

# What does stable isotope analysis reveal about trophic relationships and the relative importance of allochthonous and autochthonous resources in tropical streams? A synthetic study from Hong Kong

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## SUMMARY

1. Analysis of the stable isotope signatures of carbon (C) and nitrogen (N) of foods and consumers has led to some preliminary understanding of the relative importance of autochthonous and allochthonous resources in tropical streams. However, robust generalizations about the dynamics of food webs in these habitats, and their response to shading gradients or season, are still lacking. In addition, the feasibility of employing a baseline  $\delta^{15}\text{N}$  value for estimating trophic positions (TPs) of consumers in small tropical streams has yet to be explored.
2. We analysed data on stable isotope signatures of food sources and aquatic consumers obtained from 14 studies carried out in small streams in monsoonal Hong Kong (22°30'N, 114°10'E) between 1996 and 2006. Emphasis was placed on determining the relative importance of leaf litter and autochthonous foods in supporting consumer biomass, and the extent to which trophic base and TP vary among streams and seasons.
3. Although allochthonous leaf litter was generally  $^{13}\text{C}$ - and  $^{15}\text{N}$ -depleted relative to autochthonous foods, there were marked isotopic shifts of food sources and consumers in response to season (dry versus wet) and stream shading. Consumer taxa were generally more  $^{13}\text{C}$ - and  $^{15}\text{N}$ -enriched in the unshaded streams, but seasonal effects were more variable. Despite these changes, there was consistent evidence that stream food webs were based on periphytic algae and/or cyanobacteria with leaf litter serving as a minor food.
4. Heptageniidae (Ephemeroptera), Tipulidae (Diptera), Elmidae (Coleoptera) and shrimps (Atyidae) were used as a baseline for calculating the TPs of other consumer taxa. The maximum TPs in shaded streams remained fairly constant between seasons (dry = 3.93; wet = 3.97), while those in unshaded streams were higher and showed seasonal fluctuations (dry = 5.13; wet = 4.39).
5. Although variations in consumer isotope signatures in response to season and shading gradients did not confound our interpretation of the stream food base, changes in consumer  $\delta^{15}\text{N}$  did affect the calculation of consumer TPs. Misleading estimates of consumer TPs are likely if samples are collected from a narrow range of streams and/or during one season. Overestimation of the TPs of specialist herbivores (e.g. fish grazers) is also possible when autochthonous resources are substantially more  $^{15}\text{N}$ -enriched than allochthonous foods.

*Keywords:* energy flow, food web, riparian shading, seasonal effect, trophic position

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## Introduction

Understanding the pathways of nutrient and energy transfer and consumer–resource relationships in ecosystems has long been a major focus of ecological research (Pimm, 2002). The River Continuum Concept (RCC) suggests that food webs in shaded headwaters are principally driven by allochthonous energy, while autochthonous foods made a minor contribution to consumer biomass (Vannote *et al.*, 1980). Food web studies in temperate streams have often supported such generalizations (e.g. Winterbourn, Cowie & Rounick, 1984; Hicks, 1997; Hall, Likens & Malcolm, 2001), but the applicability of the RCC prediction that consumers depend on allochthonous than autochthonous resources in tropical headwater streams has yet to be verified.

Analyses of carbon (C) and nitrogen (N) stable isotope signatures have been employed widely in aquatic ecosystems to investigate consumer–resource interactions because they reflect food assimilation (Rounick & Winterbourn, 1986; Peterson & Fry, 1987; Fry, 2006) and thus offer an advantage over gut-content data that indicate ingestion only (Parkyn, Collier & Hicks, 2001). Stable isotope analysis (SIA) of allochthonous and autochthonous foods (which are usually distinct in both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) and consumers offers great potential for resolving issues arising from the RCC and the trophic basis of production in streams. The food base and diet of stream consumers, and thus their isotope signatures, appear prone to changes in the extent of riparian shading (e.g. Winterbourn, Rounick & Hildrew, 1986; Lester *et al.*, 1995; Hicks, 1997) and may vary seasonally (e.g. Bunn & Boon, 1993; Sheldon & Walker, 1997). These variations interact with consumer metabolic and assimilative processes and influence isotope variability through subsequent trophic transfers (Vander Zanden & Rasmussen, 2001).

Application of SIA in Neotropical streams indicates that riparian shading alters macroinvertebrate dependence on autochthonous and allochthonous foods although food webs in such streams appear predominantly algal-based (March & Pringle, 2003; Brito *et al.*, 2006). Similarly, SIA of consumers and their foods in rivers and wetlands with different riparian conditions in the Australian tropics indicates a general dependence of consumers on algal production (Bunn, Davies & Winning, 2003; Douglas, Bunn & Davies,

2005), and this dependence is high even in heavily shaded streams (Bunn, Davies & Mosisch, 1999). Investigation of a forest stream food web in Hong Kong that combined gut content analyses with SIA revealed a reliance on autochthonous foods (Mantel, Salas & Dudgeon, 2004). However, this study included only a single site in one season and does not provide a firm basis for generalization, since there is evidence that  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  signatures of mayflies and atyid shrimps in Hong Kong may vary seasonally and with riparian conditions (Salas & Dudgeon, 2001; Yam & Dudgeon, 2005). Some of this variability seems to reflect changes in food quality (e.g. C/N ratios and fatty acid content) that influence the dietary composition of consumers (Lau, Leung & Dudgeon, 2008a,b).

A major obstacle to better understanding trophic dynamics and food webs in streams is that the 'traditional' approach of grouping animals into discrete trophic levels and deriving food chain length by a simple count may underestimate the degree of complexity and prevalence of omnivory in aquatic ecosystems (Cabana & Rasmussen, 1994; Vander Zanden *et al.*, 1999; but see Schmid-Araya *et al.*, 2002). Vander Zanden & Rasmussen (1999) advocated the use of a baseline  $\delta^{15}\text{N}$  value against which  $\delta^{15}\text{N}$  values of various consumer taxa can be compared. The difference between their  $\delta^{15}\text{N}$  and the baseline  $\delta^{15}\text{N}$  would provide a non-integer value for the estimated trophic position (TP), which will be more accurate and sensitive to omnivory than the traditional approach (Cabana & Rasmussen, 1994). Thus far, application of this approach has been limited to lakes (e.g. Vander Zanden, Cabana & Rasmussen, 1997; Vander Zanden *et al.*, 1999), large rivers (Jepsen & Winemiller, 2002; Layman *et al.*, 2005; Anderson & Cabana, 2007) and coastal lagoon systems (Garcia *et al.*, 2007), although it has potential applications in studies of small streams where consumers frequently exhibit omnivory or mixed feeding modes (Mihuc, 1997; Mantel *et al.*, 2004; Douglas *et al.*, 2005).

The present study is an analysis of published and unpublished SIA data on food sources and consumers obtained from small streams in monsoonal Hong Kong over a 10-year period. The primary objective was to estimate the relative importance of autochthonous and allochthonous energy sources for aquatic consumers so as to infer the applicability of the RCC in tropical headstreams. Also, we used the SIA data to investigate whether season (wet versus dry) and

riparian shading conditions (shaded to unshaded) caused shifts in the relative importance of allochthonous and autochthonous energy to stream consumers. We also used these data to determine the extent of possible shifts in the TPs of consumer taxa in response to shading and season.

