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Effects of reach-scale canopy cover on trophic pathways of caddisfly larvae in a Japanese mountain stream

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Abstract. Differences in trophic pathways between reaches with and without tree canopy cover above the channel were assessed using stable isotopes in a 1.4-km stretch of the Kamo River, Japan. The trophic pathways of two larval trichopteran species, a grazer (*Goera japonica*, Goeridae) inhabiting stone surfaces and a net-spinning filter-feeder (*Stenopsyche marmorata*, Stenopsychidae) inhabiting interstices of the stony stream bottom, were estimated using carbon and nitrogen isotopes in reaches with and without canopy cover in winter. The $\delta^{13}C$ values of *G. japonica* were similar to those of periphyton at each station, suggesting that *G. japonica* is a grazer on periphyton. A significant positive correlation between carbon isotope values of *S. marmorata* and benthic particulate organic matter (BPOM) indicated that BPOM varied in composition according to the amount of solar energy within a reach. In addition, there was a significant positive correlation between carbon isotope values of filter-feeders and the periphytic algal contribution to BPOM using an isotope mixing model, indicating that the main food source of the filter feeders was derived from the *in situ* periphytic algae in open reaches and from a terrestrial source in canopy-covered reaches.

Additional keywords: filter-feeder, food webs, grazer, spatial scale, stable isotope.

Introduction

Lotic food webs have two main sources of energy derived from terrestrial (allochthonous) and algal (autochthonous) organic sources, and these trophic bases of food webs are often considered at comparatively large spatial scales ($>100\,m^2$) such as segment and basin scales (e.g. Vannote *et al*. 1980; Junk *et al*. 1989; Thorp and Delong 1994). Several food web models have been proposed to explain longitudinal variation in food web structure along lotic ecosystems: the river continuum concept (Vannote *et al*. 1980), the flood pulse concept (Junk *et al*. 1989), and the riverine productivity model (Thorp and Delong 1994). However, these food web models have not been thoroughly tested at the reach scale ($<10\,m^2$). Because most lotic macroinvertebrates, such as aquatic insects, live in microhabitats within the spatial scale of a reach (Allan 1995; Woodward and Hildrew 2002), it is plausible that their food webs are structured by environmental conditions at reach spatial scales (Finlay *et al*. 2002; Woodward and Hildrew 2002).

Canopy cover is a critical environmental condition for the food web structure of benthic communities in forest streams (Hill *et al*. 1995, 2001). Canopy cover suppresses primary production by periphyton and aquatic plants by decreasing light intensity; subsequently, the autochthonous trophic chain may be weaker (Hill *et al*. 1995, 2001; Lamberti and Steinman 1997). Although natural mountain forest steams in temperate regions have a high percentage of canopy cover (>90%: Davies-Colley and Quinn 1998), intermittent open reaches without canopy cover can be found, particularly along artificially altered river channels. We hypothesised that trophic pathways differ between open and canopy-covered reaches even within a stream segment. Recently, variations in lotic food web structure have been analysed using carbon and nitrogen stable isotope ratios, which are powerful tools to demonstrate trophic pathways (e.g. Thorp *et al*. 1998; Finlay *et al*. 1999, 2002). For instance, Finlay *et al*. (2002) revealed food web structure and autochthonous sources to trophic pathways in riffle and pool habitats of stream.

Thus, we hypothesised that the trophic pathways of macroinvertebrates between reaches with and without canopy cover will shift from autochthonous algal sources to allochthonous sources, due to decreasing primary production by periphyton with decreasing light availability. We tested our hypothesis by conducting a field survey that examined differences in trophic

