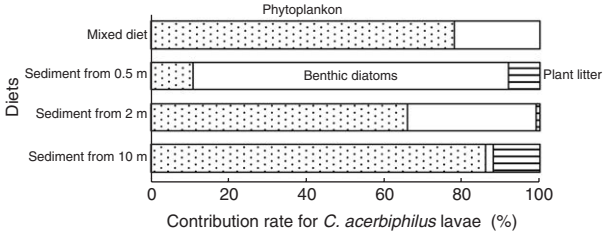

Figure 2. Contributions (%) of three food sources (phytoplankton, benthic diatoms, and plant litter) to *C. acerbiphilus* larvae fed sediments from each depth and mixed diet calculated using the isotope mixing model. Dotted, white, and crosshatched areas represent the contributions of phytoplankton, benthic diatoms, and plant litter, respectively.

Discussion

Growth of *C. acerbiphilus* larvae in the feeding experiments

In Lake Katanuma, the SOM is ingested by *C. acerbiphilus* larvae (Doi, H., per. obs.). In our experiment, *C. acerbiphilus* larvae could not grow to the third instar when fed plant litter only, indicating that terrestrial plant litter lacks sufficient nutrients to support the growth of chironomid larvae. However, feeding benthic diatoms or phytoplankton alone, we could raise chironomid larvae to the fourth instar. Terrestrial litter is composed mainly of refractory substances, such as cellulose and lignin, which are indigestible by aquatic invertebrates (Cividanes, Incera, & López, 2002). Conversely, microalgae contain large amounts of easily digestible substances, such as proteins and simple carbohydrates (Tenore, 1983), and microalgae appear to have a high potential as a food source for benthic animals. In addition, *C. acerbiphilus* larvae are confirmed surface-deposit feeders. In the feeding experiments, they ingested and assimilated SOM from surface sediments collected from Lake Katanuma, and *C. acerbiphilus* larvae were observed to ingest SOM unselectively.

Isotope fractionation of *C. acerbiphilus* larvae

We determined that the $\Delta^{13}C$ and $\Delta^{15}N$ of chironomid larvae fed on pure phytoplankton or benthic diatoms alone had similar values, as we need not consider the effect of selective assimilation when there is only one food source. Therefore, we used these mean values in isotope mixing models as the true fractionation of *C. acerbiphilus* larvae, which occurs as a result of animal metabolism.

The general $\Delta^{13}C$ and $\Delta^{15}N$ values of a species are $0.8 \pm 1.1‰$ and $3.4 \pm 1.1‰$, respectively (DeNiro & Epstein, 1978; Minagawa & Wada, 1984). Thus, many researchers have used these values, although $\Delta^{13}C$ and $\Delta^{15}N$ vary with species over fairly wide ranges ($\Delta^{13}C$ $-3‰$ to $3‰$, $\Delta^{15}N$ $0‰$ to $5.3‰$, from DeNiro & Epstein, 1978; Minagawa & Wada, 1984; McCutchan et al., 2003; Vanderklift & Ponsard, 2003). The $\Delta^{15}N$ ($5.4 \pm 1.2‰$) of chironomid larvae was near the upper limit of the general value (0–5.3‰), while the $\Delta^{13}C$ of chironomid larvae ($0.3 \pm 0.7‰$) was within the generally assumed range ($-3‰$ to $3‰$).

Selective assimilation of *C. acerbiphilus* larvae

The mixing model indicated that phytoplankton contributes the most to the biomass of the chironomid larvae, i.e., the chironomid larvae selectively assimilated more phytoplankton than benthic diatoms when fed on both in the same carbon ratio. This suggests that phytoplankton are more assimilable by the chironomid larvae, probably because the silica coat, cell size, and cell morphology were different between *C. acidophila* (a green alga) and benthic diatoms.

Chironomid larvae fed on sediments from 0.5 m selectively assimilated more benthic diatoms than phytoplankton or plant litter. In contrast, chironomid larvae fed on sediments from 10 m assimilated much more phytoplankton than plant litter and benthic diatoms. These results also indicate that the contribution of plant litter to the food of the chironomid larvae was minimal. Nevertheless, *C. acerbiphilus* larvae may assimilate plant litter as subsidiary food.

C. acerbiphilus larvae fed on sediments from 2 m assimilated more phytoplankton than benthic diatoms. In contrast, the isotope values of sediments at 2 m were closer to those of benthic diatoms (Fig. 1), suggesting that SOM at 2 m was largely composed of benthic diatoms. Therefore, the chironomid larvae assimilated more phytoplankton from sediments at 2 m irrespective of the higher content of benthic diatoms in the sediments. This discrepancy could be explained by the experimental finding, based on carbon content, that *C. acerbiphilus* larvae selectively assimilated more phytoplankton than benthic diatoms when fed equal amounts of both.

Change in the food source of *C. acerbiphilus* larvae with depth

In this study, the assimilation rate of benthic diatoms from sediments by *C. acerbiphilus* larvae tended to decrease with increasing sediment depth, while the assimilation of phytoplankton tended to increase. The decrease in the assimilation rate of benthic diatoms by chironomids with an increase in their habitat depth was also observed for chironomids collected directly from Lake Katanuma (Doi, Kikuchi, & Shikano, 2001). The production of benthic diatoms declines with increasing depth in a lake due to the decrease of light intensity with depth. Conversely, the deposition of POM originated from primary productivity (phytoplankton) tends to increase with depth for shallow

bottoms. In fact, Doi et al. (2003a, b) reported that the high production of benthic diatoms (*Pinnularia braunii* Negoro = *P. acidojaponica*) contributes to SOM in the shallow euphotic zone (less than 4 m in depth) in Lake Katanuma, while POM (mainly *C. acidophila*) is the major contribution in the aphotic zone (10 m in depth). Therefore, we hypothesized that benthic algae were the dominant source of the benthic energy flow in the shore zone in the Lake Katanuma ecosystem, while phytoplankton was the dominant energy source in the aphotic zone.

Food web analyses using carbon stable isotopes consistently demonstrate that there are two main food webs in lakes: littoral and pelagic food webs, which are energetically dependent on littoral (aquatic macrophytes and benthic algae) and pelagic (phytoplankton) producers, respectively (e.g., Bootsma et al., 1996; France, 1995; Vander Zanden & Vadeboncoeur, 2002). Benthic deposit feeders would use littoral producers more as food in the shallower parts (photic zone) of lakes, while they would use only deposited POM in deeper parts (aphotic zone). Few studies have previously demonstrated this shift in the food sources of benthic animals with their habitat depth (e.g., Grey, Kelly, & Sleep, 2004), although several investigators reported differences in the isotope values of benthic animals between shallow and deep benthic areas (e.g., Bootsma et al., 1996; Yoshii, 1999). Our results provide a clear example of the shift in the food source of a deposit feeder from benthic to pelagic algae with increasing depth in a lake ecosystem. Moreover, to our knowledge, this is the first study to demonstrate selective assimilation by a deposit feeder accurately by applying an isotope mixing model using species-specific isotope fractionation. Feeding experiments, such as those performed here, are very important for detailed food source analyses, especially for deposit feeders in aquatic ecosystems.

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