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Dietary variation and food selection by mayfly grazers in a subtropical mountain stream

Yi-Li Chuang¹, Shu-Fen Yu^{1,2} and Hsing-Juh Lin^{1*}

Abstract

Background: The classification of functional feeding groups of aquatic insects is often misleading in tropical/subtropical streams because their feeding habits are assumed to be the same as their temperate counterparts according to the mouthpart structure and foraging behavior. This study aimed to examine the diets and preferences of mayfly grazers (*Baetis* spp. and *Rhithrogena ampla*) in a subtropical mountain stream in the dry and wet seasons.

Results: In the stream, epilithic algal communities on insect-excluded bricks (as a grazer-excluded control) were dominated by small adnate diatoms, most likely due to the high current velocity. Both grazers preferred understory and small adnate diatoms, *Achnanthes* spp. and *Achnantheidium pyrenaicum*, in both seasons. However, the stalked diatoms *Gomphonema* spp. were preferred only by *Baetis*, but not by *Rhithrogena*, in the dry season when the current velocity became relatively slower. The results of pairwise tests further showed that the algal compositions on the insect-excluded bricks coincided with the diets of both grazers in the wet season but were distinct from those on the bricks in the dry season. Seasonal variations in the algal compositions of the diets of *Rhithrogena* and on the insect-excluded bricks were apparent, but not in the diets of *Baetis*.

Conclusions: The algal physiognomy was most likely attributed to the impact of the high current velocity in the stream. Our results suggest that the diet preference by mayfly grazers in the stream is potentially influenced by algal availability.

Keywords: Chesson's alpha; Diatoms; Food availability; Gut content; Mouthpart; Physiognomy

Background

Herbivory is an important factor regulating the biomass and community structure of benthic algae in streams (Feminella and Hawkins 1995; Steinman 1996). Although algal biomass can be effectively reduced by herbivores in a neotropical stream (Barbee 2005), research on the functional feeding mode of grazers in tropical and subtropical streams is still limited. Not only can they limit algal biomass, but they can also alter the physiognomy and community structure of algae, exerting strong indirect effects within food webs and on nutrient cycling (Holomuzki et al. 2010). Stream food webs rely primarily on two food sources: autochthonous primary production within the stream and allochthonous organic matter transferred to the stream as leaf litter, woody debris, and

dissolved organic carbon. Autochthonous algae may be more important than allochthonous organic matters to stream consumers in tropical forested headwater streams (March and Pringle 2003). An open forest canopy can lead to autochthonous algae becoming the dominant food source for herbivores in rainforest streams (Brito et al. 2006) and tropical Asia (Salas and Dudgeon 2001; Mantel et al. 2004; Yam and Dudgeon 2005; Lin et al. 2012). Autochthonous algae are the main energy source in some shaded tropical headwater streams (Mantel et al. 2004; Lau et al. 2008; 2009; Li and Dudgeon 2008). However, few studies have examined the diets of aquatic insects in tropical/subtropical streams (Boyero et al. 2009).

Aquatic insects have been shown to influence the community structure of benthic algae in temperate streams by food selection (Hill and Knight 1987; Feminella and Resh 1991; Feminella and Hawkins 1995; Peterson et al. 1998). A mat of benthic algae generally consists of a variety of

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algal physiognomic forms (Wellnitz and Ward 1998; Holomuzki and Biggs 2006). Algae in the upper layer of the mat or the overstory are expected to be easily accessible to most aquatic insects, whereas prostrate forms in the understory can only be ingested by aquatic insects possessing mouthparts specialized for detaching these algae from the substratum (Steinman 1996). The mouthpart structure and foraging behavior of aquatic insects vary among different taxa. They most likely select food on the basis of algal size or growth form (Tall et al. 2006).

In tropical and subtropical streams, benthic fauna such as aquatic insects have been identified as an obvious knowledge gap requiring further studies (Boyer et al. 2009). This knowledge gap constrains the ability to understand grazer community in general. Consequently, the feeding habits of aquatic insects in such streams are often assigned to functional feeding groups according to the mouthpart structure and foraging behavior for the North American stream fauna (Merritt et al. 2008). However, such classification of functional feeding groups is most likely misleading for various insect taxa (Boyer et al. 2009). Several studies have investigated the effects of mayfly grazers on algae in temperate streams or in laboratory channels (Kohler 1984; Wallace and Gurtz 1986; Lamberti et al. 1987). However, relatively little attention has been paid to the feeding behavior and diet of mayfly grazers in the tropical and subtropical streams.