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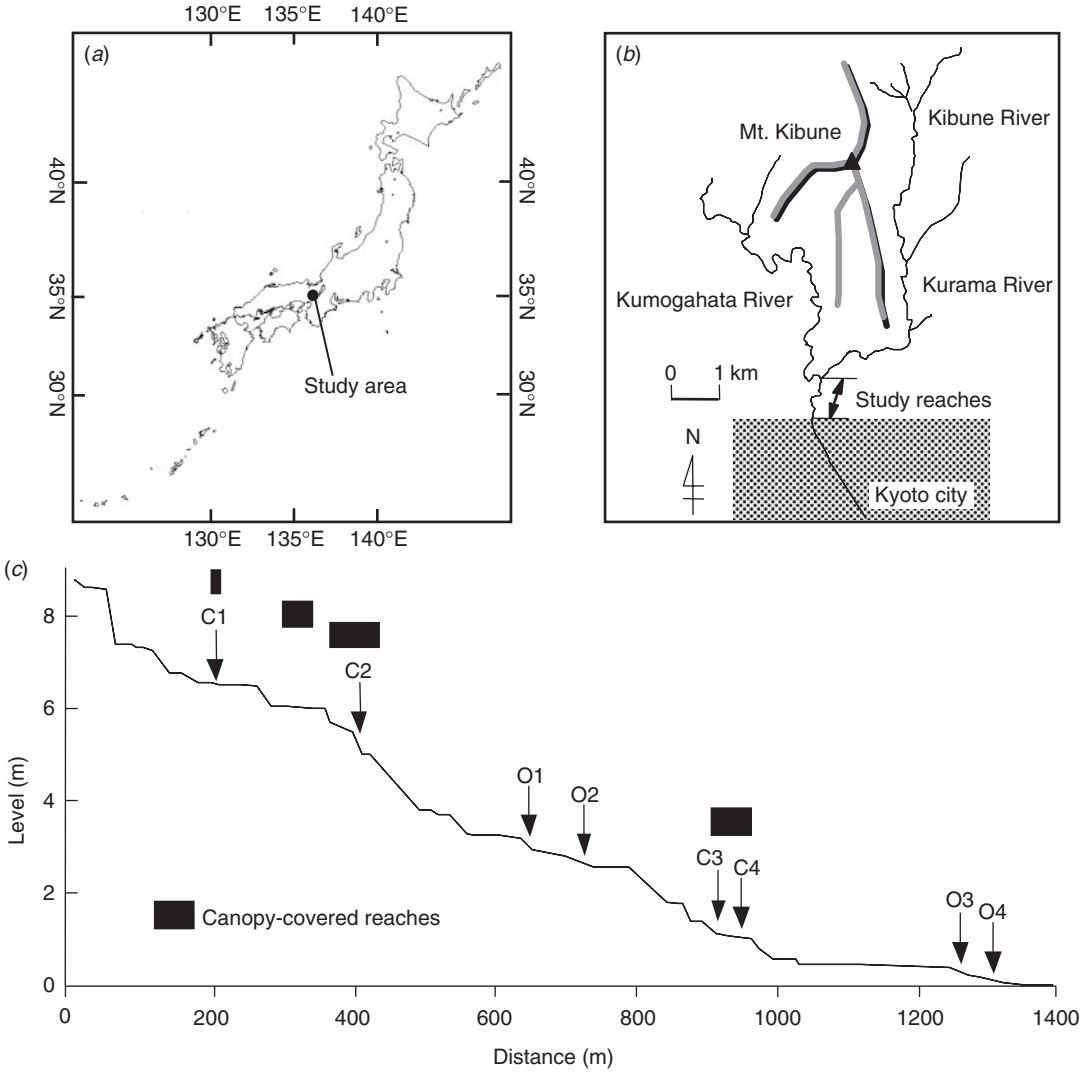

Fig. 1. (*a*) Map of the study area in Japan. (*b*) Map of the study reaches, and (*c*) longitudinal section of the study reaches. In total, eight stations were established. C1–C4 were closed stations (>50% canopy cover) and O1–O4 were open stations (no canopy cover above the river channel); arrows indicate the location of stations along the river.

pathways between open and closed-covered reaches using carbon and nitrogen stable isotope ratios. We examined invertebrates of two functional feeding groups as materials for stable isotope analysis – a grazer inhabiting stone surfaces and a filter-feeder inhabiting interstices of the stony bottom. Potential food sources for the filter-feeder and the grazer are particulate organic matter and periphyton, respectively; therefore, the carbon and nitrogen stable isotope ratios of these consumers are expected to reflect differences caused by the effects of canopy cover *in situ*.

