

Stable-isotope determination of mayfly (Insecta: Ephemeroptera) food sources in three tropical Asian streams

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With 1 figure and 3 tables

Abstract: Carbon and nitrogen stable isotopes were used to determine the foods assimilated by mayflies in three Hong Kong streams: Tai Po Kau Forest Stream (Forested 1), Shing Mun River (Forested 2), and an open (Open 1) and a shaded section (Shaded 1) of the Lam Tsuen River. Analyses were carried out during both wet and dry seasons. A total of seven mayfly species (Heptageniidae, Leptophlebiidae and Baetiidae; four or five species per site) were included. During the dry season, mayflies in the two forest streams (1 and 2) fed mainly on autochthonous foods (33–100 %) especially cyanobacteria and *Cladophora* spp. in Forested 1. During the wet season, the use of allochthonous food increased (13–100 %) in Forested 1 and 2, but the importance of autochthonous foods in shaded streams with ample stocks of detritus was nevertheless surprising. Mayflies in both Open 1 and Shaded 1 derived most of their biomass from a variety of autochthonous foods during both seasons, but epilithon was the dominant wet season food. Moreover, allochthonous foods did not appear to contribute significantly to the diet of mayflies during any season at Shaded 1. When compared with published literature, these results suggest that use of allochthonous foods in tropical streams may be greater than in temperate latitudes where allochthonous energy sources appear to dominate community energy flows in small streams.

Key words: N-15, C-13, allochthonous food, epilithon, nutrition.

Introduction

The organic carbon in streams is derived from either allochthonous (terrestrial) sources, primarily plant litter, or from autochthonous sources: i.e. primary producers within the stream itself. As in temperate regions, low-order

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streams in tropical Asia are generally considered to be heterotrophics, with high allochthonous inputs and primary production limited by riparian shading and scouring caused by spates (DUDGEON & BRETSCHKO 1996). In tropical Hong Kong, information from a single well-studied stream (Tai Po Kau Forest Stream) appears to confirm that community metabolism is heterotrophic with a P/R ratio as low as 0.17 (DUDGEON 1983, DUDGEON 1992 and references therein).

Stream discharge in Hong Kong is regulated by seasonal rainfall (DUDGEON & CORLETT 1994) as elsewhere in monsoonal Asia (DUDGEON 1995), resulting in distinct wet and dry seasons. During floods in the wet season periphyton biomass can be drastically reduced. DUDGEON (1982) measured up to a 90 % decrease in periphyton standing stock in TPKFS after a summer spate. Conversely, allochthonous material in the stream is always high, exceeding periphyton biomass by over 100 times (DUDGEON 1982). Litter input in Hong Kong streams occurs mainly from March to July when leaf-fall peaks, but there is significant leaf fall in every month and typhoons can cause short-term "pulses" of input (DUDGEON & CORLETT 1994). Based on relative biomass and availability throughout the year, it appears that allochthonous matter is the major food source available to macroinvertebrates in Hong Kong streams. However, there is evidence that the importance of autotrophs in streams is greater than their relative biomass implies (e. g., MINSHALL 1978). In addition, plant litter is not efficiently digested by most aquatic invertebrates, being suitable for consumption only after microbial conditioning (CUMMINS & KLUG 1979). This suggests that detritus does not always equal 'food' for stream invertebrates, whereas most algae are richer in protein and readily assimilated (LAMBERTI 1996).

Stable-isotope analysis has become a useful tool in determining the trophic base of food webs in aquatic systems, allowing determination of the relative contribution of allochthonous and autochthonous sources to the production of consumer biomass. However, due to the great variability of carbon signatures found in autotrophs, some authors have suggested the simultaneous use of more than one stable isotope (PETERSON & FRY 1987, FRANCE 1995). Stable isotope studies carried out in temperate regions have shown that allochthonous foods are the main source of organic carbon for macroinvertebrates in forested streams whereas, in open streams, there is an increase in the assimilation of autochthonous foods (ROUNICK et al. 1982, ROUNICK & HICKS 1985, WINTERBOURN et al. 1986, ROSENFELD & ROFF 1992, LESTER et al. 1995, HICKS 1997, THORP et al. 1998). Although there are few comparable studies from tropical streams and rivers, data from tropical and subtropical streams in Australia suggest that benthic invertebrates make considerable use of autochthonous foods (BUNN et al. 1997, 1999). Similar results were obtained from the Orinoco floodplain, where benthic animals fed primarily on phytoplankton and epiphytic

algae (HAMILTON et al. 1992). No data are currently available from tropical Asia.

The present study was undertaken to identify the principal food sources of mayflies (Ephemeroptera) in three Hong Kong streams. Food utilization was compared in the dry and wet season by measuring the stable carbon and nitrogen isotope ratios of mayflies, and comparing these values with the isotopic composition of the food sources at each site.