The Chichiawan Stream (24° 23' N, 121° 18' E) is a headwater stream of central Taiwan at an elevation of 1,770 to 2,100 m. The tropical/subtropical monsoon climate in Taiwan is characterized by abundant rainfall in the summer and a dry period in the winter (Yu and Lin 2009). The stream is characterized by short, straight, and steep channels and is often influenced by fluctuations in precipitation and typhoons. The Chichiawan Stream is the last refuge for the critically endangered Formosan salmon (*Oncorhynchus formosanus*). Due to the Formosan salmon's critically endangered status (Chung et al. 2008), there is an urgent need to identify the energy sources supporting the stream food web. Lin et al. (2012) identified mayfly grazers as an important food source for the Formosan salmon in the stream. Nevertheless, the diet of mayfly grazers in the Chichiawan Stream is still unclear. Our objectives are (1) to identify algal compositions in the gut contents of two dominant mayfly grazers (families Baetidae and Heptageniidae) in this subtropical mountain stream, (2) to determine whether there is a seasonal variation in the diet of these grazers, and (3) to assess the degree of food selectivity by these grazers by comparing the algal composition of their gut contents with the algal composition of bricks in the stream where grazing by aquatic insects was excluded.

Methods

Study site

The Chichiawan Stream (24° 23' N, 121° 18' E) is a third-order stream of central Taiwan. The stream is 15.3 km long with a high mean gradient of 130 m km⁻¹ and a catchment area of 76 km². The upper reach of the Chichiawan Stream is bordered by natural forest. Climatic data derived from a local weather station (Taichung) from 1981 to 2010 (Climatological Data Annual Report, Central Weather Bureau of Taiwan) showed that in the dry season of October to February, the mean monthly rainfall normally does not exceed 40 mm and in the wet season of May to September, the average monthly rainfall frequently exceeds 300 mm. The mean discharge and current velocity are higher in the wet season than in the dry season (Table 1). The stream bed consists of a high proportion of pebbles in the dry season but is dominated by cobbles and boulders in the wet season (Lin et al. 2006). The mean water temperature ranges from 19°C in the wet season to 9°C in the dry season. The conductivity values are slightly higher in the dry season than in the wet season. The concentration of dissolved inorganic nitrogen (DIN = NO₃⁻ + NO₂⁻ + NH₄⁺) and total phosphorous (TP) was similar in both seasons.

Sample collection

Aquatic insects are the dominant herbivores in the Chichiawan Stream. Only a small number of other herbivores, including tadpoles (*Bufo bankorensis* and *Rana sauteri*), snails (*Hippeutis* sp. and *Lymnaea* sp.), and fish (*Varicorhinus barbatulus* and *Crossostoma lacustre*), are normally observed in the stream (Lin et al. 2012). Aquatic insects living in the stream are dominated by algivorous grazers and filter feeders (Kuo and Chiu 2005). Two dominant taxa of grazers (Lin et al. 2012), the Baetidae mayfly (*Baetis* spp.) and the Heptageniidae mayfly (*Rhithrogena ampla*), were collected for examination of their gut contents in this study.

Mayfly samples were collected from randomly selected cobbles in riffles of the midstream of the Chichiawan Stream using a hand net, as riffles constituted >80% of the stream area (Yeh 2006). To reveal seasonal variations, cobbles with a diameter of approximately 20 cm were collected during the dry season of January and February and during the wet season of June and early July 2005. Only two cobbles were processed each day of each season because mayflies were collected during their feeding time (during the daytime between 1600 and 1800 hours) to minimize the percentage of empty guts (Yu 2009). The procedure was repeated for 6 days in the dry season and 5 days in the wet season. The sampling was completed before the annual typhoon season (July to October) because the high discharge levels have been shown to remarkably reduce aquatic insect populations (Chiu et al. 2008) and