Materials and methods

Study sites

This study was conducted at Hiiragino (110 m altitude) in the middle reaches of the Kamo River (35°0′N, 135°4′E; 6–48 m in width, Fig. 1), which is a tributary of the Yodo River in Kyoto, central Japan. We established four open stations (O1–O4) and four closed stations (C1–C4) within reaches of 1400 m in total length (Fig. 1). The open stations lacked tree canopy cover above the channel, whereas the closed stations had canopy cover >50% above the channel. Samples for isotope analysis were collected from the riffles at each station. River bed substrates were composed of rock, cobble, gravel, and sand. The riparian vegetation of the study area comprised planted Japanese cedar tree (*Cryptomeria japonica*), and the secondary forest with deciduous and evergreen trees, among which we selected the reaches with canopy cover of oak and bamboo. All riparian plants were C3 plants.

Measurement of environmental factors

Percentage canopy cover was measured as an indicator of solar energy abundance at each station from 180° hemispherical photos taken with a camera (Minolta α 7000, Minolta Co., Tokyo, Japan) equipped with a fisheye lens (Sigma-fisheye, Sigma Co., Kawasaki, Japan). Three hemispherical photos were taken at

each station, 15 cm above the water surface in the middle of the channel and at the right and left shore of the riffle. NIH Image 1.6 software (National Institute of Health, USA) was used to measure the area of canopy cover in the hemispherical photos. A representative value of percentage canopy cover (PC) was calculated as the average of the three photos, such that $PC = (R + L + M)/3$, where R, L, and M represent percentage canopy cover at the right shore, left shore, and middle of the channel, respectively.

Chlorophyll *a* density per unit area on a stone surface was measured using the cloth-scrub method (Tanida *et al*. 1999). Six stones were sampled at each station. The epilithon within a 25-mm diameter circle on the upper surface of each stone was scrubbed using an acrylate fibre cloth (Micro-cloth; Koyo Co., Tokyo, Japan) and a rubber plate with a hole of 25 mm in diameter. The epilithon collected on the cloth was transported to the laboratory in an ice box at 0°C under dark conditions. Chlorophyll *a* concentration was measured using the SCOR–UNESCO (1966) method, which is based on ethanol extraction. In addition, we randomly measured water velocity at 60% depth at each station ($n = 5$).

Sample collection

Two Trichoptera species, *Goera japonica* Banks (Goeridae) and *Stenopsyche marmorata* Navas (Stenopsychidae), and their potential food sources (periphyton, benthic particulate organic matter (BPOM) and suspended particulate organic matter (SPOM)) were collected on 22–24 December 2002 at each station. Larvae of *S. marmorata* are net-spinners that feed on SPOM (Nishimura 1966) – gut content analysis of *S. marmorata* has documented a variety of food items, including diatoms, blue green algae, green algae and detritus. The larvae of *G. japonica* are known as grazers of epiphytic algae, based on their gut contents and feeding behaviour (Takemon 2005). Larvae of both species were the predominant members of the benthic invertebrate community, having high biomass in the stream (Y. Takemon and T. Ohta, unpubl. data). Four replicates of fifth-instar larvae of *G. japonica* and *S. marmorata* were collected directly by hand-picking them from the stream bed and placing them in filtered stream water at 5°C for 24 h, to allow for elimination of their gut contents before freeze-drying.

Four replicate samples of periphyton, BPOM and SPOM were collected as potential food sources for the two species. Periphyton was removed from stones using a brush. BPOM was collected from the stream bed using a Surber net sampler (250-μm mesh size), and SPOM was collected using a plankton net (100-μm mesh size, net opening: diameter 30 cm) held from the surface of the stream to mid-depth for 2 min. The samples of BPOM and SPOM were sieved by 500-μm mesh; samples <500-μm were used for isotope analysis. Periphyton, BPOM, and SPOM were acidified with 1 mol L^{-1} HCl to remove carbonate before isotope measurement. All samples were freeze-dried and stored at −20°C until the isotope ratios were analysed.

Measurement of stable isotope ratios and contributions of sources

Carbon and nitrogen isotope ratios of the samples were measured with a mass spectrometer (DELTA plus, Finnigan Mat, Milan, Italy) directly connected to an elemental analyser (NA-2500, CE Instruments, Milan, Italy). All isotopic data are reported in the conventional δ notation where $\delta^{13}C$ or $\delta^{15}N = (R_{sample}/R_{standard} - 1) \cdot 1000$ (‰). R is the $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratio for $\delta^{13}C$ or $\delta^{15}N$, respectively. Pee Dee Belemnite and N_2 in air were used as standards for $\delta^{13}C$ and $\delta^{15}N$, respectively. The analyses were accurate to within ±0.2‰ for both $\delta^{13}C$ and $\delta^{15}N$.