Materials and methods

Study sites

Samples for stable isotope analysis were collected from two forested streams, Tai Po Kau Forest Stream (Forested 1) and Shing Mun River (Forested 2), and from an open (Open 1) and a shaded site (Shaded 1) in Lam Tsuen River. Tai Po Kau Forest Stream flows through a managed nature reserve located on the southwestern shores of Tolo Harbour. The stream originates 400 m above sea level, and has slightly acidic waters which are poor in dissolved minerals but high in silicates (DUDGEON 1992). The study site, a second-order stream (UTM: 50Q KK 093821), is located at approximately 200 m altitude, with riparian vegetation represented by *Machilus* sp. (Lauraceae), *Cleistocalyx operculata* (Myrtaceae), and *Ficus pyriformis* (Moraceae). Shing Mun River is situated inside Shing Mun Country Park, adjacent to Tai Po Kau Nature Reserve. Samples were collected from a second-order stream (UTM: 50Q KK 071788), which has similar water chemistry to TPKFS (SALAS 1998), and riparian vegetation represented by *Garcinia oblongifolia* (Clusiaceae), *Ficus pyriformis* (Moraceae), *Bambusa* spp. (Gramineae), *Mucuna birdwoodiana* (Papilionaceae), *Gardenia jasminoides* (Rubiaceae), and *Litsea rotundifolia* (Lauraceae). The third site was Lam Tsuen River which is a 9-km long nitrate-enriched river (~1.0–5.0 mg NO₃/l; SALAS 1998) running from southwest to northeast across central New Territories, with organic pollution affecting the middle and lower courses (DUDGEON 1984). The study site, a second-order stream (UTM: 50Q KK 040844), was located in the upper part of the river, bordering the village of Pak Ngau Shek (DUDGEON 1996). Two sections of the stream were chosen: an open area with no riparian trees surrounded by abandoned fields covered in tall grasses, and a downstream shaded area with some riparian vegetation represented by *Buettneria aspera* (Sterculiaceae), *Ficus fistulosa*, *Ficus piryformis* (Moraceae), and *Cleistocalyx operculata* (Myrtaceae). C₄ vascular plants were not present near the study reaches. The mean (± SE) biomass of epilithon standing stock (chlorophyll-*a* content: APHA 1995) measured on five stones randomly collected from each of Open 1, Shaded 1, Forested 1 and 2 during August 1996 was 4.3 ± 0.6, 2.3 ± 0.7, 2.0 ± 0.4 and 2.5 ± 0.3 mg/m², respectively.

Sample collection

Leaf litter was collected from the stream bed during August 1996 (wet season) and January 1997 (dry season) at all sites except in Open 1 where riparian trees and CPOM

were lacking. Supplementary samples of leaf litter were collected from Forested 1 and Shaded 1 on August 1999 (wet season). Fine particulate organic matter (FPOM) was collected only during the dry season (February 1997) from cages (inner area = 0.1 m²; mesh size 500 µm) that had been set up in Open and Shaded 1 and in Forested 1, and used for studies of mayfly growth (SALAS 1998). No cages were set up in Forested 2. Filamentous algae (*Cladophora* spp.) were hand-picked from along the stream margins during September 1996 in Open and Shaded 1 and in February 1997 at Open 1 and Forested 1. Cyanobacterial samples were collected during October 1998 and July 1999 from Forested 1 and during January 2000 from Open 1 and Shaded 2. Epilithon samples were obtained during September 1996 and in January 1997 from 10 to 15 stones collected at each site. Mayflies were collected in several visits during the wet season (May to September 1996) and in the dry season (November 1996 to February 1997), from individual stones sampled in a stratified manner across the stream using a 250 µm-mesh net. Not all of the seven mayfly species were present at all sites in both seasons, but at least four species were collected from each site on every sampling visit. The mayflies studied were the most abundant species at each site, comprising *Afronurus* sp., *Cinygmmina* sp. and *Afronurus* sp. 1 (Heptageniidae); *Baetiella pseudofrequenta* and *Procloeon venustum* (Baetidae); *Choroterpes* spp. (Leptophlebiidae – two unidentified species that could not be separated as larvae) and *Caenis* spp. (Caenidae). Most mayfly species were reared in the laboratory allowing us to determine whether there are one or two species present. Most of them appear to be undescribed.

Laboratory procedures

Filamentous algae were rinsed in distilled water several times and all small invertebrates were sorted from detritus under a stereo-microscope. The algae were then transferred to a Petri dish and oven dried at 60 °C for 24 hours. Epilithon was scraped from stones with a stiff brush into microcentrifuge tubes, and was subsequently pelletized by centrifugation. Epilithon was probably contaminated by FPOM as no further separation of the pellets was carried out. Cyanobacterial films were collected from emergent boulders by scraping black algal patches with a razor blade into plastic vials filled with stream water. *Phormidium* and *Calothryx* species were the major components of cyanobacterial films in Forested 1, while *Microcystis*, *Phormidium* and diatom species were dominant in samples collected in Open and Shaded 1.