## Methods

The research consisted of an analysis of published and unpublished SIA results (henceforth, the data set) obtained from 14 studies carried out in second- to fourth-order streams during the dry and wet seasons in monsoonal Hong Kong (22°30'N, 114°10'E) between 1996 and 2006. The wet season in Hong Kong lasts from May to September when c. 80% of the total annual rainfall (mean = 2214 mm) is received; November to February is the dry season (Dudgeon & Corlett, 2004). The data set consisted of published studies of stable isotope signatures of consumers and food sources reported in Salas (1998), Salas & Dudgeon (2001), Mantel (2003), Mantel & Dudgeon (2004), Mantel *et al.* (2004), Yam & Dudgeon (2005), Chan (2008), Lau (2008), Lau *et al.* (2008a,b), Li (2008), Li & Dudgeon (2008), and unpublished studies undertaken by F.K.W. Cheung and D.C.P. Lau. All stream sites included in the data set were either shaded (with >70% riparian coverage over their channels) or unshaded (with <30% riparian coverage, flowing through abandoned farmland). They were generally unpolluted, although two received minor nutrient enrichment from agricultural runoff (Yam & Dudgeon, 2005; Li and Dudgeon, 2008).

Whole animals (singly or in groups) were used for SIA of all consumers except fish, for which only lateral white muscle tissues without bones and scales were analysed. To avoid sample contamination by non-assimilated materials, guts were removed by dissection or the animals were kept without food in the laboratory to allow gut clearance. Shells of all snails and shrimps were removed by hand in most studies. Hydrochloric acid was used by Mantel (2003) and Mantel *et al.* (2004) to decalcify snails, but no significant effect of this decalcification on isotope signatures was detected (Mantel, 2003).

Since variations in isotope signatures probably occur among major lineages (Vuorio, Meili & Sarvala, 2006; Anderson & Cabana, 2007), a range of consumer

taxa (i.e. species, families, orders and phyla) were included in TP component of the present study. For each shading condition in each season, the taxonomic group with the lowest  $\delta^{15}\text{N}$  was selected as the baseline for estimating the TPs of other taxa (Vander Zanden *et al.*, 1999; Anderson & Cabana, 2007):  $\text{TP}_{\text{TA}} = [\delta^{15}\text{N}_{\text{TA}} - \delta^{15}\text{N}_{\text{baseline}}]/3.4 + 2$ , where  $\delta^{15}\text{N}_{\text{TA}}$  is the  $\delta^{15}\text{N}$  of the taxon for which the TP is estimated,  $\delta^{15}\text{N}_{\text{baseline}}$  is the baseline  $\delta^{15}\text{N}$ , 3.4 is the typical  $\delta^{15}\text{N}$  fractionation per trophic level (Minagawa & Wada, 1984), and 2 is the expected TP of the baseline. Corrections used in Vander Zanden & Rasmussen (1999) and Anderson & Cabana (2007) were not performed since it was assumed that  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values were directly influenced by C/N ratios of consumers rather than correlated with each other (see Adams & Sterner, 2000; Matthews & Mazumder, 2005). Employing  $\delta^{15}\text{N}$  for a single taxon as the baseline is preferable to using the mean  $\delta^{15}\text{N}$  value of primary consumers, because it reduces the bias caused by the high  $\delta^{15}\text{N}$  variability among different taxa (Anderson & Cabana, 2007).

Seasonal and shading effects on the C and N isotope signatures of food sources and consumer taxa were investigated using two-way analysis of variance (ANOVA) and followed by Tukey multiple comparisons. Within each season and shading condition, isotope signatures of the taxa were also compared by one-way ANOVA. Independent-sample Student's *t*-tests (with equal/unequal variances) were used for comparisons when stable isotope data of food sources or consumers were not available from all sites or seasons. Data for ANOVA were  $\log_{10}$ -transformed if the variances among groups were heterogeneous. Power analysis was also employed for ANOVA and *t*-tests. All statistical analyses were performed with SPSS [Release 14.0.0 (2005); SPSS Inc., Chicago, IL, U.S.A.] at  $\alpha = 0.05$ .

## Results

There were a total of 780 stable isotope samples from 30 stream sites: 295 samples were basal food sources, and 485 were consumers identified mainly to species or genus (Table 1). Data were available from 16 shaded sites in the dry season, and 10 in the wet season. The equivalent totals for unshaded streams were 10 and four. There were more samples available from shaded streams in the dry season, while data

**Table 1** A summary of the data set extracted from previous studies (A–L) with the number of samples of food sources and aquatic taxa

	Abbreviation	Shaded		Unshaded		Total	References
		Dry	Wet	Dry	Wet		
<i>Food source</i>							
Leaf litter	LL	49 (13)	6 (5)	21 (8)	2 (2)	78	A, B, C, D, E, F, H, I, J, L, M, N
Fine particulate organic matter	FPOM	33 (12)	14 (4)	18 (9)	8 (3)	73	A, B, C, D, E, F, H, J, L, M, N
Periphyton	PP	22 (12)	31 (9)	14 (9)	9 (3)	76	B, D, E, F, G, H, I, J, L, M, N
Cyanobacteria	CY	21 (9)	3 (1)	11 (6)	3 (1)	38	B, D, E, H, J, L, M, N
Filamentous algae	FAI	16 (5)	1 (1)	12 (6)	1 (1)	30	B, D, E, H, J, L, M, N
Total		141	55	76	23	295	
<i>Taxonomic group</i>							
Mollusca: Gastropoda							
<i>Brotia hainanensis</i> (Brot, 1872) (Pachychilidae)	BH	27 (7)	15 (1)	14 (4)	2 (1)	58	D, E, H, I, J, L, M, N
Crustacea: Decapoda							
<i>Caridina</i> spp. (Atyidae)	Aty	20 (4)	11 (3)	22 (3)	6 (2)	59	D, E, F, H, J, N
<i>Macrobrachium hainanense</i> (Parisi, 1919) (Palaemonidae)	MH	22 (2)	22 (1)	15 (1)	– (–)	59	D, E, H, J, N
Ephemeroptera							
Baetidae	Bae	12 (9)	5 (3)	2 (2)	1 (1)	20	B, C, D, E, K, L, M
Caenidae	Cae	1 (1)	1 (1)	1 (1)	1 (1)	4	B
Ephemerellidae	Eph	5 (4)	– (–)	1 (1)	– (–)	6	D, E, L, M
Heptageniidae	Hep	31 (9)	5 (3)	8 (4)	1 (1)	45	B, C, D, E, L, M
Leptophlebiidae	Lep	5 (5)	3 (3)	3 (3)	1 (1)	12	B, D, E, K, L
Odonata: Zygoptera							
<i>Euphaea decorata</i> (Selys, 1853) (Euphaeidae)	ED	8 (4)	2 (2)	– (–)	– (–)	10	B, C, D, E, L
Plecoptera							
Perlidae	Per	4 (1)	– (–)	– (–)	– (–)	4	C, D, E
Tricoptera							
Calamoceratidae	Cal	8 (6)	– (–)	4 (4)	– (–)	12	D, E, K, L
Glossosomatidae	Glo	5 (3)	– (–)	– (–)	– (–)	5	C, K, L, M
Hydropsychidae	Hyd	12 (6)	– (–)	4 (4)	– (–)	16	C, K, L, M
Philopotamidae	Phi	3 (3)	– (–)	2 (2)	– (–)	5	L, M
Polycentropodidae	Pol	3 (3)	– (–)	2 (2)	– (–)	5	D, E, L, M
Coleoptera							
Elmidae	Elm	2 (2)	– (–)	3 (3)	– (–)	5	L
Eulichadidae	Eul	3 (3)	– (–)	– (–)	– (–)	3	L
Psephenidae	Pse	13 (6)	3 (3)	3 (3)	1 (1)	20	B, C, D, E, L, M
Scirtidae	Sci	2 (2)	– (–)	– (–)	– (–)	2	L
Diptera							
Chironominae (Chironomidae)	Chi	5 (4)	– (–)	2 (2)	– (–)	7	C, D, E, K, L, M
Orthoclaadiinae (Chironomidae)	Ort	3 (2)	– (–)	– (–)	– (–)	3	C, D, M
Tanypodinae (Chironomidae)	Tan	4 (3)	– (–)	– (–)	– (–)	4	C, D, E, L
<i>Eusimulium</i> spp. (Simuliidae)	Sim	1 (1)	– (–)	– (–)	– (–)	1	K
<i>Antocha</i> and <i>Tipula</i> spp. (Tipulidae)	Tip	3 (3)	– (–)	– (–)	– (–)	3	C, L, M
Heteroptera							
<i>Ptilomera tigrina</i> (Uhler, 1860) (Gerridae)	PT	6 (1)	– (–)	– (–)	– (–)	6	G
Pisces							
<i>Liniparhomaloptera disparis</i> (Lin, 1934) (Balitoridae)	LD	7 (2)	6 (2)	5 (1)	2 (1)	20	C, D, E, N