BPOM was considered to be composed of two sources: periphytic algae and allochthonous sources (Vannote *et al*. 1980; Allan 1995). We calculated the contribution of each source to BPOM composition using a two-source carbon isotope mixing model. We used mean values of terrestrial litters in the study stream (−27.0‰, H. Doi, unpubl. data) as terrestrial sources. The model was defined as:

$$\delta^{13}C_b = f_t\delta^{13}C_t + f_b\delta^{13}C_a \quad (1)$$

$$f_t + f_a = 1 \quad (2)$$

The subscripts t, a, and b refer to mean values of terrestrial litters (−27.0‰), periphyton, and BPOM, respectively, from the results of this study; f_t and f_b are the contributions of SPOM and periphyton, respectively, to the BPOM as organic sources.

Results

Environmental factors

Environmental factors at each station in the Kamo River are shown in Table 1. Percentage canopy cover ranged from 54.1 to 79.9% at closed and from 19.9 to 39.9% at open stations, and was significantly lower at open than at closed stations (t-test, $t = 4.90$, $P < 0.01$, $n = 4$). Despite the expectation of lower algal productivity at closed stations owing to lower light intensity, there was no significant difference in the chlorophyll *a* on the stone surfaces among stations (multiple comparison Holm test, $P > 0.05$, $n = 6$). Current velocity, which ranged from 44.4 to 77.4 cm s^{-1}, did not differ significantly among all of stations (multiple comparison Holm test, $P > 0.05$, $n = 5$).

Isotopic signatures of periphyton and SPOM

The mean $\delta^{13}C$ values of periphyton ranged from −20.3 to −15.3‰ (Fig. 2) and were significantly different among stations (Holm test, $P < 0.05$, $n = 4$). The mean $\delta^{13}C$ values of SPOM ranged from −25.9 to −25.0‰ and were not significantly different among stations (Holm test, $P > 0.05$, $n = 4$). They exhibited less variation among stations than those of periphyton and BPOM (Fig. 2). The mean $\delta^{15}N$ values of SPOM ranged from 2.2 to 2.9‰, and there were no significant differences among stations for either of these materials (Holm test, $P > 0.05$, $n = 4$; Fig. 3). The mean $\delta^{15}N$ values of periphyton ranged from 2.2 to 3.2‰, and there were no significant differences among stations for either of these materials (Holm test, $P > 0.05$, $n = 4$; Fig. 3).

The isotope signatures and components of BPOM

The $\delta^{13}C$ values of BPOM varied widely, from −25.0 to −20.7‰ (Fig. 2). Those at C1–C4 were significantly lower than those

Table 1. Environmental factors measured at stations in the Kamo River including percentage canopy cover, chlorophyll *a* density on the stone surface, current velocity and maximum depth

Chlorophyll *a* and current velocity values are mean ± 1 s.e. ($n = 6$ and 5, respectively)

Station	Cover (%)	Chlorophyll *a* ($mg\,m^{-1}$)	Velocity ($cm\,s^{-1}$)	Stream depth (cm)
C1 (closed)	74.2	44.8 ± 7.4	70.7 ± 11.6	25
C2 (closed)	79.9	52.9 ± 8.7	65.6 ± 6.9	35
O1 (open)	39.6	75.5 ± 6.6	63.1 ± 8.5	45
O2 (open)	38.7	43.8 ± 18.9	64.8 ± 8.2	35
C3 (closed)	61.1	53.6 ± 13.1	68.7 ± 5.1	39
C4 (closed)	54.1	43.0 ± 17.8	77.4 ± 8.1	74
O3 (open)	19.9	71.5 ± 19.6	44.4 ± 2.9	14
O4 (open)	21.4	35.1 ± 8.7	64.8 ± 6.4	43