FPOM was washed several times inside a large Petri dish by suspending in distilled water. Associated macroinvertebrates were removed by hand under the stereo-microscope, and the excess water was filtered. FPOM on filters was transferred to a Petri dish using a spatula. Leaf litter collected from the stream was identified to genus or species and each taxon was processed separately. An additional sample of a mixture of litter from the stream bed collected during both wet and dry seasons was also analyzed. After drying, leaf litter and other potential food sources were ground in a small coffee mill. The resulting material was powdered with a pestle and mortar, and stored in sealed plastic vials. Before analysis, leaf samples were further processed through a ball mill to produce a homogeneous powder.

Table 1. Stable carbon and nitrogen isotope signatures of allochthonous and autochthonous foods collected from four sites in three Hong Kong streams during the wet and dry seasons. Forested 1 = Tai Po Kau Forest stream, Forested 2 = Shing Mun River, Open 1 and Shaded 1 = Open and Shaded sites in the Lam Tsuen River.

Food sources	Forested 1		Forested 2		Open 1		Shaded 1	
	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$
Dry season								
Allochthonous foods								
Terrestrial leaves								
<i>Cleistocalyx operculata</i>	-31.7	-0.7						
<i>Machilus</i> sp.	-32.1	-3.4						
<i>Ficus fistulosa</i>							-31.0	0.4
<i>Buettneria aspera</i>							-30.0	2.3
<i>Bambusa</i> spp.			-29.0	-0.2				
<i>Garcinia oblongifolia</i>			-28.8	0.0				
<i>Mucuna birdwodiana</i>			-28.1	-2.0				
<i>Gardenia jasminoides</i>			-32.7	-2.0				
<i>Litsea rotundifolia</i>			-33.2	-1.7				
Mixed leaf litter	-31.8	-1.1	-28.6	2.2			-30.0	0.6
FPOM	-29.3	0.6			-26.9	6.4	-26.9	6.4
Autochthonous foods								
Periphyton								
<i>Cladophora</i> spp.	-22.0	1.2	-22.5	0.7	-22.0	8.6	-27.8	7.0
Cyanobacteria*	-21.0	0.9					-24.3	7.3
	-21.5	-0.2			-16.8	8.1	-20.5	5.9
Wet season								
Allochthonous foods								
Terrestrial leaves								
<i>Cleistocalyx operculata</i> **	-33.3	-0.4						
<i>Machilus longipedunculata</i> **	-31.1	-6.2						
<i>Ficus fistulosa</i> **							-30.3	4.8
<i>Buettneria aspera</i> **							-29.7	1.2
Mixed leaf litter	-32.0	-2.0	-28.7	-1.6			-30.0	0.3
Autochthonous foods								
Periphyton								
<i>Cladophora</i> sp.	-24.9	1.3	-23.2	1.7	-24.3	6.8	-23.6	7.1
Cyanobacteria**					-27.2	6.0	-27.2	6.0
	-18.1	-0.5						

* = supplementary samples collected on October 1998 and January 2000

** = supplementary samples collected on July 1999

Mayflies were dissected in the laboratory and their digestive tracts removed to avoid contamination with non-assimilated materials still present in the gut. However, due to their small size (<5.0 mm body length), it was not possible to remove the guts of *B. pseudofrequenta*, *Caenis* spp. and *P. venustum*. After dissection, mayflies were oven dried at 60 °C for 24 hours, and then ground to a fine powder in a small mortar. Stable isotope analysis of each mayfly species for each site in each season was per-

formed on pooled samples (40 to 150 individuals) because at least 100 mg dry weight was required for analysis of both carbon and nitrogen isotopes.

Analysis of samples

Due to resource constraints only one sample of each food source and mayfly species was analyzed. Dry samples (each of at least 100 mg DW) were shipped to the Stable Isotope Research Facility for Environmental Research, in the Department of Biology of the University of Utah, where they were analyzed for $^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$. The analytical precision of this method was $\pm 0.3\text{‰}$. Results are reported as $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, which represents the difference between the sample and an international standard, air for nitrogen and Peedee Belemnite for carbon (PETERSON & FRY 1987). Units are parts per thousand (‰):

$$\delta X = [(R_{\text{sample}} / R_{\text{standard}}) - 1] \times 1000$$

where $X = ^{13}\text{C}$ or ^{15}N , and $R = ^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$. Positive ratios between the heavy and light isotope indicated enrichment in the heavy isotope, and negative values revealed depletion in the heavy isotope relative to a standard reference sample. A simple two-source model was used to determine the proportion of organic carbon derived from each food sampled (BUNN et al. 1999): $P_A = (\delta_{\text{consumer}} - f - \delta_B) / (\delta_A - \delta_B)$, where P_A = proportion of food A, f = isotopic fractionation (‰) (consumer – diet), $\delta_A = \delta^{13}\text{C}$ or $\delta^{15}\text{N}$ (‰) of food source A and $\delta_B = \delta^{13}\text{C}$ or $\delta^{15}\text{N}$ (‰) of food source B. An isotopic fractionation (f) of 1‰ was used for ^{13}C and 1–3‰ for ^{15}N (PETERSON & FRY 1987, BUNN & BOON 1993, BUNN et al. 1997).