Table 1 Physico-chemical variables in the Chichiawan Stream in the dry and wet seasons

Variables	Dry season	Wet season	Reference
Chl <i>a</i> concentration on insect-excluded bricks (mg m ⁻²)	0.4 to 14.4	4.3 to 49.9	Present study
Chl <i>a</i> concentration on grazed cobbles (mg m ⁻²)	1.9 to 53.5	6.1 to 182.7	Present study
Conductivity (μS cm ⁻¹)	287.0 ± 11.0	222.5 ± 1.5	Present study
Current velocity (m s ⁻¹)	0.87 ± 0.10	1.10 ± 0.05	Present study
Discharge (m ³ s ⁻¹)	1.84 to 2.30	2.58 to 2.96	Chung et al. (2008)
Dissolved inorganic nitrogen (DIN, mg L ⁻¹)	1.81 ± 1.61	1.57 ± 0.85	Lin et al. (2006)
Substrate type	Pebbles (42%)	Cobbles (26%), boulders (21%)	Lin et al. (2006)
Total phosphorus (TP, mg L ⁻¹)	0.03 ± 0.02	0.02 ± 0.02	Lin et al. (2006)
Water temperature (°C)	9.1 ± 0.4	18.8 ± 0.8	Present study

Physico-chemical variables (mean ± standard deviation, range, or proportion) in the Chichiawan Stream in the dry and wet seasons before the occurrence of typhoons.

algal biomass (Tsai et al. 2014) in the stream. In total, 12 and 10 cobbles were collected during the dry and wet seasons, respectively.

The mayflies living on each cobble were carefully removed with forceps and placed in a squirt bottle filled with 75% ethanol. Prior to gut removal, the total body length and head capsule width of the mayflies were recorded. The entire gut was removed under a stereomicroscope. The entire gut content was fixed in Lugol's solution for algal taxa identification. After removal of aquatic insects, the epilithic algal samples on the cobbles were also collected for further analyses.

During the two study periods, aquatic insects were excluded from epilithic algae by field caging in the Chichiawan Stream (Figure 1). The size of the cages was 36 cm in length, 23 cm in width, and 18 cm in height and covered with fine nets (mesh = 0.025 mm) to exclude insects and falling leaf litter. We put one brick in each cage ($n = 10$) and incubated them in riffles for 40 days prior to the sampling in the stream. The epilithic algal samples were collected after the incubation of the insect-excluded bricks in the field. However, one brick was lost upon retrieval in the wet season. Caging to exclude insects was assessed as the control of potentially available algal community to grazers by comparing the algal abundance and composition found on the bricks with those on the cobbles collected after removal of aquatic insects in riffles.

For collecting epilithic algal samples, a frame made of steel was used to define an algal patch sampling area of 12.5 cm². Four algal patches were scraped off a surface area on each brick or cobble of 50 cm² with a toothbrush. The scraped algae were washed off from the toothbrush and the brick or cobble with filtered stream water, and the algae/water mixture was poured into a 100-mL graduated sample bottle. The sample bottles were transferred to a refrigerator on ice and stored in the dark until the samples were processed. In the laboratory, the algal samples were centrifuged for 10 min to concentrate them to 5 mL. A 3-

mL subsample was filtered using a GF/F glass fiber filter (Whatman, GE Healthcare UK Limited, Buckinghamshire, UK), and chlorophyll *a* (Chl *a*) was extracted from the filter using a solution of 90% acetone (Lobban et al. 1988). The other 2-mL subsample was fixed in Lugol's solution for taxa identification.

Epilithic algal compositions were identified and counted using a light microscope of differential interference contrast (Zeiss Axioplan 2, Göttingen, Germany). Filamentous