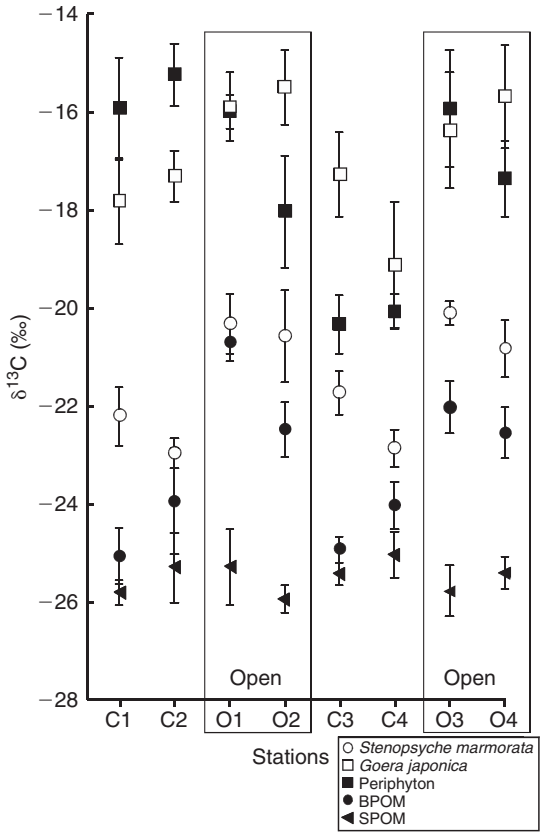

Fig. 2. Carbon isotope ratios of two Trichoptera species, SPOM, BPOM, and periphyton. The Trichoptera *Goera japonica* and *Stenopsyche marmorata* are a grazer and a filter-feeder, respectively. Symbols indicate mean ± 1 s.e. ($n = 4$).

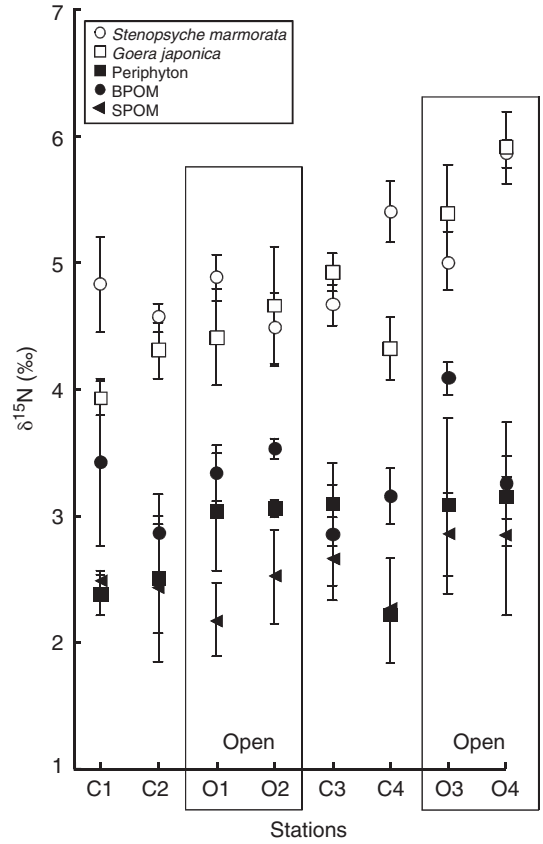

Fig. 3. Nitrogen isotope ratios of *Goera japonica*, *Stenopsyche marmorata*, SPOM, BPOM, and periphyton. Symbols indicate mean ± 1 s.e. ($n = 4$).

at O1–O4 (Holm test, $P < 0.01$, $n = 4$). The $\delta^{13}C$ values of BPOM were lower at closed sites than at open sites, suggesting that canopy cover might be affecting the BPOM component, through changes in the production of autochthonous periphytic algal matter. To estimate the autochthonous contribution to the $\delta^{13}C$ values of BPOM, we calculated the periphytic algal and SPOM contributions using the isotope mixing model (Fig. 4). At the open stations, the periphytic algal and terrestrial matter contributions to BPOM were 70.5 ± 5.3% and 29.5 ± 5.3% (mean ± 1 s.e., $n = 4$), respectively. In contrast, at the covered stations, the periphytic algal and terrestrial matter contributions were 50.2 ± 2.8% and 49.8 ± 2.8%, respectively. The periphytic