Results

Isotopic composition of food sources

The mixed sample of allochthonous leaf litter showed little temporal or spatial variation in $\delta^{13}\text{C}$ -values (-28.7 to -32.0‰), while $\delta^{15}\text{N}$ -signatures varied from -2.0 to 2.2‰ (Table 1). The $\delta^{13}\text{C}$ of individual leaves from terrestrial species collected from all sites during the dry season ranged from -33.2 to -28.1‰ , while $\delta^{15}\text{N}$ varied from -3.4 to 2.3‰ . Supplementary samples of individual leaves collected during the wet season of 1999 showed $\delta^{13}\text{C}$ that ranged from -33.3 to -29.7‰ and $\delta^{15}\text{N}$ that varied from -6.2 to 4.8‰ . Mixed allochthonous leaf litter from Forested 2 was slightly more ^{13}C -enriched (2.0‰) than mixed litter from Forested 1 and Shaded 1. FPOM collected in Forested 1 during the dry season showed similar $\delta^{13}\text{C}$ -values to allochthonous matter, while FPOM from Shaded 1 had similar $\delta^{13}\text{C}$ - and $\delta^{15}\text{N}$ -values to epilithon. Although FPOM collected from Open 1 was more $\delta^{13}\text{C}$ -depleted than epilithon and *Cladophora* spp., the high $\delta^{15}\text{N}$ signature suggested an autochthonous origin.

Table 2. Ranges of relative contribution of autochthonous foods (%) to the support of mayfly biomass in three Hong Kong streams during the dry and wet seasons. E = epilithon, Cy = cyanobacteria, C = *Cladophora* spp. and FPOM = fine particulate autochthonous organic matter.

Site	Forested 1			Forested 2	Open 1				Shaded 1			
	E	Cy	C	E	E	Cy	C	FPOM	E	Cy	C	FPOM
Dry season												
<i>Afronurus</i> sp.	●	●	●	100	28	14	0	72–86	50	11	–	35
<i>Cinygmina</i> sp.	#	#	#	33	6	3	0	94–97	*	*	–	*
<i>Afronurus</i> sp. 1	●	●	97	●	–	–	–	–	–	–	–	–
<i>P. venustum</i>	●	●	●	#	0	56–67	44	33	–	–	–	–
<i>B. pseudofrequenta</i>	79	70	74	100	67	10–32	90	33–67	0	43	–	0
<i>Choroterpes</i> sp.	–	–	–	33	92	25–54	75	8–45	0	61	–	0
Wet season												
<i>Afronurus</i> sp.	53	46–75	–	43	●	–	●	–	73	–	0	–
<i>Cinygmina</i> sp.	49	27	–	0	–	–	–	–	–	–	–	–
<i>Caenis</i> spp.	–	–	–	–	93	–	7	–	95	–	0	–
<i>P. venustum</i>	–	–	–	#	–	–	–	–	–	–	–	–
<i>B. pseudofrequenta</i>	59	32	–	49	83	–	17	–	69	–	0	–
<i>Choroterpes</i> spp.	87	47	–	0	83	–	17	–	65	–	0	–

● = more ^{13}C -enriched than food

* = more ^{15}N -depleted than food

= more ^{15}N -enriched than food

– = not present

Epilithon samples were more ^{13}C -enriched than allochthonous sources in all sites ($>-2.0\text{‰}$), and higher ^{15}N values ($>6.0\text{‰}$) were measured for epilithon at both Open and Shaded 1 compared to both Forested 1 and 2 (Table 1). Epilithon collected during the dry season from Open 1 was more ^{13}C -enriched (-5.8‰) than samples from Shaded 1. However, during the wet season there was little difference in the isotopic carbon composition of epilithon from these two sites. Epilithon samples collected during the dry season at all sites were slightly more ^{13}C -enriched (-1 to -3‰) than in the wet season, except at Shaded 1 where wet-season samples were more carbon-enriched ($\sim-4.0\text{‰}$). *Cladophora* spp., a filamentous alga collected from Open 1 followed a similar seasonal pattern to epilithon, with $\delta^{13}\text{C}$ -enrichment during the dry season. *Cladophora* spp. sampled during the dry season in Forested 1 was more ^{13}C -enriched and ^{15}N -depleted than in Open 1, *Cladophora* spp. was not present in Forested 1 during the wet season. Cyanobacteria collected at Forested 1, Open 1 and Shaded 1 were one of the most ^{13}C -enriched materials sampled.