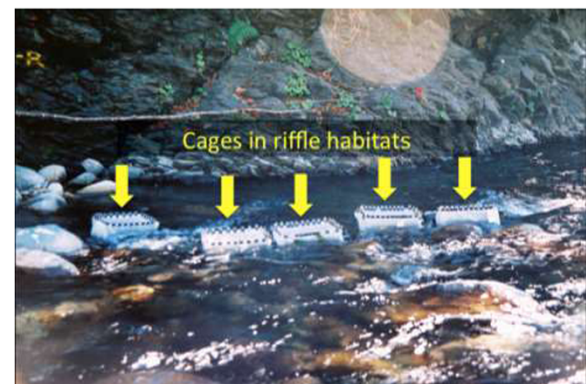
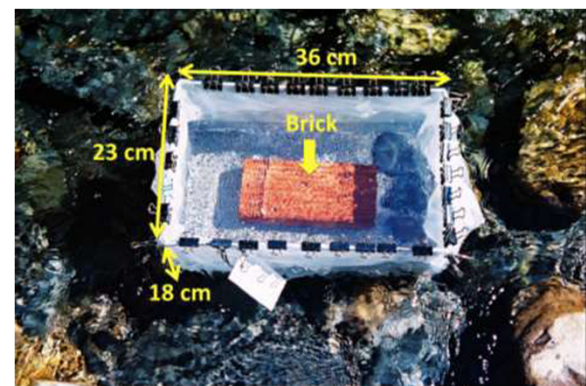


Figure 1 Steel cages for insect-excluded bricks were set in the riffles of the Chichiawan Stream.

algae such as *Oscillatoria* and *Cladophora* were counted in every cell using a hemocytometer. Diatom samples were further treated with H₂SO₄ and KNO₃ (Sabater et al. 1990) and mounted with Naphrax. At least 500 diatom valves were counted per sample. Identification was carried out according to Patrick and Reimer (1966), Patrick and Reimer (1975), Round et al. (1990), Vyverman (1991), Yamagishi (1992), Round and Bukhtiyarova (1996), and Krammer and Lange-Bertalot (1997). The relative abundance (%) of algal cells was calculated for analysis of the algal composition.

Data analysis

A two-tailed Student's *t* test was used to compare epilithic Chl *a* concentrations between the insect-excluded bricks and grazed cobbles and to compare head capsule width of mayflies between the wet and dry season. Epilithic algal species were categorized into five physiognomic groups, including adnate, stalked, erect, motile, and filamentous, using a modified version of the methods of Wellnitz and Ward (1998) and Holomuzki and Biggs (2006). To assess seasonal changes in the diets of the mayflies, variations in the algal compositions of their gut contents were examined using multivariate analyses in PRIMER v6 (Clarke and Gorley 2006, PRIMER-E Ltd, Plymouth, UK). Empty guts were excluded from this analysis. Dissimilarity coefficients of untransformed cell numbers of algal communities on the insect-excluded bricks and in the gut contents were computed using Gower's distance, which takes into account the joint absence of food items in the samples. The dissimilarity matrix was first classified by hierarchical agglomerative clustering using the unweighted pair group mean arithmetic (UPGMA) linking method and was then ordinated using the non-metric multidimensional scaling (MDS) techniques. Using season and mayfly taxon as two grouping variables, we assessed the dispersion of algal composition within each group in ordination space using permutational multivariate analysis of variance (PERMANOVA). A one-way PERMANOVA analysis of Gower's distance was used to determine the seasonal effect on algal compositions of the insect-excluded bricks, and a two-way PERMANOVA was used to determine the interactive effect of season and mayfly taxon on algal compositions in the gut contents and pooled samples of insect-excluded bricks and gut contents. Effects were considered to be significant at the 0.05 probability level by comparing the observed statistic to its 9999 permutations distribution in the absence of differences. In cases of a restricted number of possible permutations in pairwise tests, *p* values were obtained from Monte Carlo samplings (Anderson and Robinson 2003). Similarity of percentages (SIMPER) was employed to reveal which algal species contributed most to the dissimilarities between the groups for each

season or the most common algal species in replicate samples for each group. All the multivariate analyses were performed using the PRIMER v6 with PERMANOVA + add-on software package (Anderson et al. 2008).

We further used Chesson's alpha (α) (Chesson 1978; Alverson and Courtney 2002) to determine algal species preference by comparing the algal species in the gut contents of both mayflies to the relative availability of epilithic algae on the insect-excluded bricks.

$$\alpha = (r_i/p_i) / \Sigma(r_i/p_i)$$

where r_i is the relative abundance of algal species *i* in the gut content and p_i is the relative abundance of algal species *i* in the source sample. The index returns a proportion coefficient from 0 to 1, with values exceeding $1/n$ indicating a preference for algal species *i* and values less than $1/n$ indicating an avoidance of algal species *i*, where *n* is the total number of algal species included in the analysis. In this study, only the most 20 common algal species (each contributes >2% to the similarity by using the SIMPER analysis) in replicate samples on the insect-excluded bricks were included for the analysis in each season (i.e., *n* = 20, $1/n$ = 0.05).