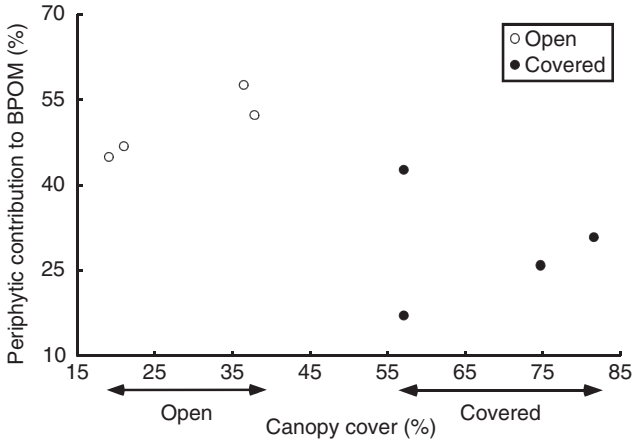

Fig. 4. Relationship between the contribution of periphytic algae to BPOM calculated by the isotope mixing model and percentage canopy cover.

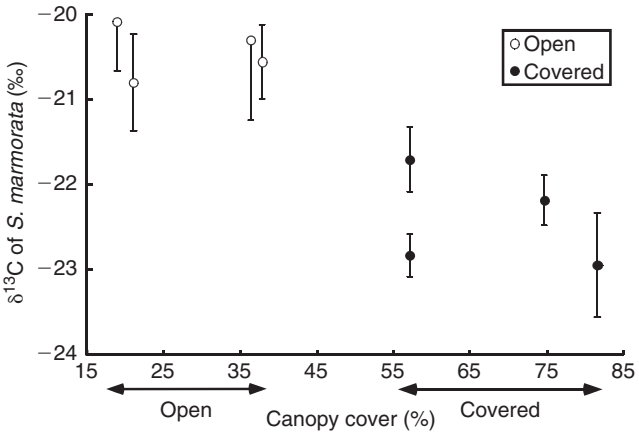

Fig. 5. Relationship between δ^{13}C values of *Stenopsyche marmorata* and percentage canopy cover. Symbols indicate mean ± 1 s.e. ($n = 4$).

algal contribution to BPOM at the open stations was significantly higher than at the closed stations (t-test, $t = 3.38$, $P < 0.05$, $n = 4$), showing that canopy cover was affecting the periphytic algal contribution to BPOM.

Isotopic signatures of macroinvertebrates

The mean δ^{13}C values of *G. japonica* at each station ranged from −19.1 to −15.5‰ (Fig. 2) and did not differ significantly among stations (Holm test, $P > 0.05$, $n = 4$). When compared with the δ^{13}C values of potential food sources, the values of *G. japonica* were similar to those of periphyton at each station. However, no significant relationship was found between the δ^{13}C values of *G. japonica* and periphyton ($r = 0.356$, $P > 0.05$, $n = 8$), probably owing to their long isotopic turnover time.

The δ^{13}C values of *S. marmorata* ranged from −22.9 to −20.1‰. The δ^{13}C values of *S. marmorata* were similar to those of BPOM at each station. In addition, the δ^{13}C values of *S. marmorata* at the open stations was significantly higher than at the closed stations (t-test, $t = 6.01$, $P < 0.01$, $n = 4$; Figs 5 and 6). Thus, to ascertain whether the BPOM components affected the food sources for *S. marmorata*, we compared the δ^{13}C values of *S. marmorata* with the contribution of periphytic algal matter to BPOM using the isotope mixing model. There was a significant positive correlation between δ^{13}C values of *S. marmorata* and the periphytic algal contribution to BPOM ($r = 0.705$, $P < 0.01$, $n = 8$; Fig. 6).

The δ^{15}N values of *G. japonica* ranged from 3.9 to 5.9‰ and were significantly higher than those of periphyton by 1.4–2.8‰ at each station (Fig. 3). The δ^{15}N values of *S. marmorata* ranged from 4.5 to 5.9‰ and were significantly higher than those of BPOM and SPOM by 0.9–3.0‰ at each site (Holm test, $P < 0.05$, $n = 4$). This indicated that *G. japonica* and *S. marmorata* were at a higher trophic level than the potential food sources, periphyton, BPOM and SPOM. Variations in the δ^{15}N enrichment of the macroinvertebrates ranged from 1.4 to 5.9‰ among stations and could not be explained in relation to the canopy cover ($r = 0.252$, $P > 0.05$, $n = 8$).

