

Article

Evaluation of algal contribution to aquatic insects based on $^{13}\text{C}/^{12}\text{C}$ and C/N ratios

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Abstract

The traditional two-source linear mixing model, based on distinct $^{13}\text{C}/^{12}\text{C}$ signatures among sources and samples, has been widely used in a number of ecological dietary studies. This model is especially useful in the case that the elemental compositions (i.e. H, C, N, and O) are similar among sources (i.e. diets) and samples (i.e. animals). However, elemental compositions (e.g. C/N ratio) are commonly widely variable, which often leads to large errors in observed results and subsequent interpretations. In this study, we propose a modified mixing model, employing the $^{13}\text{C}/^{12}\text{C}$ ratio combined with the C/N ratio, to better evaluate the dietary properties of animals. As a case study, we investigate the relative contributions of riverine algae and terrestrial plants to the diets of aquatic insects, including five grazers and four filterers, in the Chikuma River, Japan. The traditional mixing model indicates that the algal contribution to aquatic insects ranges from 45% to 92%, whereas the modified mixing model yields values ranging from 64% to 97%. These results are in agreement with the C/N ratios of algae, plants, and aquatic insects (5.4 ± 0.7 , 13.4 ± 3.3 , and 4.9 ± 0.5 , respectively), indicating that the traditional mixing model may underestimate the algal contribution to aquatic insects. We suggest that the modified mixing model, using $^{13}\text{C}/^{12}\text{C}$ in combination with C/N, is better than traditional mixing models in terms of evaluating the contribution of various sources in animals, even in the case that elemental compositions are markedly different between animals and their diets.

1. Introduction

Stable isotope analysis has been widely used in geochemical and ecological studies to evaluate energy flow, trophic structure, and the nutrient cycle in natural environments (e.g. Peterson and Fry, 1987; Dawson and Siegwolf, 2007). In fact, the isotopic compositions (e.g. $^{13}\text{C}/^{12}\text{C}$, $^{15}\text{N}/^{14}\text{N}$, and $^{34}\text{S}/^{32}\text{S}$) of organisms

reflect two factors: 1) the isotopic composition of the organism's diet, and 2) isotopic fractionation during feeding-assimilation processes in animals (e.g. DeNiro and Epstein, 1978; Minagawa and Wada, 1984; Post, 2002; McCutchan et al., 2003). The recent development of a continuous-flow connection between an elemental analyzer and an isotope ratio mass spectrometer (EA/IRMS) enables the simple and rapid analysis

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of isotopic compositions from very small samples (e.g. Hoefs, 2004). Consequently, in contrast to traditional approaches (e.g. direct anatomical observations of gut contents), isotope analysis enables investigations of the dietary properties of organisms by analyzing a large number of samples.

The traditional two-source linear mixing model, based on the distinct $^{13}\text{C}/^{12}\text{C}$ signatures of various food sources, has been frequently employed in quantifying the relative contributions of various food sources to animals' diets (e.g. Schwarcz, 1991; Phillips, 2001; Doi et al., 2006; Akamatsu et al., 2007). For example, Akamatsu et al. (2007) compared the $^{13}\text{C}/^{12}\text{C}$ ratio of riparian spiders and their diet (i.e. aquatic and terrestrial insects), and found that the contribution of aquatic insects to the spiders varied widely from 4% to 51%. Thus, the traditional mixing model is useful in the case that the elemental composition (i.e. H, C, N, and O) is similar between animals and their diets (in the case of Akamatsu et al. (2007), between spiders and the insects that form their diet).