Results

Insect-excluded bricks

Epilithic Chl *a* concentrations on the insect-excluded bricks ranged from 0.4 to 14.4 mg m⁻² in the dry season and 4.3 to 49.9 mg m⁻² in the wet season in the Chichiawan Stream (Table 1). Diatoms were the most abundant taxa of the algal communities on the insect-excluded bricks. Of the 58 taxa identified, 52 taxa were diatoms, followed by cyanobacteria and green algae (Additional file 1). Diatoms contributed 85% of the total cell numbers in the algal communities. *Achnanthes* spp., *Achnanthidium pyrenaicum* (Hustedt) H. Kobayasi, *Achnanthidium minutissimum* (Kützinger) Czarnecki, *Cocconeis placentula* var. *euglypta* (Ehrenberg) Grunow, *Gomphonema* spp., and *Planothidium lanceolatum* (Brébisson ex Kützinger) Lange-Bertalot were the most abundant species. Algal compositions on the bricks in the dry season differed significantly from the wet season (Figure 2, Table 2). *Achnanthes* spp. were the most abundant diatom species in the dry and wet seasons (Figure 3a).

Seasonal variations in the algal composition were further determined using the SIMPER analysis (Figure 3b). Approximately, 11% of the variations between the dry and wet seasons were caused by *Cocconeis placentula* var. *euglypta*, which was more abundant during the wet season. *A. pyrenaicum* (10.6%) and *Gomphonema* spp. (9.6%) were also significant contributors to the variation and were also more abundant during the wet season.

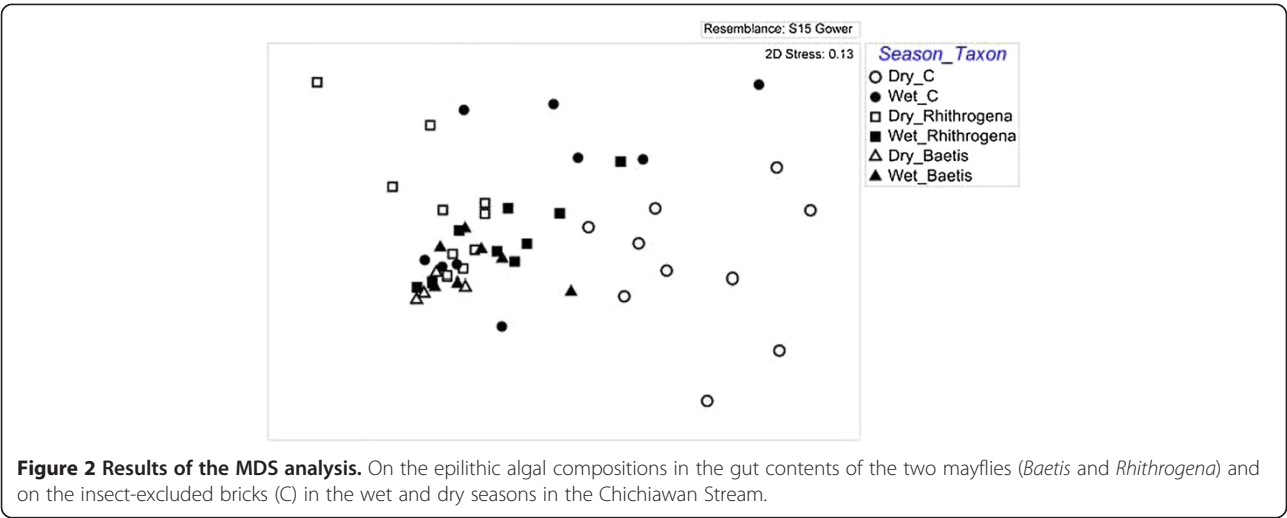


Figure 2 Results of the MDS analysis. On the epilithic algal compositions in the gut contents of the two mayflies (*Baetis* and *Rhithrogena*) and on the insect-excluded bricks (C) in the wet and dry seasons in the Chichiawan Stream.