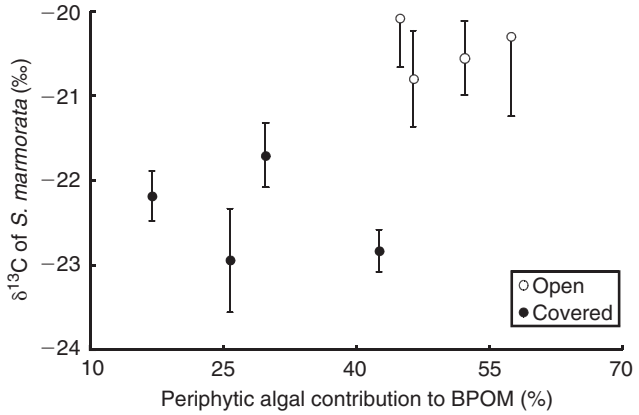

Fig. 6. Relationship between δ^{13}C values of *Stenopsyche marmorata* and the contribution of periphytic algae to BPOM calculated by the isotope mixing model. Symbols indicate mean ± 1 s.e. ($n = 4$).

Discussion

Effect of canopy cover on periphyton biomass and isotopic signature

Photosynthetic rates of periphyton will decrease with increased canopy cover because of decreased light penetration and colder temperatures (Behmer and Hawkins 1986; Lamberti and Steinman 1997). However, we found that periphyton biomass, measured as chlorophyll *a* concentration, did not differ significantly between closed and open stations. This is probably caused by higher feeding pressure by the grazing caddisflies *G. japonica* and *Glossosoma* sp., which had significantly higher densities and biomass at open than at closed stations (Y. Takemon and T. Ohta, unpubl. data). Feminella *et al*. (1989) similarly found that the biomass of periphyton was limited by grazers, rather than by light intensity. However, in spite of lower periphyton biomass, net primary productivity can be higher at open than at closed stations (Behmer and Hawkins 1986; Feminella *et al*. 1989; Lamberti and Steinman 1997).

Periphyton will increase its $\delta^{13}C$ values under increased light intensity (MacLeod and Barton 1998). The $\delta^{13}C$ values of periphyton at low photosynthetic rates have been shown to be highly depleted relative to the $\delta^{13}C$ values of dissolved inorganic carbon (DIC) (Fry 1996; Finlay 2001). We found that the $\delta^{13}C$ values of periphyton were high at the open stations with more light availability, but were in the low range of −20.3 to −15.3‰ at the closed stations. Between reaches C1–C2 and C3–C4, variation in the $\delta^{13}C$ values may be explained by the thickness of the algal mat and the current velocity. The $\delta^{13}C$ values of periphyton may be higher in a thick algal mat and under slow current velocities because of limited supplies of DIC (Fry 1996; Finlay *et al*. 1999; Doi *et al*. 2003; Trudeau and Rasmussen 2003). However, chlorophyll *a* concentrations and current velocity were not higher at C1–C2 than at C3–C4; thus, both of these hypotheses can be rejected. Therefore, the $\delta^{13}C$ values of periphyton increase under higher light intensity at the open stations, whereas those at the closed stations are affected by the other factors such as inflowing of groundwater DIC (e.g. Finlay 2004). To explain the $\delta^{13}C$ values of periphyton, another possibility is that the abundance of terrestrially-derived POM in periphyton mats influenced the $\delta^{13}C$ values of periphyton. However, the chlorophyll *a* content of the periphyton at closed sites was similar to those at open sites – thus, the presence of POM in periphyton mats did not strongly affect the $\delta^{13}C$ values of periphyton.

Effect of canopy cover on SPOM and BPOM isotopic signatures

SPOM showed little variation in the $\delta^{13}C$ and $\delta^{15}N$ values, indicating that SPOM components were not affected by canopy cover, and did not change along the river drift at a reach scale. The $\delta^{13}C$ values of SPOM ranged from −25.9 to −25.0‰, suggesting that SPOM mainly consisted of riverine C_3 terrestrial detritus (terrestrial litters: −27.0‰), and also consisted of periphytic matter, as the $\delta^{13}C$ values of SPOM were higher than those of terrestrial litters. The $\delta^{13}C$ values of BPOM were lower at closed than at open stations, suggesting contributions by different organic sources to the composition of BPOM at closed and open stations. At open stations, the contribution of periphytic algae to BPOM was significantly higher than at closed stations (Fig. 4), suggesting that high microalgal productivity may increase the periphytic algal contribution to BPOM *in situ*. The BPOM at closed stations may consist mainly of SPOM from terrestrial materials because of low algal productivity *in situ* (Behmer and Hawkins 1986; Feminella *et al*. 1989; Lamberti and Steinman 1997).