Table 1 (Continued)

	Abbreviation	Shaded		Unshaded		Total	References
		Dry	Wet	Dry	Wet		
<i>Pseudogastromyzon myersi</i> (Herre, 1932) (Balitoridae)	PM	8 (2)	8 (2)	– (–)	4 (1)	20	C, D, E, N
<i>Schistura fasciolata</i> (Nichols & Pope, 1927) (Balitoridae)	SF	7 (2)	6 (2)	2 (1)	– (–)	15	C, D, E, G, N
<i>Parazacco spilurus</i> (Günther, 1868) and <i>Zacco platypus</i> (Temminck & Schlegel, 1846) (Cyprinidae)	Cyp	12 (2)	7 (3)	6 (1)	4 (1)	29	D, E, G, N
<i>Ctenogobius duospilus</i> (Herre, 1935) (Gobiidae)	CD	10 (2)	8 (2)	5 (1)	4 (1)	27	C, D, E, N
Total		252	102	104	27	485	

Values in parentheses are the number of sites where samples were obtained.

A, Salas (1998); B, Salas & Dudgeon (2001); C, Mantel (2003); D, Mantel & Dudgeon (2004); E, Mantel *et al.* (2004); F, Yam & Dudgeon (2005); G, Chan (2008); H, Lau (2008); I, Lau *et al.* (2008a); J, Lau *et al.* (2008b); K, Li (2008); L, Li & Dudgeon (2008); M, F.K.W. Cheung (unpubl. data); N, D.C.P. Lau (unpubl. data).

from the wet season, especially unshaded streams, were relatively limited. Food sources included in the data set were leaf litter (mixed litter and single species), fine particulate organic matter (FPOM), periphyton, cyanobacteria and filamentous algae, each with at least 30 samples in total. Among the consumer taxa, 58 samples were gastropods (one species), 118 were decapods (two families), 87 were Ephemeroptera (five families), 43 were Trichoptera (five families), 30 were Coleoptera (four families), 18 were Diptera (three families), 111 were fishes (three families), while Odonata (Zygoptera), Plecoptera and Heteroptera were represented by one family and  $\leq 10$  samples each.

Allochthonous leaf litter was generally more  $^{13}\text{C}$ - and  $^{15}\text{N}$ -depleted than autochthonous periphyton and epilithic cyanobacteria in every season and shading condition, while FPOM and filamentous algae generally showed intermediate C and N signatures between litter and periphyton as well as cyanobacteria (one-way ANOVA: all  $P \leq 0.001$ ; Table 2; Fig. 1; Appendix S1). There were no interactions between seasonal and shading effects on food-source C signatures (Tables 2 & 3; Fig. 1). Leaf-litter C signatures were consistent among seasons and shading conditions, while  $\delta^{13}\text{C}$  values of FPOM and cyanobacteria were significantly higher during the wet season and in unshaded streams (Tables 2 & 3; Fig. 1). Periphyton was similarly more  $^{13}\text{C}$ -enriched in unshaded than shaded streams, but had lower  $\delta^{13}\text{C}$  values during the wet season that were similar to values for FPOM under all shading conditions. No significant difference in the  $\delta^{13}\text{C}$  signals of

filamentous algae between shaded and unshaded streams was observed (Student's *t*-test:  $t = 0.57$ , d.f. = 14.6,  $P > 0.05$ ; Fig. 1). Mean  $\delta^{13}\text{C}$  differences between periphyton and leaf litter ranged from 4.80‰ (wet, shaded) to 10.06‰ (dry, unshaded); for cyanobacteria and leaf litter the mean differences were 5.86‰ (dry, shaded) to 12.68‰ (wet, unshaded).

Significant interactions between season and shading effects on  $\delta^{15}\text{N}$  signatures were apparent for leaf litter only, which had highest  $\delta^{15}\text{N}$  in unshaded streams during the dry season but lowest during the wet season (Tables 2 & 3; Fig. 1). FPOM, periphyton and cyanobacteria were more  $^{15}\text{N}$ -enriched in unshaded than shaded streams, and during the wet season compared to the dry season. Filamentous algae from unshaded and shaded streams did not differ in  $\delta^{15}\text{N}$  (Student's *t*-test:  $t = 0.80$ , d.f. = 28,  $P > 0.05$ ; Fig. 1). Periphyton was, on average, 2.49‰ (dry, shaded) to 8.53‰ (wet, unshaded) more  $^{15}\text{N}$ -enriched than leaf litter; mean  $\delta^{15}\text{N}$  differences between cyanobacteria and leaf litter ranged from 2.35‰ (dry, shaded) to 10.43‰ (wet, unshaded) (Fig. 1).