However, the elemental composition (e.g. the C/N ratio) is strongly variable among different food sources in many cases in the natural environment (Elser et al., 2000; Sterner and Elser, 2002). In fact, the C/N ratio of heterotrophs (animals) is generally 4-10, which is commonly lower than that of photoautotrophs; even within photoautotrophs, the C/N ratio of aquatic algae (5-20) is much lower than that of terrestrial plants (5-100) (e.g. Elser et al., 2000). Such differences in elemental composition should be considered in relevant studies because the transfer (or assimilation) of these elements from the source to the animal is potentially complex, or at least not as simple as that assumed by the traditional mixing model, which is based solely on the $^{13}\text{C}/^{12}\text{C}$ ratio of animals and their diet (Sterner and Elser, 2002; Dodds et al., 2004). In fact, animals consist mainly of biomolecules with a low C/N ratio, such as proteins, nucleotides, and polymers of amino sugars (e.g. chitin), which may indicate that they require food with a low C/N ratio, in contrast to the nitrogen-free cellulose in terrestrial plants. Thus, a marked difference in the elemental composition of animals and their diets

may generate an error in the relative contributions of different food sources estimated by the traditional two-source mixing model (e.g. Post, 2002).

To obtain more accurate estimates of the relative contributions of various food sources, several studies have proposed alternative approaches; e.g. a combination of multi-element (C, H, N, O) and isotope analyses (e.g. Phillips and Koch, 2002; Doi et al., 2006), and compound-specific stable isotope analyses (CSIA) of organic molecules (e.g. Naito et al., 2010). For example, Phillips and Koch (2002) investigated differences in the $^{13}\text{C}/^{12}\text{C}$ ratio and in C and N contents between bears and their diets (i.e. salmon, terrestrial plants, and meat), revealing that a modified mixing model, based on the $^{13}\text{C}/^{12}\text{C}$ ratio combined with C and N contents, yields better estimates (compared with the traditional two-source mixing model) of the diet of omnivorous animals. In addition, Naito et al. (2010) investigated the nitrogen isotopic composition of bone collagen amino acids in ancient humans and related faunas, and was able to accurately estimate the relative contributions of terrestrial and marine proteins to the ancient human diets.

However, compared with the traditional model, the above approaches require additional analyses (or instruments), representing greater investments in money and time. Therefore, in the present study, as an alternative approach, we propose a modified mixing model, based on the $^{13}\text{C}/^{12}\text{C}$ ratio combined with the C/N ratio, to obtain better evaluations of the relative contributions of various sources to the sample of interest. This new model has a significant advantage in that it does not require additional analyses (or instruments) because the C/N ratio can be simultaneously obtained from the $^{13}\text{C}/^{12}\text{C}$ analysis by EA/IRMS. In addition, the model used to combine the $^{13}\text{C}/^{12}\text{C}$ data with the C/N ratio is simpler than that employed in the model proposed by Phillips and Koch (2002), which employs C and N contents as independent factors. Although CSIA is currently the most accurate tool available, our model would be useful in a strategy in which CSIA is only employed as a decisive tool for specific samples of high interest after screening of a large number of samples using the proposed model. As a case study, we investigate

the relative contributions of riverine algae and terrestrial plants to the diet of aquatic insects, including grazers and filterers, in the Chikuma River, Japan.

2. Materials and methods

Aquatic insects, riverine algae (attached algae), terrestrial plant leaves, and particulate organic matter (POM) were collected from five sites along the Chikuma River (between 250.5 and 357.0 km upstream from the mouth of the Shinano River), Japan, in April 2004 (Fig. 1; Table 1). The riverbed was covered with cobbles at all sites, and the concentrations of nitrate and chlorophyll *a* showed a gradual increase from the upper to lower reaches (from 0.16 to 1.33 mg L⁻¹ for nitrate, and from 9.0 to 133.3 mg m⁻² for Chlorophyll *a*; unpublished data). Aquatic insects (Fig. 2; including the grazers *Baetis* sp., *Cincticostella nigra*, *Ecdyonurus yoshidaei*, *Epeorus aesculus*, and *Epeorus latifolium*,