Effect of canopy cover on trophic pathways of stream benthic invertebrates

The larvae of *G. japonica* are known as grazers of epiphytic algae. The $\delta^{13}C$ values of *G. japonica* were similar to those of periphyton at each station (Fig. 2), suggesting that *G. japonica* is a grazer on periphyton. However, there was no significant correlation between the $\delta^{13}C$ values of *G. japonica* and periphyton; probably a result of their long isotopic turnover time. In freshwater ecosystems, the $\delta^{13}C$ values of periphyton may fluctuate temporally over several hours to several days (e.g. Fry 1996; Finlay *et al*. 2002), while those of *G. japonica* may be indicative of their food materials over the previous few weeks to several months. Thus, the $\delta^{13}C$ values of *G. japonica* would be determined by the mean $\delta^{13}C$ values of periphyton during their isotopic turnover time over a period of weeks to months.

The larvae of *S. marmorata* are net-spinning caddisflies that have been shown to feed on suspended POM (Nishimura 1966), along with a variety of food items including diatoms, blue green algae, green algae and detritus (Nishimura 1966; Takemon 2005). Results of the present study showed that the $\delta^{13}C$ values of *S. marmorata* clearly changed in parallel with, and were quite similar to, those of BPOM. This indicates that the larvae of *S. marmorata* might be feeding on suspended POM derived from the BPOM upstream within the reach. Moreover, the $\delta^{13}C$ values of *S. marmorata* clearly changed with the periphytic algal matter contents of BPOM (Fig. 6). Consequently, *S. marmorata* in the open reaches may assimilate greater amounts of periphytic algal matter in BPOM than those in the closed reaches. This would result in higher $\delta^{13}C$ values, because the $\delta^{13}C$ values of periphyton (−20.3 to −15.3‰) were higher than those of SPOM (−25.9 to −25.0‰). Therefore, the trophic status of filter-feeders might be highly influenced by the *in situ* periphytic algal productivity at the reach scale.

Within the closed reaches, the $\delta^{13}C$ values of *S. marmorata* were more ^{13}C-enriched at stations C1 and C3 than at C2 and C4. In other words, at upper closed stations, $\delta^{13}C$ values of *S. marmorata* were more similar to those of periphyton, while at lower closed stations, their $\delta^{13}C$ values were more similar to those of BPOM. This indicated that periphyton contributed more to the diet of *S. marmorata* at upper than at lower closed stations. Plausible implications of the phenomenon might be the influence of open and closed upstream reaches that are different in light availability: i.e. the stations C1 and C3 would have received SPOM derived from the upstream reaches with higher primary production, whereas the stations C2 and C4 would not because of the closed upstream reaches. Although it has been believed that the structure of stream food webs is highly influenced by processes occurring upstream in segment scales (e.g. river continuum concept: Vannote *et al*. 1980; Wallace *et al*. 1997), the effective distance of the upstream influence might be limited to reach scales.

The river continuum concept describes stream networks that are characterised by the downstream progression of the trophic base from terrestrial to algal sources (Vannote *et al*. 1980). However, we found that filter-feeders and BPOM were highly influenced by the variation in *in situ* periphytic algal productivity, with variation in light intensity caused by canopy cover at the reach scale (i.e. 100 m level). This indicates that trophic pathways of macroinvertebrates should vary at the reach scale. Variations in trophic pathways have also been found to occur between riffle and pool habitats because of low light penetration into the deep water of the pools (Finlay *et al*. 1999, 2002). Thus, both stream bed geomorphology (i.e. riffle–pool structure) and light availability at reach scales will influence trophic pathways via changes in primary production and the abundance of POM of terrestrial origin. In this study, we showed the canopy cover effects on trophic pathways at reach scale. Further study is required in order to estimate the effects of canopy cover at multiple spatial scales.

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