The balitorid fishes *Liniparhomaloptera disparis* and *Pseudogastromyzon myersi* (benthic grazers) were usually the most  $^{13}\text{C}$ -enriched consumer taxa, although caenid mayflies (grazer-collectors) had high  $\delta^{13}\text{C}$  in shaded streams during the dry season (one-way ANOVA: all  $P \leq 0.001$ ; Table 2; Fig. 1; Appendix S2). During the wet season, most consumer taxa were more  $^{13}\text{C}$ -enriched than periphyton with mayflies (e.g. Leptophlebiidae and Baetidae) exhibiting the



**Table 2** Sample sizes and means ( $\pm$  SE) for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of food sources and consumer taxa in shaded and unshaded Hong Kong streams during the dry and wet seasons

	Dry shaded			Wet shaded			Dry unshaded			Wet unshaded		
	<i>n</i>	Mean	SE	<i>n</i>	Mean	SE	<i>n</i>	Mean	SE	<i>n</i>	Mean	SE
$\delta^{13}\text{C}$												
Food source												
LL	49	-30.46	0.17	6	-30.20	0.44	21	-29.81	0.27	2	-29.55	0.75
FPOM	33	-28.34	0.40	14	-24.46	0.73	18	-25.61	0.58	8	-21.97	0.54
PP	22	-23.10	0.72	31	-25.39	0.43	14	-19.75	1.23	9	-22.54	0.46
CY	21	-24.60	0.54	3	-20.53	1.17	11	-22.90	0.98	3	-16.87	1.22
FAI	16	-27.73	0.59	1	-27.20	–	12	-28.84	1.84	1	-27.20	–
Taxonomic group												
BH	27	-25.37	0.42	15	-25.44	0.32	14	-21.66	0.43	2	-21.20	0.00
Aty	20	-23.45	0.36	11	-23.79	0.51	22	-23.89	0.27	6	-22.20	0.58
MH	22	-24.15	0.12	22	-24.50	0.13	15	-21.16	0.11	–	–	–
Bae	12	-23.08	1.02	5	-24.24	1.05	2	-21.26	1.34	1	-23.80	–
Cae	1	-22.16	–	1	-22.90	–	1	-25.39	–	1	-23.50	–
Eph	5	-23.26	2.20	–	–	–	1	-27.06	–	–	–	–
Hep	31	-24.12	0.68	5	-25.36	1.34	8	-22.44	1.38	1	-21.00	–
Lep	5	-25.49	0.69	3	-26.07	1.27	3	-21.45	1.57	1	-23.80	–
ED	8	-25.84	0.47	2	-24.00	0.40	–	–	–	–	–	–
Per	4	-26.42	0.26	–	–	–	–	–	–	–	–	–
Cal	8	-29.10	0.44	–	–	–	4	-25.23	2.11	–	–	–
Glo	5	-26.14	2.38	–	–	–	–	–	–	–	–	–
Hyd	12	-25.80	0.79	–	–	–	4	-26.23	0.74	–	–	–
Phi	3	-25.73	1.61	–	–	–	2	-23.63	0.76	–	–	–
Pol	3	-23.11	1.71	–	–	–	2	-20.59	3.03	–	–	–
Elm	2	-26.60	1.16	–	–	–	3	-24.14	1.47	–	–	–
Eul	3	-29.03	0.49	–	–	–	–	–	–	–	–	–
Pse	13	-25.39	0.97	3	-24.17	1.20	3	-21.07	1.10	1	-22.20	–
Sci	2	-27.04	0.05	–	–	–	–	–	–	–	–	–
Chi	5	-24.94	1.34	–	–	–	2	-23.08	2.23	–	–	–
Ort	3	-24.23	1.83	–	–	–	–	–	–	–	–	–
Sim	1	-26.34	–	–	–	–	–	–	–	–	–	–
Tan	4	-24.78	1.99	–	–	–	–	–	–	–	–	–
Tip	3	-24.89	2.66	–	–	–	–	–	–	–	–	–
PT	6	-26.27	0.14	–	–	–	–	–	–	–	–	–
LD	7	-22.87	0.76	6	-18.60	0.92	5	-17.43	0.26	2	-16.75	0.15
PM	8	-23.93	0.46	8	-20.78	0.58	–	–	–	4	-19.68	0.64
SF	7	-24.72	0.17	6	-24.14	0.53	2	-20.28	0.04	–	–	–
Cyp	12	-25.13	0.14	7	-23.90	0.60	6	-19.11	0.19	4	-22.03	0.31
CD	10	-24.54	0.17	8	-23.06	0.40	5	-21.36	0.07	4	-20.98	0.46
$\delta^{15}\text{N}$												
Food source												
LL	49	-2.08	0.29	6	-1.30	0.76	21	0.70	0.45	2	-2.30	1.40
FPOM	33	0.47	0.34	14	4.71	0.75	18	3.28	0.49	8	5.93	0.84
PP	22	0.41	0.44	31	3.00	0.48	14	4.70	0.89	9	6.23	0.82
CY	21	0.27	0.33	3	5.97	0.62	11	4.54	1.00	3	8.13	0.82
FAI	16	1.85	0.54	1	6.00	–	12	2.61	0.87	1	6.00	–
Taxonomic group												
BH	27	0.79	0.13	15	1.91	0.10	14	7.34	0.42	2	7.71	0.12
Aty	20	3.40	0.40	11	2.66	0.17	22	5.69	0.44	6	4.02	0.71
MH	22	4.96	0.11	22	5.25	0.15	15	8.65	0.34	–	–	–
Bae	12	2.58	0.70	5	4.42	1.95	2	6.31	2.19	1	8.70	–
Cae	1	5.97	–	1	8.70	–	1	7.13	–	1	8.20	–
Eph	5	0.93	0.27	–	–	–	1	6.22	–	–	–	–

Table 2 (Continued)

	Dry shaded			Wet shaded			Dry unshaded			Wet unshaded		
	<i>n</i>	Mean	SE	<i>n</i>	Mean	SE	<i>n</i>	Mean	SE	<i>n</i>	Mean	SE
Hep	31	2.52	0.44	5	2.00	1.69	8	6.96	0.96	1	9.90	–
Lep	5	4.07	1.47	3	4.27	2.37	3	5.41	2.80	1	8.40	–
ED	8	5.23	0.64	2	6.35	3.05	–	–	–	–	–	–
Per	4	4.87	0.26	–	–	–	–	–	–	–	–	–
Cal	8	1.60	0.37	–	–	–	4	3.52	1.29	–	–	–
Glo	5	0.97	0.20	–	–	–	–	–	–	–	–	–
Hyd	12	3.07	0.42	–	–	–	4	4.99	1.36	–	–	–
Phi	3	2.86	0.18	–	–	–	2	6.32	1.76	–	–	–
Pol	3	5.39	1.31	–	–	–	2	6.65	2.91	–	–	–
Elm	2	0.75	0.08	–	–	–	3	1.23	0.55	–	–	–
Eul	3	0.07	0.21	–	–	–	–	–	–	–	–	–
Pse	13	0.69	0.53	3	2.17	2.32	3	3.38	1.77	1	6.70	–
Sci	2	2.10	0.40	–	–	–	–	–	–	–	–	–
Chi	5	3.18	0.94	–	–	–	2	3.15	0.34	–	–	–
Ort	3	1.76	0.30	–	–	–	–	–	–	–	–	–
Sim	1	1.89	–	–	–	–	–	–	–	–	–	–
Tan	4	4.46	1.06	–	–	–	–	–	–	–	–	–
Tip	3	0.13	0.60	–	–	–	–	–	–	–	–	–
PT	6	3.53	0.37	–	–	–	–	–	–	–	–	–
LD	7	5.57	0.40	6	7.07	1.31	5	11.57	0.14	2	9.95	0.05
PM	8	5.14	0.56	8	7.95	1.00	–	–	–	4	10.98	0.16
SF	7	6.71	0.53	6	6.41	0.19	2	10.39	0.25	–	–	–
Cyp	12	5.66	0.28	7	7.44	1.06	6	11.87	0.16	4	11.38	0.14
CD	10	5.93	0.49	8	8.70	1.06	5	10.60	0.04	4	12.13	0.17

See Table 1 for abbreviations.