and the filterers *Cheumatopsyche brevilineata*, *Hydropsyche orientalis*, *Isonychia japonica*, and *Stenopsyche marmorata*) were collected using a square-framed net (25 × 25 cm, 0.5-mm mesh); two to five individuals were collected for each taxon (Table 2). The insects were identified according to Merritt and Cummins (1996) and Takemon (2005). The gut contents were removed before analysis. As food sources of aquatic insects, attached algae (mainly diatoms, although species were not identified in this study) were collected from three cobbles (~10 cm in diameter) within stretches of riffles (~30 cm water depth) at each site. Each cobble was rinsed gently with river water to remove contamination such as sludge, debris, and invertebrates, and then algal colonies in an area of 25 cm² were brushed and collected. Terrestrial plant leaves (*Artemisia capillaries*, *Artemisia vulgaris*, *Betula platyphylla*, *Equisetum arvense*, *Larix kaempferi*, *Miscanthus sinensis*, *Phragmites japonica*, *Polygonum nodosum*, *Rumex japonicus*, *Salix gilgiana*, *Tsuga diversifolia*, and *Typha latifolia*) were collected from riverside areas at the same sites; two to five leaves were collected for each plant. The surfaces of the leaves were cleaned with distilled water to remove contaminants such as dust, and the plants were identified according to

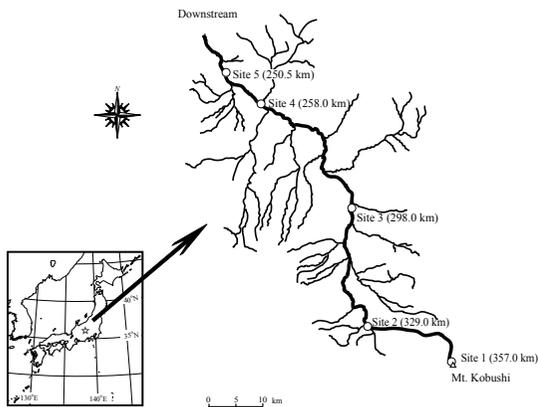


Fig. 1. Locations of five sampling sites along the Chikuma River. Numbers in parentheses indicate the distance (km) from the mouth of the Shinano River.

Table 1. Sampling sites along the Chikuma River.

Site	Location	Latitude	Longitude	Elevation above sea level (m)	Bed slope	Distance from river mouth (km)
1	Igura	35°55'N	138°42'E	1978	1/5	357.0
2	Hisawa	35°58'N	138°42'E	1119	1/87	329.0
3	Usuda	36°11'N	138°31'E	707	1/104	298.0
4	Ikuta	36°22'N	138°28'E	449	1/192	407.0
5	Nezumi	36°25'N	138°11'E	407	1/200	250.5

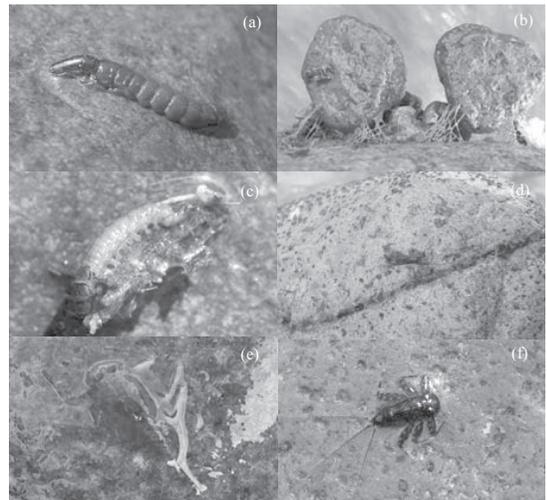


Fig. 2. Photographs of aquatic insects analyzed in this study: (a) *S. marmorata* and (b) its nest, (c) *H. orientalis* and (d) its nest, (e) *E. latifolium*, and (f) *E. yoshidaei*.

Table 2. $\delta^{13}\text{C}$ and C/N ratios of aquatic insects, attached algae, terrestrial plant leaves, and particulate organic matter, and the contribution of attached algae to aquatic insects, as estimated using the traditional (f_T) and modified (f_M) stable isotope mixing models for samples from the Chikuma River, Japan.