Isotopic composition of mayflies and potential food sources

The isotopic composition of mayflies showed greater temporal and spatial variation in both $\delta^{13}\text{C}$ - and $\delta^{15}\text{N}$ -values than their food sources. All mayfly species were more ^{13}C -enriched during the dry season at all stream sites, except for

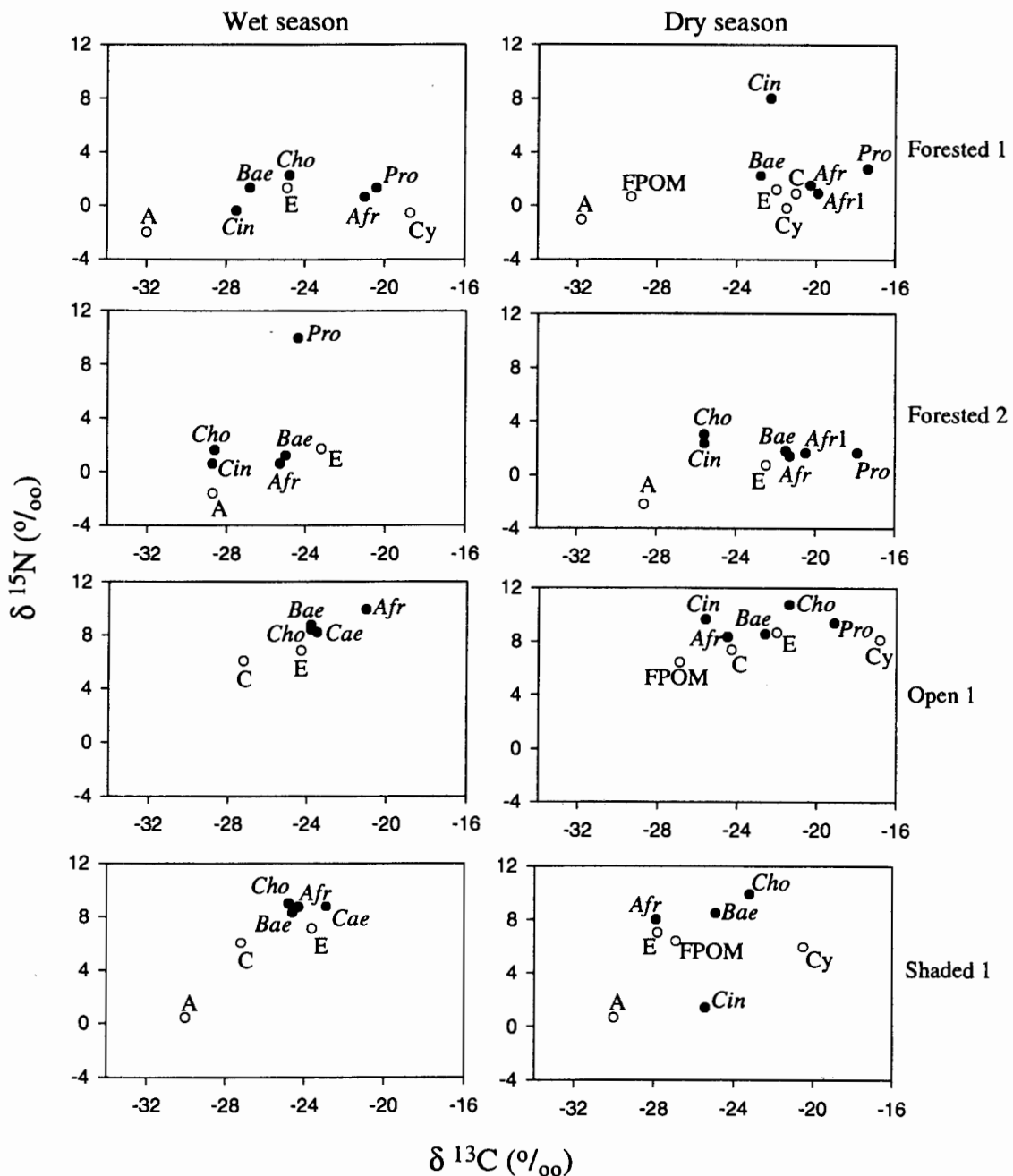


Fig. 1. Stable carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) ratios of mayflies (full circles) and food sources (open circles) in four stream sites sampled during the wet and dry seasons. Afr = *Afronurus* sp., Cin = *Cinygmmina* sp., Bae = *Baetiella pseudofrequenta*, Pro = *Procladius venustum*, Afr1 = *Afronurus* sp. 1, Cho = *Choroterpes* spp., C = *Cladophora* spp., Cy = Cyanobacterial films, E = epilithon, A = mixed allochthonous matter, FPOM = fine particulate organic matter.