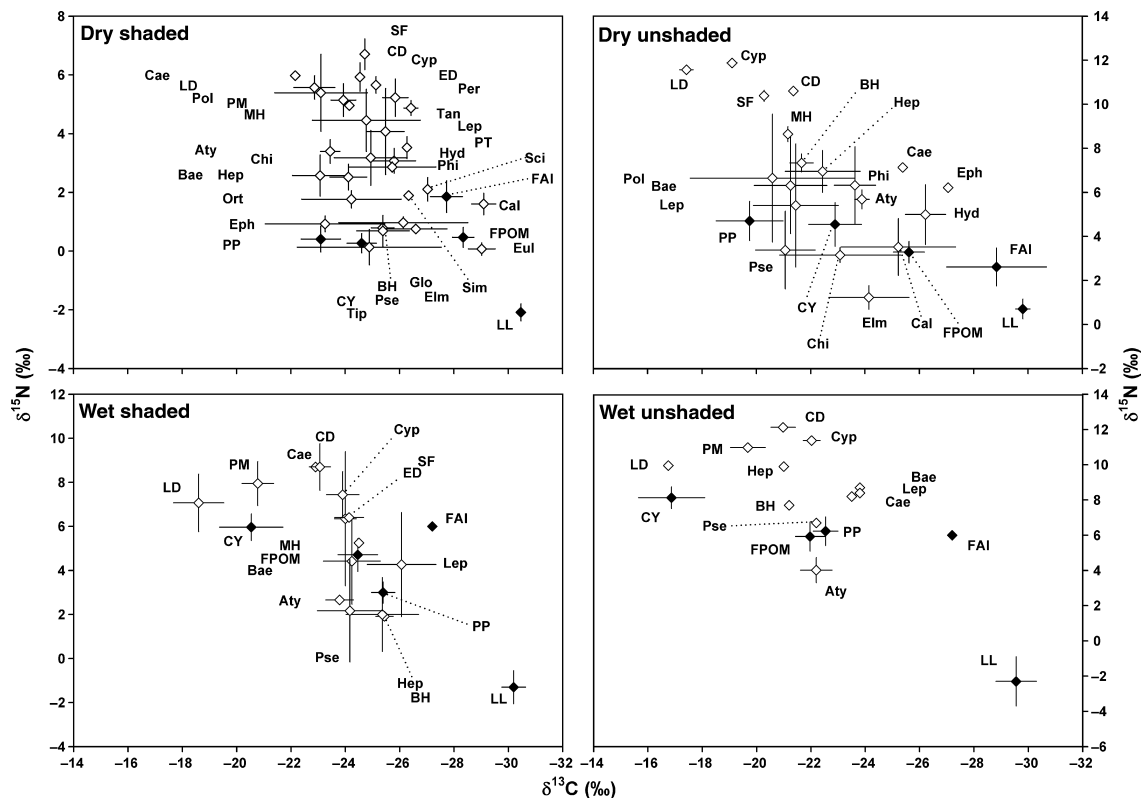
lowest  $\delta^{13}\text{C}$ . Shredders such as Calamoceratidae (*Anisocentropus* and *Ganonema*) and Eulichadidae (*Eulichas*) were the most  $^{13}\text{C}$ -depleted taxa in shaded streams during the dry season when Ephemerellidae (grazer-collectors) and Hydropsychidae (filter-feeders) were most  $^{13}\text{C}$ -depleted taxa in unshaded streams (one-way ANOVA: all  $P \leq 0.001$ ; Table 2; Fig. 1; Appendix S2).

*Brodia hainanensis* (a snail that feeds as a grazer-shredder) and *Schistura fasciolata* (a predatory benthic fish) were more  $^{13}\text{C}$ -enriched in unshaded than shaded streams, but their C signatures did not change with season (Tables 2 & 4; Fig. 1). In contrast, *P. myersi* was more  $^{13}\text{C}$ -enriched during the wet season, although shading condition had no effect on its C signature. *Macrobrachium hainanense* (a benthic predatory shrimp), *L. disparis* and *Ctenogobius duospilus* (a predatory benthic fish) had higher  $\delta^{13}\text{C}$  in unshaded streams during the dry season (*M. hainanense*) and the wet season (both fishes). There was a significant interaction between season and shading effects on the C signatures of atyid shrimps (*Caridina* spp.; grazer-collectors) and cyprinid fish (*Parazacco spilurus* and *Zacco platypus*; nektonic

predators): atyids in unshaded streams had higher  $\delta^{13}\text{C}$  during the wet season; during the dry season, both cyprinids were  $^{13}\text{C}$ -enriched in unshaded streams but  $^{13}\text{C}$ -depleted in shaded streams.  $\delta^{13}\text{C}$  values of other insect taxa did not differ significantly among seasons or between shaded and unshaded streams ( $P > 0.05$  in all cases; Tables 2 & 4; Fig. 1) but statistical power was relatively low (0.5–0.8).

In general, fishes (especially *C. duospilus* and predatory cyprinid minnows) were the most  $^{15}\text{N}$ -enriched consumers in all streams and seasons (one-way ANOVA: all  $P \leq 0.001$ ; Table 2; Fig. 1; Appendix S2). *Macrobrachium hainanense* also showed high  $\delta^{15}\text{N}$  values during the dry season when compared with other taxa. In contrast, Psephenidae (benthic grazers) and *B. hainanensis* were  $^{15}\text{N}$ -depleted relative to other consumers in shaded streams, as were grazer-collectors such as Elmidae (dry season) and Atyidae (wet season) (one-way ANOVA: all  $P \leq 0.001$ ; Table 2; Fig. 1; Appendix S2).

*Brodia hainanensis*, shrimps (Atyidae and *M. hainanense*) and *S. fasciolata* were more  $^{15}\text{N}$ -enriched in unshaded than shaded streams, especially during the



**Fig. 1**  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values (mean  $\pm$  SE) of food sources (solid diamonds) and consumer taxa (open diamonds) in shaded and unshaded Hong Kong streams during the dry and wet seasons. Food sources: CY, cyanobacteria; FAI, filamentous algae; FPOM, fine particulate organic matter; LL, leaf litter; PP, periphyton. Consumers: Aty, *Caridina* spp.; Bae, Baetidae; BH, *Brotia hainanensis*; Cae, Caenidae; Cal, Calamoceratidae; CD, *Ctenogobius duospilus*; Chi, Chironominae; Cyp, *Parazacco spilurus* and *Zacco platypus*; ED, *Euphaea decorata*; Elm, Elmidae; Eph, Ephemerellidae; Eul, Eulichadidae; Glo, Glossosomatidae; Hep, Heptageniidae; Hyd, Hydropsychidae; LD, *Liniparhomaloptera disparis*; Lep, Leptophlebiidae; MH, *Macrobrachium hainanense*; Ort, Orthocladiinae; Per, Perlidae; Phi, Philopotamidae; PM, *Pseudogastromyzon myersi*; Pol, Polycentropodidae; Pse, Psephenidae; PT, *Ptilomera tigrina*; Sci, Scirtidae; SF, *Schistura fasciolata*; Sim, *Eusimulium* spp.; Tan, Tanypodinae; Tip, *Antocha* and *Tipula* spp.

dry season (Tables 2 & 4; Fig. 1). *Pseudogastromyzon myersi* and *C. duospilus* similarly showed higher  $\delta^{15}\text{N}$  in unshaded streams, but were more  $^{15}\text{N}$ -enriched during the wet season. N signatures of Heptageniidae (grazer-collectors), Psephenidae, *L. disparis* and predatory minnows were consistent among seasons but were higher in unshaded streams. No significant effects of season and/or shading condition on N signatures of other taxa were detected ( $P > 0.05$  in all cases; Tables 2 & 4; Fig. 1).