Site	Sample	Feeding type	$\delta^{13}\text{C}$ (‰)	C/N ratio	f_T^1	f_M^2	n		
1	Aquatic insects	<i>Ecdyonurus yoshidae</i>	Grazer	-26.1 ± 0.9	6.3 ± 0.3	0.45 ± 0.19	0.64 ± 0.18	4	
		<i>Epeorus aesculus</i>	Grazer	-25.1 ± 1.3	5.3 ± 0.7	0.67 ± 0.29	0.81 ± 0.18	3	
		<i>Isonychia japonica</i>	Filterer	-24.3 ± 0.9	5.4 ± 0.8	0.81 ± 0.16	0.90 ± 0.09	5	
	Attached algae			-24.5 ± 3.0	6.1 ± 1.1			3	
		Terrestrial plants	<i>Betula platyphylla</i>		-30.1 ± 2.5	14.8 ± 2.3			2
	<i>Larix kaempferi</i>			-28.5 ± 1.1	15.2 ± 2.7			3	
	<i>Tsuga diversifolia</i>			-29.3 ± 1.3	13.1 ± 2.2			2	
	Particulate organic matter			-28.9 ± 0.2	14.0 ± 0.2	0.06 ± 0.04	0.13 ± 0.09	3	
	2	Aquatic insects	<i>Baetis</i> sp.	Grazer	-23.2 ± 1.3	4.4 ± 0.2	0.58 ± 0.15	0.69 ± 0.14	5
<i>Epeorus latifolium</i>			Grazer	-21.8 ± 0.4	4.5 ± 0.2	0.75 ± 0.05	0.83 ± 0.03	5	
<i>Cheumatopsyche brevilineata</i>			Filterer	-20.1 ± 2.1	5.0 ± 0.3	0.88 ± 0.14	0.96 ± 0.15	3	
<i>Hydropsyche orientalis</i>			Filterer	-21.1 ± 1.3	5.0 ± 0.1	0.82 ± 0.15	0.86 ± 0.10	5	
<i>Stenopsyche marmorata</i>			Filterer	-21.1 ± 1.8	5.0 ± 0.3	0.82 ± 0.20	0.87 ± 0.15	5	
Attached algae				-20.7 ± 1.4	5.8 ± 0.3			3	
		Terrestrial plants	<i>Artemisia capillaris</i>		-27.1	10.7			1
<i>Artemisia vulgaris</i>				-30.1	8.0			1	
<i>Equisetum arvense</i>				-28.9	12.1			1	
<i>Phragmites japonica</i>				-30.3	8.5			1	
<i>Polygonum nodosum</i>				-29.2 ± 2.5	9.5 ± 5.2			3	
Particulate organic matter				-25.9 ± 0.2	8.3 ± 0.3	0.38 ± 0.02	0.50 ± 0.02	3	
3		Aquatic insects	<i>Baetis</i> sp.	Grazer	-17.9 ± 0.4	4.4 ± 0.2	0.80 ± 0.03	0.90 ± 0.02	5
			<i>Cincticostella nigra</i>	Grazer	-16.5 ± 2.3	4.3 ± 0.1	0.90 ± 0.17	0.94 ± 0.10	5
			<i>Ecdyonurus yoshidae</i>	Grazer	-18.1 ± 0.9	3.9 ± 0.3	0.78 ± 0.07	0.89 ± 0.02	2
	<i>Epeorus latifolium</i>		Grazer	-16.6 ± 1.1	4.7 ± 0.2	0.91 ± 0.09	0.95 ± 0.05	5	
	<i>Cheumatopsyche brevilineata</i>		Filterer	-21.2 ± 0.9	4.8 ± 0.4	0.53 ± 0.07	0.70 ± 0.07	5	
	<i>Isonychia japonica</i>		Filterer	-18.1 ± 0.4	5.1 ± 0.7	0.78 ± 0.03	0.89 ± 0.02	2	
	<i>Stenopsyche marmorata</i>		Filterer	-18.1 ± 0.4	4.7 ± 0.3	0.78 ± 0.03	0.88 ± 0.02	5	