Afronurus sp. in both Open and Shaded 1, where lower $\delta^{13}\text{C}$ values were measured during the dry season (Fig. 1). Most mayflies from Open and Shaded 1 were far more ^{15}N -enriched (8.0 to 10.7‰) than mayflies in either Forested 1 or 2. However, high nitrogen enrichment was also recorded in *Ci-*

nygmina sp. (8.0‰) collected during the dry season in Forested 1 and in *P. venustum* (10.0‰) from Forested 2 during the wet season. During both seasons, *Cinygmina* sp. and *B. pseudofrequenta* were the most ^{13}C -depleted species in Forested 1 while, in Forested 2, *Cinygmina* sp. and *Choroterpes* spp. had the lowest $\delta^{13}\text{C}$ values. *P. venustum* in Forested 1 was more ^{13}C -enriched (-17.4 to -20.4‰) than conspecifics from Forested 2 (-20.5 to -24.4‰), during both seasons. *Afronurus* sp. 1 was the most ^{13}C -enriched species in Forested 2 and the most ^{15}N -depleted in Forested 1. During the dry season, *Choroterpes* spp. from Open 1 showed higher $\delta^{13}\text{C}$ values than *Afronurus* sp., but the opposite was observed during the wet season.

The isotopic signatures of mayflies suggested that there was temporal variation in the utilization of available food sources at all stream sites (Fig. 1; Table 2). Using a two-source mixing model the percentage of mayfly biomass derived from autochthonous foods was calculated for each mayfly per site and season. During the dry season *Afronurus* species and *B. pseudofrequenta* from Forested 1 and 2 derived 70–100 % of their biomass from autochthonous foods, while *Cinygmina* sp. and *Choroterpes* spp. from Forested 2 derived only 33 % of their biomass from epilithon. In Open and Shaded 1 mayflies derived 0–92 % of their biomass from epilithon, 0–90 % from *Cladophora* spp., 3–67 % from cyanobacteria and 0–97 % from autochthonous FPOM (Table 2). However, during the wet season, mayflies collected from forest streams (1 and 2) showed an increase in the use of allochthonous foods. In Forested 1, 27–87 % of mayfly carbon was derived from autochthonous foods (cyanobacteria and epilithon), while in Forested 2, 57–100 % of their biomass was obtained from allochthonous foods. On the contrary, mayflies from Open and Shaded 1 derived most of their biomass from autochthonous foods (0–95 %).

For some mayfly species potential food sources could not be found. During the dry season, *Afronurus* sp. and *P. venustum* from Forested 1 and *Afronurus* sp. 1 from Forested 2 were more ^{13}C -enriched, while *Cinygmina* sp. (Forested 1) and *P. venustum* (Forested 2) were more ^{15}N -enriched than the foods sampled. By contrast, *Cinygmina* sp. in Shaded 1 had lower ^{15}N -values than the foods collected.

Discussion

Allochthonous and autochthonous food sources

The allochthonous food sources examined in this study showed little temporal variation in carbon at any stream site, although greater variations in nitrogen signatures were observed. Similar variations in nitrogen signatures for allochthonous detritus have been reported in related studies (BUNN & BOON 1993, HICKS 1997, HICKS & LABOYRIE 1999), where leaves conditioned for 28 days

had $\delta^{15}\text{N}$ values that were 0.7 to 3.0‰ higher than fresh leaves (HICKS & LABOYRIE 1999), and autochthonous carbon was more ^{13}C -enriched than allochthonous sources. LESTER et al. (1995), THORP et al. (1998) and BUNN et al. (1999) have observed a similar $\delta^{13}\text{C}$ -enrichment of autochthonous sources compared to allochthonous material. However, the opposite was found by ROUNICK et al. (1982), ROSENFELD & ROFF (1992), BUNN et al. (1997), and HICKS (1997). Such variation in the isotopic composition of aquatic autotrophs reflects the variability in $\delta^{13}\text{C}$ -values of inorganic carbon sources in different habitats, as well as taxonomic differences in photosynthetic fractionation (FRY & SHERR 1984).

Epilithon showed temporal variation in carbon signatures, with higher $\delta^{13}\text{C}$ -values during the dry season in all stream sites except in Shaded 1 where it was more ^{13}C -enriched during the wet season. Isotopic changes in benthic algae are dependent on factors affecting their growth rate, such as water temperature, light, nutrients and water velocity (HICKS 1997, MACLEOD & BARTON 1998, FINLAY et al. 1999). As stream discharge decreases during the dry season the concentrations of phosphate and nitrate rise (DUDGEON 1984, 1992, DUDGEON & CORLETT 1994). Higher nutrient availability is known to enhance primary production of filamentous algae and periphyton (THOMAS & DALDORPH 1994) increasing the assimilation of dissolved inorganic carbon (CO_2). This may explain the rise in $^{13}\text{C}/^{12}\text{C}$ -ratios found in epilithon collected during the dry season at Open 1, Forested 1 and 2. Although water current was not measured during the collection of samples, seasonal differences in carbon signatures of epilithon could be explained by changes in water velocity between seasons (FINLAY et al. 1999). ^{13}C -enrichment of periphyton at Shaded 1 during the wet season may reflect the combined effect of light and nutrients on algal growth in an environment where nutrients are not strongly limiting. Epilithon collected during the dry season from Open 1 was more ^{13}C -enriched than epilithon from Shaded 1, although little difference was observed during the wet season. Similar ^{13}C -depletion in periphyton from shaded sites has been reported previously (ROSENFELD & ROFF 1992, LESTER et al. 1995, HICKS 1997) and may reflect an increase in the allochthonous carbon present in periphyton samples, either in the form of FPOM or through the uptake of dissolved organic carbon by microheterotrophs.