Tipulidae (*Antocha* and *Tipula* spp.; grazers and shredders respectively) (dry, shaded), Heptageniidae (wet, shaded), Elmidae (dry, unshaded) and Atyidae (wet, unshaded) were selected as the baseline for TP estimation as they were relatively low in  $\delta^{15}\text{N}$  and their samples were obtained from most stream sites (Table 2). Using these baselines, it was evident that TPs of fishes were always high irrespective of the

feeding modes (i.e. herbivorous or predatory). The maximum TPs in the shaded streams during the dry and wet seasons were similar: 3.93 (*S. fasciolata*) and 3.97 (*C. duospilus*). TPs in unshaded streams were higher: 4.39 (*C. duospilus*) during the dry season, increasing to 5.13 (predatory minnows) during the wet season (Table 5).

## Discussion

The results of the present study showed that C and N stable isotope signatures of consumers and their foods varied with season and shading conditions in small tropical headstreams. Autochthonous sources such as periphyton and cyanobacteria were consistently more  $^{13}\text{C}$ - and  $^{15}\text{N}$ -enriched (by  $>4\text{‰}$  and  $>2\text{‰}$  respectively) than allochthonous leaf litter, with these differences usually more pronounced ( $>8\text{‰}$ ) in unshaded



**Table 3** Two-way ANOVA for seasonal (dry and wet) and shading (shaded and unshaded) effects on  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of food sources in Hong Kong streams

ANOVA		d.f. 1	d.f. 2	F	P-value	Tukey multiple comparison		
$\delta^{13}\text{C}$								
LL	Season	1	74	0.272	0.604	N.A.		
	Shading	1	74	1.655	0.202	N.A.		
	Interaction	1	74	<0.001	0.991			
FPOM	Season	1	69	36.517	<0.001	Wet	>	Dry
	Shading	1	69	17.512	<0.001	Unshaded	>	Shaded
	Interaction	1	69	0.037	0.848			
PP*	Season	1	72	7.423	0.013	Dry	>	Wet
	Shading	1	72	14.500	<0.001	Unshaded	>	Shaded
	Interaction	1	72	0.046	0.668			
CY	Season	1	34	17.552	<0.001	Wet	>	Dry
	Shading	1	34	4.964	0.033	Unshaded	>	Shaded
	Interaction	1	34	0.661	0.422			
$\delta^{15}\text{N}$								
LL	Season	1	74	1.620	0.207	N.A.		
	Shading	1	74	1.051	0.309	N.A.		
	Interaction	1	74	4.719	0.033			
FPOM <sup>†</sup>	Season	1	69	31.815	<0.001	Wet	>	Dry
	Shading	1	69	13.026	0.001	Unshaded	>	Shaded
	Interaction	1	69	2.978	0.089			
PP <sup>†</sup>	Season	1	72	11.040	0.001	Wet	>	Dry
	Shading	1	72	30.868	< 0.001	Unshaded	>	Shaded
	Interaction	1	72	1.156	0.286			
CY <sup>†</sup>	Season	1	34	19.348	< 0.001	Wet	>	Dry
	Shading	1	34	8.282	0.007	Unshaded	>	Shaded
	Interaction	1	34	1.772	0.192			

See Table 1 for abbreviations.

\* $\text{Log}_{10}(\delta^{13}\text{C} + 38)$ -transformed.<sup>†</sup> $\text{Log}_{10}(\delta^{15}\text{N} + 8)$ -transformed.

streams. This is probably due to substantial isotopic enrichment of in-stream primary production with decreasing riparian coverage and increasing light (see also March & Pringle, 2003). Despite such inter-stream differences, isotope signatures of allochthonous and autochthonous foods in Hong Kong streams were generally within the same range as those reported from other tropical areas. For instance, mean  $\delta^{13}\text{C}$  ranges of leaf litter and periphyton in streams in Puerto Rico were  $-30.0\text{‰}$  to  $-28.6\text{‰}$  and  $-24.1\text{‰}$  to  $-20.2\text{‰}$  (March & Pringle, 2003); while mean  $\delta^{15}\text{N}$  ranges were  $0.1\text{‰}$  to  $1.5\text{‰}$  and  $3.4$  to  $6.4\text{‰}$ , respectively. Mean  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of periphytic microalgae in Brazilian streams similarly ranged from  $-25.6\text{‰}$  to  $-19.0\text{‰}$  and  $1.4$  to  $2.5\text{‰}$  (Brito *et al.*, 2006). In tropical Australasian streams, C signatures of leaf litter were generally around  $-30\text{‰}$ , while autochthonous algal foods were more variable with  $\delta^{13}\text{C}$  values ranged from  $-35.0\text{‰}$  to  $-18.3\text{‰}$  (Douglas *et al.*, 2005).

Isotopic shifts of consumers due to season and shading effects were usually more apparent for taxa feeding higher in the food web, such as predatory fishes (i.e. *S. fasciolata*, *C. duospilus* and cyprinid minnows) or predatory invertebrates such as *M. hainanense* and *Euphaea decorata*, but they were also observed in a few primary consumers such as Heptageniidae and Psephenidae. Although these taxa generally showed higher  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in unshaded streams, the overall pattern was that they had C signatures well within or occasionally greater than the ranges for sources such as periphyton and cyanobacteria, indicating a dependence on such autochthonous foods regardless of shading conditions. Autochthonous foods are of higher quality (with lower C/N ratios and higher essential fatty acid contents) than leaf litter (Lau *et al.*, 2008a,b) which probably accounts for their importance to consumers. Algal foods also tend to be the main energy source of stream consumers in the Neotropics (Bunn

**Table 4** Two-way ANOVA for seasonal (dry and wet) and shading (shaded and unshaded) effects on  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of consumer taxa in Hong Kong streams