	Attached algae			-16.5 ± 3.4	5.3 ± 0.4			3	
		Terrestrial plants	<i>Artemisia capillaris</i>		-29.3	10.8			1
	<i>Phragmites japonica</i>			-27.7 ± 1.5	11.3 ± 2.5			5	
	<i>Polygonum nodosum</i>			-31.6	12.8			1	
	Particulate organic matter			-24.0 ± 1.6	8.0 ± 0.2	0.38 ± 0.13	0.56 ± 0.15	3	
	4	Aquatic insects	<i>Baetis</i> sp.	Grazer	-17.7 ± 1.4	5.0 ± 0.3	0.68 ± 0.10	0.87 ± 0.06	5
			<i>Cincticostella nigra</i>	Grazer	-14.6 ± 0.3	4.7 ± 0.1	0.92 ± 0.03	0.97 ± 0.01	4
			<i>Epeorus latifolium</i>	Grazer	-14.8 ± 0.8	5.0 ± 0.2	0.90 ± 0.06	0.97 ± 0.02	5
<i>Cheumatopsyche brevilineata</i>			Filterer	-16.1 ± 0.4	5.2 ± 0.2	0.80 ± 0.03	0.93 ± 0.01	5	
<i>Isonychia japonica</i>			Filterer	-18.8 ± 0.3	4.6 ± 0.2	0.59 ± 0.02	0.83 ± 0.01	2	
Attached algae				-17.5 ± 0.5	5.0 ± 0.4	0.69 ± 0.04	0.89 ± 0.02	5	
		Terrestrial plants			-14.6 ± 4.3	4.8 ± 0.4			3
<i>Miscanthus sacchariflorus</i>				-12.8 ± 0.9	21.4 ± 10.8			3	
<i>Polygonum nodosum</i>				-30.8 ± 0.6	16.3 ± 2.0			4	
<i>Rumex japonicus</i>				-31.0	15.8			1	
<i>Typha latifolia</i>				-30.8 ± 0.2	11.6 ± 2.8			2	
Particulate organic matter				-23.7 ± 0.5	7.3 ± 0.1	0.29 ± 0.04	0.58 ± 0.05	3	
5		Aquatic insects	<i>Baetis</i> sp.	Grazer	-16.7 ± 1.2	5.0 ± 0.6	0.73 ± 0.07	0.88 ± 0.04	5
			<i>Cincticostella nigra</i>	Grazer	-15.8 ± 0.4	4.0 ± 0.1	0.78 ± 0.03	0.91 ± 0.01	2
			<i>Epeorus latifolium</i>	Grazer	-16.1 ± 0.7	4.6 ± 0.1	0.76 ± 0.04	0.90 ± 0.02	5
	<i>Cheumatopsyche brevilineata</i>		Filterer	-17.5 ± 0.7	4.7 ± 0.2	0.67 ± 0.04	0.86 ± 0.02	5	
	<i>Hydropsyche orientalis</i>		Filterer	-16.8 ± 0.2	5.1 ± 0.2	0.72 ± 0.01	0.88 ± 0.01	5	
	<i>Isonychia japonica</i>		Filterer	-17.7 ± 0.4	4.7 ± 0.2	0.66 ± 0.02	0.85 ± 0.01	5	
	<i>Stenopsyche marmorata</i>		Filterer	-16.8 ± 0.6	5.0 ± 0.2	0.72 ± 0.04	0.88 ± 0.02	5	
	Attached algae				-13.1 ± 1.5	5.2 ± 0.4			3
		Terrestrial plants	<i>Phragmites japonica</i>		-28.1 ± 1.1	15.2 ± 3.4			8
	<i>Salix gilgiana</i>			-30.7 ± 2.2	2.2 ± 0.9			2	
	Particulate organic matter				-22.6 ± 0.4	6.8 ± 0.1	0.43 ± 0.03	0.68 ± 0.02	3