In both Open and Shaded 1, autochthonous sources were at least 6‰ more ^{15}N -enriched than autochthonous material collected in either Forested 1 or 2. ^{15}N -enrichment of autochthonous sources has been found also for autotrophs in water bodies surrounded by grazing pasture (BOON & BUNN 1994, HICKS 1997), and for filamentous algae and macrophytes in a tropical lowland stream where the riparian vegetation was cleared for sugar cane (BUNN et al. 1997). Such ^{15}N -enrichment can also be explained by nitrogen inputs from agricultural land (MACKO & OSTROM 1994). Additionally, evidence from nitrogen-en-

riched sites with high terrestrial inputs shows that heterotrophic microbes depending on allochthonous organic matter can become increasingly ^{15}N -enriched by assimilating their nitrogen from nitrate (CARACO et al. 1998). Significantly, Lam Tsuen River had higher mean (\pm range) concentrations of dissolved nitrate ($1.2 \pm 4.7 \text{ mg NO}_3/\text{l}$) than either Forested 1 or 2 (0.1 ± 0.92 ; $0.1 \pm 0.4 \text{ mg NO}_3/\text{l}$, respectively) (SALAS 1998).

Food sources of consumers

Data on seasonal variations in algal abundance and possible changes in tissue support trophic structure in streams are scarce. Seasonal changes in the consumption of allochthonous and autochthonous foods were observed during the present study. During the dry season, mayflies in both Open and Shaded 1 assimilated autochthonous foods almost exclusively. Allochthonous foods did not appear to contribute significantly to the diet during any season at Shaded 1. Mayflies in both Forested 1 and 2 showed an increase in the assimilation of allochthonous foods during the wet season, and this was more evident in Forested 2. Seasonal and site-specific differences in the assimilated foods of macroinvertebrates have been reported from billabongs in southern Australia (BUNN & BOON 1993). For instance, during the spring, atyid shrimps appeared to ingest a mixture of foods at one site, and largely filamentous algae at another. However, in late summer, atyids were too ^{13}C -depleted to have fed on any of the foods sampled (BUNN & BOON 1993). By contrast, no seasonal variation was found in the foods used by macroinvertebrates in a New Zealand willow-shaded stream (Styx Creek), where animals fed on willow leaves during all seasons (LESTER et al. 1995). The isotopic composition of benthic animals from a second willow-shaded site also indicated use of allochthonous foods during summer and autumn, but in winter *Deleatidium* (Leptophlebiidae), *Olinga* (Conoesucidae) and *Aoteapsyche* (Hydropsychidae) were more ^{13}C -enriched than the food sources sampled (LESTER et al. 1995). Data from another New Zealand site – which was unshaded – indicated that benthic herbivores derived little of their carbon (0–14 %) from allochthonous foods in summer, but usage increased in autumn (13–23 %). In winter, they utilized autochthonous foods only (data from Table 1; in LESTER et al. 1995).

Some mayfly species in Hong Kong were more ^{13}C - and ^{15}N -enriched than the potential food sources sampled. For example, during the dry season, *Afronurus* sp. and *P. venustum* in Forested 1 and *Afronurus* sp. 1 in Forested 2 were more ^{13}C -enriched than the foods sampled. Likewise, the $\delta^{15}\text{N}$ -values of *Cinygmmina* sp. at Forested 1 during the dry season, and of *P. venustum* at Forested 2 during the wet season, were greater than the expected trophic enrichment (~ 3 – 5%) associated with the isotopic fractionation of food sources (MINAGAWA & WADA 1984). Coprophagy may increase ^{15}N -levels, because faeces

are $\delta^{15}\text{N}$ -enriched compared to the animal's diet (PETERSON & FRY 1987), but this does not seem a likely explanation of the present work. Fasting or starvation has been shown to increase ^{15}N (MINAGAWA & WADA 1984). However, this seems unlikely to occur in Hong Kong streams as secondary production estimates for both *Cinygmmina* sp. in Forested 1 and *P. venustum* in Forested 2 showed no evidence of food limitation (SALAS 1998). Another possible explanation might be variable levels of fractionation in autochthonous foods (PENNOCK et al. 1996) or the selective assimilation of ^{13}C -enriched algae from epilithon that also contains plant detritus.