ANOVA		d.f. 1	d.f. 2	F	P-value	Tukey multiple comparison		
$\delta^{13}\text{C}$								
BH	Season	1	54	0.242	0.788	N.A.		
	Shading	1	54	93.760	<0.001	Unshaded	>	Shaded
	Interaction	1	54	0.422	0.722			
Aty	Season	1	55	2.344	0.132	N.A.		
	Shading	1	55	1.714	0.196	N.A.		
	Interaction	1	55	5.298	0.025			
MH	Season	1	56	4.194	0.045	Dry	>	Wet
	Shading	1	56	251.495	<0.001	Unshaded	>	Shaded
	Interaction	N.A.	N.A.	N.A.	N.A.			
Bae	Season	1	16	0.759	0.397	N.A.		
	Shading	1	16	0.281	0.603	N.A.		
	Interaction	1	16	0.105	0.750			
Hep	Season	1	41	0.002	0.964	N.A.		
	Shading	1	41	1.922	0.173	N.A.		
	Interaction	1	41	0.380	0.541			
Lep	Season	1	8	1.086	0.328	N.A.		
	Shading	1	8	5.019	0.055	N.A.		
	Interaction	1	8	0.394	0.548			
Pse	Season	1	16	<0.001	0.983	N.A.		
	Shading	1	16	2.242	0.154	N.A.		
	Interaction	1	16	0.314	0.583			
LD	Season	1	16	7.562	0.014	Wet	>	Dry
	Shading	1	16	16.441	0.001	Unshaded	>	Shaded
	Interaction	1	16	3.985	0.063			
PM	Season	1	17	18.902	< 0.001	Wet	>	Dry
	Shading	1	17	1.527	0.233	N.A.		
	Interaction	N.A.	N.A.	N.A.	N.A.			
SF	Season	1	12	1.330	0.271	N.A.		
	Shading	1	12	37.447	< 0.001	Unshaded	>	Shaded
	Interaction	N.A.	N.A.	N.A.	N.A.			
Cyp	Season	1	25	5.628	0.026	Dry	>	Wet
	Shading	1	25	123.073	< 0.001	Unshaded	>	Shaded
	Interaction	1	25	33.938	< 0.001			
CD*	Season	1	23	8.711	0.007	Wet	>	Dry
	Shading	1	23	66.638	< 0.001	Unshaded	>	Shaded
	Interaction	1	23	3.634	0.069			
$\delta^{15}\text{N}$								
BH <sup>†</sup>	Season	1	54	5.016	0.029	Dry	>	Wet
	Shading	1	54	223.489	<0.001	Unshaded	>	Shaded
	Interaction	1	54	1.908	0.173			
Aty <sup>†</sup>	Season	1	55	5.523	0.022	Dry	>	Wet
	Shading	1	55	13.791	<0.001	Unshaded	>	Shaded
	Interaction	1	55	0.789	0.378			
MH <sup>†</sup>	Season	1	56	1.493	0.227	Dry	>	Wet
	Shading	1	56	161.072	<0.001	Unshaded	>	Shaded
	Interaction	N.A.	N.A.	N.A.	N.A.			
Bae	Season	1	16	1.071	0.316	N.A.		
	Shading	1	16	3.838	0.068	N.A.		
	Interaction	1	16	0.018	0.896			
Hep	Season	1	41	0.614	0.438	N.A.		
	Shading	1	41	15.916	< 0.001	Unshaded	>	Shaded
	Interaction	1	41	1.258	0.269			

Table 4 (Continued)

ANOVA		d.f. 1	d.f. 2	F	P-value	Tukey multiple comparison		
Lep	Season	1	8	0.352	0.570	N.A.		
	Shading	1	8	1.034	0.339	N.A.		
	Interaction	1	8	0.271	0.617			
Pse	Season	1	16	2.219	0.156	N.A.		
	Shading	1	16	5.033	0.039	Unshaded	>	Shaded
	Interaction	1	16	0.328	0.575			
LD	Season	1	16	0.004	0.950	N.A.		
	Shading	1	16	21.286	< 0.001	Unshaded	>	Shaded
	Interaction	1	16	2.617	0.125			
PM	Season	1	17	7.181	0.016	Wet	>	Dry
	Shading	1	17	5.570	0.030	Unshaded	>	Shaded
	Interaction	N.A.	N.A.	N.A.	N.A.			
SF	Season	1	12	0.270	0.612	Dry	>	Wet
	Shading	1	12	19.598	0.001	Unshaded	>	Shaded
	Interaction	N.A.	N.A.	N.A.	N.A.			
Cyp <sup>†</sup>	Season	1	25	1.213	0.281	N.A.		
	Shading	1	25	61.446	<0.001	Unshaded	>	Shaded
	Interaction	1	25	3.043	0.093			
CD <sup>†</sup>	Season	1	23	6.347	0.019	Wet	>	Dry
	Shading	1	23	24.683	<0.001	Unshaded	>	Shaded
	Interaction	1	23	0.891	0.355			

See Table 1 for abbreviations.

\*Log<sub>10</sub>( $\delta^{13}\text{C} + 38$ )-transformed.

<sup>†</sup>Log<sub>10</sub>( $\delta^{15}\text{N} + 8$ )-transformed.

*et al.*, 1999; March & Pringle, 2003; Brito *et al.*, 2006) and even in some temperate lotic ecosystems (Bunn *et al.*, 2003; Delong & Thorp, 2006; Torres-Ruiz, Wehr & Perrone, 2007), despite predictions of the RCC that small forested streams should depend mainly on allochthonous energy.

Balitorid fish such as *L. disparis* and *P. myersi* showed notably high  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values and were even more  $^{15}\text{N}$ -enriched than predatory fish such as *S. fasciolata* or predatory invertebrates. Raised C and N signatures of herbivorous balitorids is probably a result of selective consumption and assimilation of  $^{13}\text{C}$ - and  $^{15}\text{N}$ -enriched cyanobacteria, since gut content analyses revealed that these fishes fed primarily on filamentous N-fixing cyanobacteria such as *Calothrix* and *Phormidium* (Dudgeon, 1987) with limited occasional ingestion of chironomid larvae (Mantel *et al.*, 2004). Assimilation of cyanobacteria resulting in high  $^{15}\text{N}$ -enrichment has been noted in other lotic consumers (Lau *et al.*, 2008b). The N signatures of herbivorous balitorids were similar to that of the algivorous goby *Sicydium plumieri* (Bloch, 1786), which also exhibits predator-like  $\delta^{15}\text{N}$  values

(6.5–8.7‰) in Puerto Rican streams (March & Pringle, 2003). The similarity of signatures reported for omnivorous fishes in streams in Venezuela (mean  $\delta^{15}\text{N}$  ranges: 7.7–9.6‰; Jepsen & Winemiller, 2002) and Brazil (7.5–8.1‰; Brito *et al.*, 2006) suggests that selective assimilation of cyanobacteria by tropical stream fishes may be more widespread than has been supposed previously (see also Mantel *et al.*, 2004).

Both atyid shrimps and the snail *B. hainanensis* exhibited obvious dependence on autochthonous production and, although they showed significant isotopic shifts in response to season and shading, their C signatures were nonetheless generally similar to those of periphyton and/or cyanobacteria. Gut-content analysis revealed that atyids in forest streams consume substantial quantities of algae (Mantel *et al.*, 2004), and it has been estimated that periphyton contributes to 35–60% (depending on site) of shrimp biomass in shaded streams and >60% in unshaded streams (Yam & Dudgeon, 2005). Although *B. hainanensis* ingests a considerable amount of FPOM (Mantel *et al.*, 2004), the stable isotope results presented here indicate that FPOM may not be assimilated efficiently

**Table 5** Trophic position (TP) estimates for consumer taxa in shaded and unshaded Hong Kong streams during the dry and wet seasons

	Shaded		Unshaded	
	Dry	Wet	Dry	Wet
BH	2.19	1.97	3.80	3.09
Aty	2.96	2.19	3.31	(2.00)
MH	3.42	2.96	4.18	–
Bae	2.72	2.71	3.50	3.38
Cae	3.72	3.97	3.74	3.23
Eph	2.23	–	3.47	–
Hep	2.70	(2.00)	3.68	3.73
Lep	3.16	2.67	3.23	3.29
ED	3.50	3.28	–	–
Per	3.39	–	–	–
Cal	2.43	–	2.68	–
Glo	2.25	–	–	–
Hyd	2.86	–	3.11	–
Phi	2.80	–	3.50	–
Pol	3.55	–	3.59	–
Elm	2.18	–	(2.00)	–
Eul	1.98	–	–	–
Pse	2.16	2.05	2.63	2.79
Sci	2.58	–	–	–
Chi	2.90	–	2.57	–
Ort	2.48	–	–	–
Sim	2.52	–	–	–
Tan	3.27	–	–	–
Tip	(2.00)	–	–	–
PT	3.00	–	–	–
LD	3.60	3.49	5.04	3.75
PM	3.47	3.75	–	4.05
SF	3.93	3.30	4.69	–
Cyp	3.62	3.60	5.13	4.16
CD	3.70	3.97	4.76	4.39

Taxa in parentheses were used as the baselines to estimate TPs of other taxa. See Table 1 for abbreviations.