Comparison of caged bricks with grazed cobbles

Epilithic Chl *a* concentrations on the insect-excluded or caged bricks incubated for 40 days were lower but comparable to those on the grazed cobbles after removal of aquatic insects in riffles of the Chichiawan Stream (Table 1, Student's *t* test, *p* = 0.013 for the dry season and *p* = 0.075 for the wet season). Communities of epilithic algae on the caged bricks and grazed cobbles were dominated by stalked or adnate form of diatoms both in the dry and wet seasons. Using the SIMPER analysis, the

Table 2 Results of the multivariate permutational analysis

Samples	df	SS	MS	Pseudo-F	<i>p</i> (perm)
Insect-excluded bricks					
Season	1	2,046	2,046.1	6.775	0.0005
Residuals	17	5,134	302.0		
Total	18	7,180			
Gut contents of mayflies					
Season	1	1,198	1,198.4	3.995	0.0078
Treatment	1	873	873.3	2.911	0.0309
Season × Treatment	1	318	318.3	1.061	0.3698
Residuals	25	7,500	300		
Total	28	10,443			
Insect-excluded bricks + gut contents of mayflies					
Season	1	389	388.7	2.810	0.0141
Treatment	2	2,753	1,376.4	9.948	0.0001
Season × treatment	2	1,447	723.3	5.228	0.0001
Residuals	43	5,949	138.4		
Total	48	10,816			

Results of the multivariate permutational analysis (PERMANOVA) of effects of season and treatment (mayfly taxon or insect-exclusion) on the algal communities in the Chichiawan Stream. *p* (perm), possible permutations. Statistically significant *p* values (<0.05) are highlighted in bold font.

community structures of epilithic algae on the caged bricks and grazed cobbles were slightly different (Table 3). The algal composition responded to seasonal variation more than the variation caused by grazing. *Achnanthes* spp. and *Planothidium lanceolatum* were frequently observed on the caged bricks and grazed cobbles both in the dry and wet seasons. However, *A. pyrenaicum* and *Gomphonema* spp. occurred more frequently in the wet season. While *Cocconeis placentula* and *Diatoma vulgaris* occurred more frequently on the grazed cobbles in the dry season, *Achnanthidium minutissimum* was more frequently observed on the caged bricks in the wet season. It appears that algal composition on the grazed cobbles can be approximated by the composition found on the caged bricks; therefore, caging to exclude insects was used as a grazer-excluded control of algal composition.

Gut contents of mayflies

In total, 11 and 19 individuals of *Baetis* and *Rhithrogena* were examined for their gut contents and their head capsule widths (mean ± standard deviation) were 0.99 ± 0.19 and 2.74 ± 0.67 mm, respectively. No significant difference in head capsule width was detected between the wet and dry seasons for both mayflies (Student's *t* test, *p* = 0.84 for *Baetis* and *p* = 0.96 for *Rhithrogena*). However, the algal composition of the gut contents of both mayflies between the two seasons was significantly distinct (Table 2). Algal compositions between the gut contents of two mayflies also significantly differed. The composition of the gut contents of *Rhithrogena* was more variable than that of *Baetis* (Figure 2).

The seasonal differences in the preference of diatom species by *Baetis* and *Rhithrogena*, assessed using Chesson's *α*, were apparent between the dry and wet seasons. Both *Baetis* and *Rhithrogena* preferred adnate diatoms, *Achnanthes* spp. and *Achnanthidium pyrenaicum*, in the dry and wet