Despite the apparent abundance of allochthonous foods in the majority of the study sites, the present study showed the relative importance of autochthonous sources in the assimilated diet of stream macroinvertebrates even at the shaded sites. It also revealed seasonal changes in the exploitation of autochthonous and allochthonous energy sources. Data from a temperate second-order stream have shown that algae support a high proportion of the macroinvertebrate production (SMOCK & ROEDING 1986) but, unfortunately, no stable isotope analysis was undertaken during that study. Isotopic data that are available for carbon sources in small streams are summarized in Table 3. Most studies suggest that benthic macroinvertebrates utilize allochthonous foods in forest streams but, at open sites, the assimilation of autochthonous matter generally increases. The importance of cyanobacteria, as shown by the present study, is not apparent elsewhere but whether this reflects inadequate sampling or a real difference is not clear. Although the database is limited, stable-isotope studies in tropical and subtropical streams (Table 3) indicate that autochthonous foods, especially epilithic algae, are the main source of carbon for benthic animals even at forested sites. The present study is the first to show the dominant importance of autochthonous foods in the assimilated carbon of primary consumers in forested Asian tropical streams, and this result is interesting in the light of the apparent paucity of shredders in these streams (DUDGEON 1995, DUDGEON & BRETSCHKO 1996). Unfortunately, there are insufficient data to determine whether the results from Hong Kong and elsewhere in the tropics (Table 3) are indicative of a widespread use of autochthonous foods, especially cyanobacteria, in lower latitude streams. However, community energy flow in small temperate streams evidently depends more on allochthonous inputs (Table 3; see also VANNOTTE et al. 1980).

This is the first study undertaken in tropical Asian streams using stable isotopes and, as such, there lacks a detailed analysis of all potential autochthonous food sources or isotopic identification of the individual components of the epilithon. A similar lack of resolution is apparent in studies undertaken elsewhere (ROSENFELD & ROFF 1992, LESTER et al. 1995). The mayflies studied obtained much of their energy from autochthonous sources, and unpublished data on *Psephenoides* sp. (*Psephenidae*: Coleoptera) larvae from the Shaded 1

Table 3. Allochthonous and autochthonous foods used by macroinvertebrates in temperate and tropical streams.

	Riparian vegetation	Main food assimilated	Source
Tropical			
Forest streams (Australia)	Tropical, subtropical rainforest	Epilithic algae	BUNN et al. 1999
Orinoco River floodplain (Venezuela)	Floodplain forest	Phytoplankton and epiphytic algae	HAMILTON et al. 1992
Bamboo Creek Lowland stream (Australia)	Sugarcane, paragrass	Epiphytic microalgae on aquatic plants	BUNN et al. 1997
Tai Po Kau Forest stream (Hong Kong)	Secondary forest	Dry season: autoch.; Wet season: autoch. but increased alloch. assimilation	Present study
Shing Mun River (Hong Kong)	Secondary forest	Dry season: autoch.; Wet season: allochthonous	Present study
Lam Tsuen River (Hong Kong)	Grasses (open site) Secondary forest (shaded site)	Autochthonous foods in both seasons Autochthonous foods in both seasons	Present study
Temperate			
Middle Bush stream	Beech	Allochthonous detritus	ROUNICK et al. 1982
Grasmere stream (New Zealand)	Tussock	Variable utilization of autoch. and alloch. foods	
Ashdown streams (England)	Woodland, grassland	Alloch. foods in small forest streams, but increased use of autoch. foods in open sites	WINTERBOURN et al. 1986
River Usk (Wales)	Woodland, grass		
Unforested streams		Epilithon and algae	ROSENFELD & ROFF 1992
Forest streams (Canada)	Beech, Oak, Ash, Poplar	Substantial use of algal carbon during spring	
Billabongs (Australia)	River red-gum, grass	Probably on planktonic Chlorophyta	BUNN & BOON 1993
Styx and Henney Creek (New Zealand)	Willow trees (two shaded sites) Tussock (two open sites)	Alloch. in shaded sites, but autoch. at open sites	LESTER et al. 1995
Ohio River (United States)	Willow, water mapple, grasses	Autoch. component of the seston (phytoplankton or detritus)	THORP et al. 1998
Forest and pasture streams	Native forest, exotic forest and pasture lands	Alloch. in forest streams; a mixture of both foods in unshaded pasture streams	HICKS 1997
Waikato region (New Zealand)			

showed that their organic carbon was mostly derived from autochthonous foods also (SALAS 1998). The high nutritive value and fast turnover rates of algae (LAMBERTI 1996) allows rapid growth and development of consumers. Indeed, Hong Kong mayflies can complete from four to 10 generations each year (SALAS 1998). However, it remains to be seen whether the dependence of mayflies on autochthonous sources is paralleled by use of such foods by other primary consumers.

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