(see also Lau *et al.*, 2008b). Another possibility, as shown by the similar C signatures of FPOM and periphyton during wet season, is that much of the FPOM originates from periphyton (see also Delong & Thorp, 2006) and, if so, it would be difficult to distinguish from the C derived from autochthonous sources that *B. hainanensis* had assimilated during its lifetime. Studies in the Neotropics using SIA have also shown that atyid shrimps (*Atya* spp. and *Potimirim glabra* [Kingsley, 1878]) and prosobranch snails (*Neritina* spp. and *Thiara granifera* [Lamarck, 1822]) depended more on algal food than FPOM or leaf litter in both shaded and unshaded streams (March & Pringle, 2003; Brito *et al.*, 2006).

Like baetid and heptageniid mayflies, other mayfly families (Caenidae and Ephemerellidae) had

C signatures indicating dependence on periphyton, cyanobacteria and filamentous algae. Studies in New Zealand suggest that mayflies depend mainly on allochthonous foods in shaded sites and autochthonous foods at unshaded sites (Lester *et al.*, 1995), whereas research in Hong Kong has indicated that the contribution of autochthonous C sources to mayfly biomass in forest streams can be substantial (Salas & Dudgeon, 2001; Li & Dudgeon, 2008). Our findings also suggested that Hong Kong mayflies (e.g. Baetidae and Leptophlebiidae) turn to allochthonous resources in the wet season when algae are depleted by spates (see also Salas & Dudgeon, 2001).

Shredders are scarce and constitute only <10% the total abundance of benthos in Hong Kong and they do not appear to play an important role in leaf litter breakdown (Dudgeon, 1989, 1999; Li & Dudgeon, 2008). Our SIA results also indicated that only two calamoceratid species and one Eulichadidae were obligate shredders that made use of allochthonous litter (see also Li and Dudgeon, 2008). Because of their relative scarcity these shredders were not important prey taxa. Accordingly,  $\delta^{13}\text{C}$  differences between them and predators such as *M. hainanense* and *C. duospilus* were usually >2‰ rather than the more typical value of 1‰ associated with direct trophic transfer (Peterson & Fry, 1987).

The high seasonal and inter-stream variation in  $\delta^{15}\text{N}$  of foods had consequences for primary consumer  $\delta^{15}\text{N}$  and hence affected the baselines for consumer TP estimations. Maximum TPs did not change substantially with season in shaded streams but tended to increase (c. 20%) during the wet season in unshaded streams. Furthermore they were higher in unshaded streams than shaded streams (wet season values were 3.97 versus 5.13) corresponding to the N isotopic shifts seen in autochthonous foods and many consumer taxa in response to reduced riparian coverage. The effect was such that primary consumers such as mayflies, *B. hainanensis* and atyid shrimps in unshaded streams were sufficiently  $^{15}\text{N}$ -enriched to attain a TP exceeding 3, similar to those of typical predators (see also Jaarsma *et al.*, 1998). For instance, TPs of *B. hainanensis* in shaded streams were 1.97 (wet) to 2.19 (dry), but equivalent values in unshaded sites were 3.09 (wet) to 3.80 (dry). Caenidae TPs were 2.71 (wet) to 2.72 (dry) in shaded streams, but 3.38 (wet) to 3.50 (dry) in unshaded streams. The effects of riparian-shading and, less conspicuously, season on

$\delta^{15}\text{N}$  variations may lead to misinterpretations of consumer TPs or underestimations of the extent of variation in food chain lengths if samples were only collected from streams of one shading type and/or in one season. Our findings also seem to indicate that TP estimates of consumers from autotrophic streams are generally higher than those from heterotrophic streams (see also Jaarsma *et al.*, 1998; Townsend *et al.*, 1998), and the baseline-calculation approach may overestimate the TPs of specialist herbivores (e.g. fish grazers) when in-stream algal sources are substantially more  $^{15}\text{N}$ -enriched than allochthonous foods.

Estimates of food chain length derived by using gut-content data yielded a mean chain length of 2.04 and a maximum of 3.00 for a Hong Kong forest stream (Mantel *et al.*, 2004), which are lower than the maximum estimated TPs calculated using the current approach. Our estimates of TPs for shaded streams (maximum TPs 3.93–3.97) are comparable to those in tributaries of the St Lawrence River (Canada) where the mean TP ( $\pm$  SD) of the highest trophic level (*Cottus bairdi* [Girard, 1850], a non-piscivorous predatory fish) was  $4.08 \pm 0.47$  (Anderson & Cabana, 2007); similar values (mean TPs *c.* 3.6) were reported for top predators (piscivorous fishes) in tropical Venezuelan rivers (Jepsen & Winemiller, 2002; Layman *et al.*, 2005). The methods used to derive TPs in these studies were somewhat different from ours which may explain why TPs from unshaded streams (up to 5.13) were higher in the present study. This TP value for predatory cyprinid minnows is comparable to other cyprinids such as *Rhinichthys cataractae* (Valenciennes, 1842) (maximum TP = 4.54) in St Lawrence River tributaries (Anderson & Cabana, 2007), lake-dwelling salmonids such as *Coregonus clupeaformis* (Mitchill, 1818) (mean TP =  $4.52 \pm 0.29$  SD; Vander Zanden *et al.*, 1999), and the characid *Oligosarcus robustus* (Menezes, 1969) (mean TP *c.* 4.6) in a subtropical Brazilian lagoon (Garcia *et al.*, 2007). Nevertheless, the current study may still have underestimated the actual consumer TPs as the benthic meiofauna (organisms with a maximum size  $<500\ \mu\text{m}$ ), which usually account for most of the lotic animal diversity, were not included sufficiently in the past food web investigations of Hong Kong streams (see also Schmid-Araya *et al.*, 2002).

This SIA study has shown that, contrary to the RCC prediction, stream food webs in tropical Hong Kong were predominantly dependent on autochthonous

sources, especially cyanobacteria and periphyton, notwithstanding some marked isotopic shifts of basal foods and consumers in response to season and shading conditions. These seasonal and riparian-shading effects on  $\delta^{15}\text{N}$  variations of consumers also influenced the estimated TPs. Differential assimilation of basal food sources probably leads to variable fractionation of  $\delta^{15}\text{N}$  within and among consumer taxa (Vander Zanden & Rasmussen, 2001), and it is likely that such variability is responsible for the observed differences in TP estimates under different shading conditions and between seasons in unshaded streams.

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## Supporting Information

Additional supporting Information may be found in the online version of this article.

**Appendix S1.** Tukey multiple comparisons for differences in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  among food sources in shaded and unshaded Hong Kong streams during the dry and wet seasons.

**Appendix S2.** Tukey multiple comparisons for differences in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  among consumer taxa in shaded and unshaded Hong Kong streams during the dry and wet seasons.

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