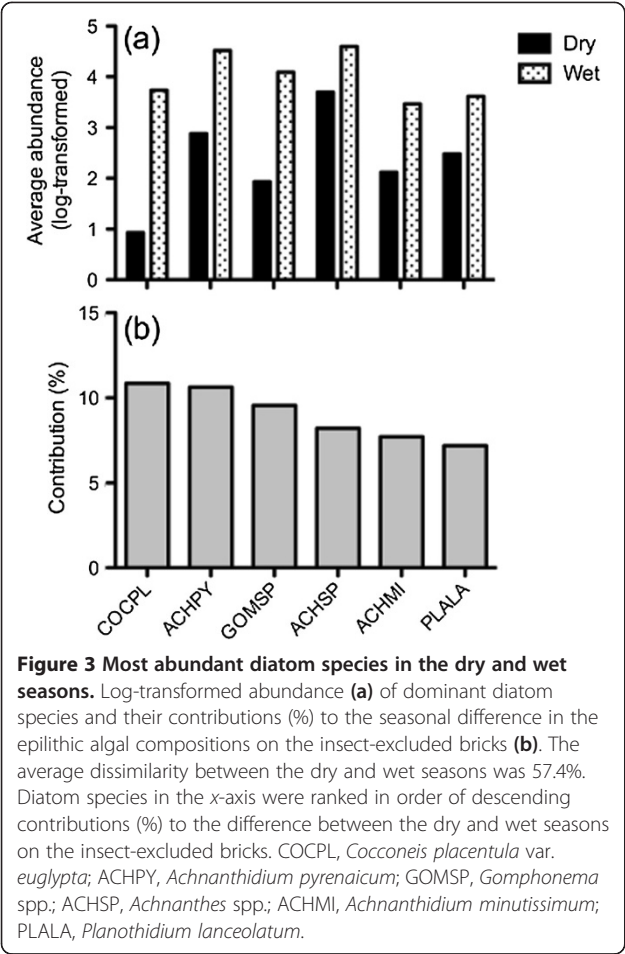


Table 3 SIMPER analysis of contribution of species

Algal species	Dry season		Wet season	
	Caged	Grazed	Caged	Grazed
<i>Achnanthes</i> spp.	35.50	51.87	2.67	2.26
<i>Achnanthyidium minutissimum</i> (Kützing) Czarnecki	13.13	5.49	2.74	
<i>Achnanthyidium pyrenaicum</i> (Hustedt) H. Kobayasi			55.04	71.74
<i>Cocconeis placentula</i> var. <i>euglypta</i>		7.77	2.26	7.19
<i>Diatoma vulgaris</i> Bory de Saint-Vincent		2.79		
<i>Gomphonema</i> spp.			18.53	13.19
<i>Planothidium lanceolatum</i> (Brébisson ex Kützing) Lange-Bertalot	41.48	24.08	5.31	2.58
Total contribution	90.11	92.00	86.55	96.96

SIMPER (similarity of percentage) analysis of contribution (%) of species occurrence in epilithic algal community respectively sampled on the caged bricks and the grazed cobbles in the dry and wet seasons in the Chichiawan Stream.

seasons (Figure 4). The stalked diatoms, *Gomphonema* spp., were preferred by *Baetis*, but not by *Rhithrogena*, in the dry season (Figure 4a). In the wet season, two mayflies preferred similar species of adnate diatoms with the exception of *A. minutissimum*, which was also preferred only by *Baetis* (Figure 4b).

Pooled analysis of insect-excluded bricks and gut contents of mayflies

When analyzing pooled algal compositions on the insect-excluded bricks and in the gut contents of both mayflies, the interactive effects of season and mayfly taxon on algal communities were significant (Table 2). The results of pairwise tests further showed that the algal compositions on the insect-excluded bricks (as a grazer-excluded control) coincided with the gut contents in the wet season (Table 4). In the dry season, however, the algal composition of the gut contents and on the insect-excluded bricks was highly variable and showed a widespread in the MDS ordination (Figure 2). The algal composition of the gut contents of *Baetis* and *Rhithrogena* was distinct from that of insect-excluded bricks in the dry season. Furthermore, the algal composition of the gut contents of *Rhithrogena*