Each value is shown the average and 1σ.

1. f_T represents the fractional contribution of algae to aquatic insects or POM estimated by the traditional mixing model.

2. f_M represents the fractional contribution of algae to aquatic insects or POM estimated by the modified mixing model.

Makino (2000). POM samples were collected from the surface of the river using a conical-shaped plankton net (25 cm in diameter, 90 cm in length; $100\ \mu\text{m}$ mesh), and the coarse fraction was removed using a stainless-steel sieve ($1000\ \mu\text{m}$ mesh). The samples were kept cool with ice during transportation to the laboratory, and were dried at 60°C for 48 hours.

All samples were ground to fine powder before analysis. Carbon isotopic composition was determined by elemental analyzer/isotope ratio mass spectrometer (EA/IRMS) using a Thermo Fisher Scientific Flash EA (EA1112) coupled to a Delta^{plus} IRMS via a ConFlo II Interface. Approximately 1 mg aliquots of the powdered samples were used for isotope analysis. Isotope data are reported using δ notation relative to the Vienna Pee Dee Belemnite (VPDB). A working standard of known δ value (glycine: -33.0‰) was analyzed every 5-8 runs to confirm the reproducibility and accuracy of the isotope measurements. The analytical errors (1σ) for the standards were always better than 0.1‰. The C/N ratio (wt/wt) was obtained from the peak areas of nitrogen (m/z 28) and carbon (m/z 44), which were simultaneously determined during the EA/IRMS analysis.

The traditional two-source linear mixing model, based on the distinct values of $\delta^{13}\text{C}$ between attached algae ($\delta^{13}\text{C}_{\text{algae}}$) and terrestrial plants ($\delta^{13}\text{C}_{\text{plant}}$), is defined by the following mass balance calculation:

$$\delta^{13}\text{C}_{\text{sample}} - \alpha = f_{\text{T}} \times \delta^{13}\text{C}_{\text{algae}} + (1 - f_{\text{T}}) \times \delta^{13}\text{C}_{\text{plant}} \quad (1)$$

where $\delta^{13}\text{C}_{\text{sample}}$ represents the isotopic composition of aquatic insects or POM, α is the ^{13}C -enrichment factor during feeding processes (assuming a value of +1.0 for aquatic insects and ± 0.0 for POM in this study, following DeNiro and Epstein, 1978; Wada and Yoshioka, 1995), and f_{T} is the fractional contribution of algae to aquatic insects or POM. The modified mixing model, based on $\delta^{13}\text{C}$ combined with C/N, employs f_{M} as an alternative proxy for the contribution of algae, with the result produced by the traditional mixing model (i.e. f_{T}) being weighted by the relative nitrogen content (as the C/N ratio):

$$f_{\text{M}} = (f_{\text{T}} \times \text{C/N}_{\text{algae}}^{-1}) / \{f_{\text{T}} \times \text{C/N}_{\text{algae}}^{-1} + (1 - f_{\text{T}}) \times \text{C/N}_{\text{plant}}^{-1}\} \quad (2)$$

where $\text{C/N}_{\text{algae}}$ and $\text{C/N}_{\text{plant}}$ represent the C/N ratio of attached algae and terrestrial plants, respectively. This equation is simply designed based on the fact that f_{M} is equal to f_{T} in the case that attached algae and terrestrial plants have the same C/N ratio. f_{M} values of 1 (the maximum value) or zero (the minimum value) indicate no contribution of terrestrial plants or attached algae to the diet of aquatic insects, respectively. In nature, the value of f_{M} is expected to be intermediate between these two extreme cases. In the present study, we assumed that the values of $\delta^{13}\text{C}$ and the C/N ratio for attached algae and terrestrial plants at each site are adequately represented by the average value obtained for the collected algae and plant leaves, respectively (Table 2).

3. Results and discussion

3.1. $\delta^{13}\text{C}$ and C/N ratio

The carbon isotopic composition ($\delta^{13}\text{C}$) and C/N ratio of aquatic insects, attached algae, terrestrial plants, and POM are summarized in Table 2 and Fig. 3. The mean $\delta^{13}\text{C}$ values of aquatic insects (i.e. both grazers and filters) and POM show a gradual downstream increase, from the upper (-27.0‰ and -28.9‰ , respectively)

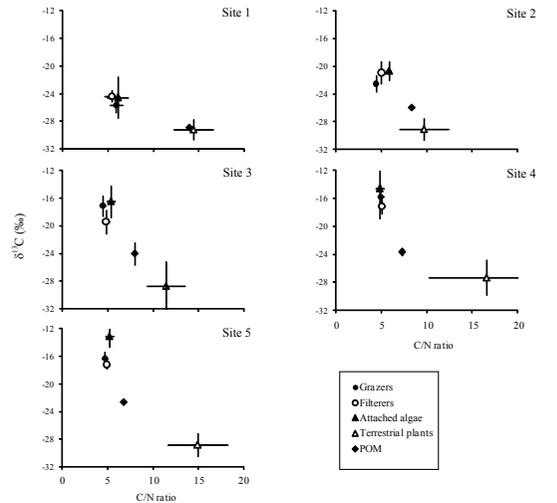


Fig. 3. Relationships between $\delta^{13}\text{C}$ and the C/N ratio of aquatic insects, attached algae, terrestrial plants, and particulate organic matter (POM) in the Chikuma River. Symbols and error bars represent the average and 1σ , respectively.

to lower reaches (-13.5‰ and -22.6‰ , respectively), similar to the trend for attached algae (from -24.5‰ to -13.1‰). The attached algae are enriched in $\delta^{13}\text{C}$ by $1.9 \pm 1.8\text{‰}$ and $7.2 \pm 2.3\text{‰}$ compared with aquatic insects and POM, respectively. In contrast, terrestrial plant leaves show minor variations in $\delta^{13}\text{C}$ for all sites ($-28.6 \pm 0.8\text{‰}$), which are the lowest values obtained in this study.

The aquatic insects and attached algae show minor variations in the C/N ratio at all sites (5.4 ± 0.4 and 5.0 ± 0.2 , respectively), which are much smaller than the values obtained for terrestrial plant leaves (13.4 ± 3.3). The C/N ratio of POM shows a gradual downstream decrease from 14.0 to 6.8.

3.2. Traditional and modified mixing models

As shown in Fig. 4, the contribution of algae to the diets of aquatic insects varies from 45% to 92%, as calculated using the traditional mixing model. For example, the contribution of algae to the diets of grazers and filterers is 55% and 82%, respectively, at site 1, but 75% and 69%, respectively, at site 5. A similar trend is observed in the results obtained using the modified mixing model; for example, the contribution of algae to the diet of grazers and filterers is 71% and 90%, respectively, at site 1, but 89% and 87%, respectively, at site 5. The contribution of algae estimated using the modified mixing model is $\sim 12\%$ greater than that estimated using the traditional mixing model; thus, the traditional model may underestimate the contribution of algae to aquatic insects. These results indicate that the studied insects feed mainly on attached algae rather than on terrestrial plant detritus, probably because of the similar C/N ratio between the insects and algae (Sterner and Elser, 2002).

Given the difference in elemental composition (e.g. N) between animals and their diets, the present results obtained using the traditional mixing model may include large errors. In contrast, the modified mixing model considers the C/N ratio, meaning that it may be better evaluations than the traditional mixing model in evaluating the contribution of various sources to animal diets, even in the case of markedly different element

compositions between the animals and their diet. Nevertheless, there still exists the potential for large uncertainty (i.e. error) in the results obtained from the modified mixing model, given the large variations in $\delta^{13}\text{C}$ and the C/N ratio among aquatic insects, attached algae, and terrestrial plants. Moreover, the applicability of the modified mixing model should be verified future by, for example, controlled-feeding experiments using model animals and ^{13}C or ^{15}N labeled diets.

3.3. Diets of grazers and filterers

Although the modified mixing model shows that both types of aquatic insects (i.e. grazers and filterers) feed mainly on attached algae in the river, the downstream trend in algal contribution shows slight differences between these two types of insects (Fig. 4). The contribution of algae to grazers shows a gradual increase from the upper (71%) to lower reaches (89%), whereas

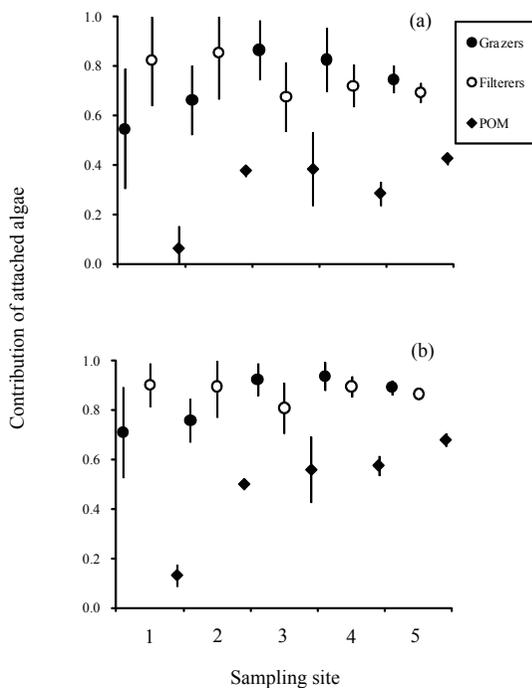


Fig. 4. Relative contribution of attached algae to the diets of aquatic insects and to POM in the Chikuma River, as estimated using (a) the traditional model and (b) the modified isotope-mixing model. Symbols and error bars represent the average and 1σ , respectively.

the contribution of algae to filterers is largely constant along the river ($87 \pm 4\%$). Consequently, the difference in the contribution of algae to grazers and filterers is relatively large in the upper reaches (e.g. 19% at site 1). These results suggest that grazers feed not only on algae but also other food sources derived from terrestrial plant detritus, particularly where the algal population is limited in the upper reaches of the river.

On the other hand, it is generally considered that filterers feed on POM in the river within natural environments (Wallace and Merritt, 1980). In fact, the gut contents of filterers in this study consist mainly of an admixture of algae and terrestrial plant detritus (e.g. POM), as indicated by anatomical observations (unpublished data). However, the contribution of algae to POM is between 13% and 68%, which is much smaller than the contribution of algae to the diet of filterers (Fig. 4). In particular, a large difference in the contribution of algae to POM and filterers is seen in the upper reaches of the river (site 1). These results may suggest that filterers feed mainly on POM but can selectively assimilate algal material from the complex mixture that constitutes POM.

4. Summary and implications

The relative contribution of attached algae to the diet of aquatic insects (grazers and filterers) in the Chikuma River, Japan, is estimated to be 64% to 97%, respectively, as calculated using a modified mixing model that considers $\delta^{13}\text{C}$ and the C/N ratio. These values are $\sim 12\%$ larger than those obtained using the traditional mixing model. Considering the large variation in the C/N ratio among aquatic insects, attached algae, and terrestrial plants, we suggest that the modified mixing model is superior to the traditional mixing model in estimating the contribution of various sources to the analyzed samples.

The results obtained using the modified mixing model also show that grazers can feed not only on attached algae but also on other food sources derived from terrestrial plants in the case that the algal population is limited, and that filterers can selectively

assimilate algal material from the complex mixture that makes up their diet. Although these results seem to be inconsistent with empirical observations, they are in agreement with recent findings that filterers in various taxa can selectively assimilate specific materials from the complex mixtures of their diet (Wallace and Merritt, 1980; Edler and Georgian, 2004; Kitazawa et al., 2007; Naddafi et al., 2007). If such selective feeding is common in natural ecosystems, it further suggests that carbon and nutrient cycling is more complex than currently assumed. Therefore, both qualitative and quantitative assessments based on chemical analyses are required to gain a better understanding of carbon and nutrient cycling, in which regard we hope the modified mixing model proposed in the current study will make a contribution.

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