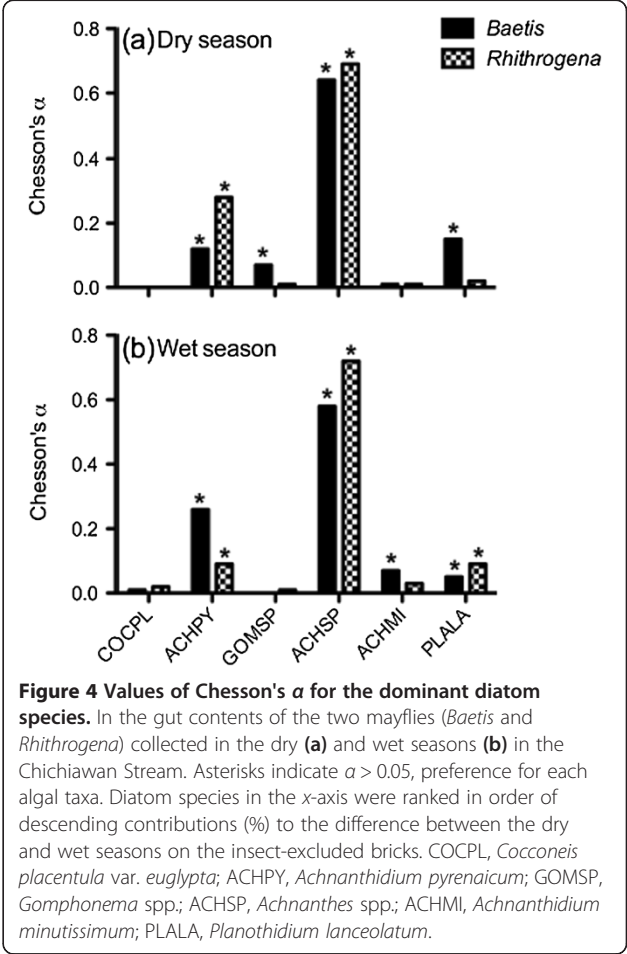


Table 4 Pair-wise tests of the effects of season and treatment

Groups	t	p (perm)	p (MC)
Groups within level 'wet season'			
<i>Rhithrogena</i> vs. <i>Baetis</i>	1.086	0.3071	0.2986
<i>Rhithrogena</i> vs. control	1.246	0.1740	0.1936
<i>Baetis</i> vs. control	1.473	0.0911	0.1083
Groups within level 'dry season'			
<i>Rhithrogena</i> vs. <i>Baetis</i>	1.960	0.0246	0.0307
<i>Rhithrogena</i> vs. control	4.263	0.0001	0.0001
<i>Baetis</i> vs. control	3.663	0.0008	0.0001
Groups within level 'treatment'			
Dry vs. wet (<i>Rhithrogena</i>)	2.070	0.0006	0.0066
Dry vs. wet (<i>Baetis</i>)	1.401	0.1323	0.1509
Dry vs. wet (control)	2.589	0.0005	0.0005

Pair-wise tests of the effects of season and treatment (mayfly taxon or insect-exclusion) on the algal communities on the insect-excluded bricks (control) and in the gut contents of the two mayflies (*Baetis* and *Rhithrogena*). p (perm), possible permutations. p (MC), Monte Carlo p values. Statistically significant p values (<0.05) are highlighted in bold font.

significantly differed from that of *Baetis* in the dry season (Table 4). The algal compositions of the gut contents of *Rhithrogena* and on the insect-excluded bricks in the dry season were distinct from those in the wet season. However, the algal composition of the gut contents of *Baetis* was similar between both seasons.

Discussion

Gut contents of mayflies

In temperate streams, while *Baetis* was categorized as collectors-gatherers and facultative scrapers, *Rhithrogena* was assigned as scrapers and facultative collectors-gatherers (Merritt et al. 2008). Such classifications were mainly based on examination of the mouthpart structure and foraging behavior for mayflies, rather than on the gut contents. Consequently, both mayflies in different taxa were considered to feed on the same food sources: detritus and diatoms (Merritt et al. 2008). Our results of gut content analysis demonstrate that both mayflies preferred understory and small adnate diatoms, *Achnanthes* spp. and *A. pyrenaicum* in the Chichiawan Stream. We also discovered that the preference of diatom species by *Baetis* and *Rhithrogena* was different; stalked diatoms *Gomphonema* spp. were preferred by *Baetis*, but not by *Rhithrogena*, in the dry season. In the wet season, however, when the current velocity became relatively faster, the distinctness in preference by *Baetis* and *Rhithrogena* decreased.

In the dry season, diatoms in the gut contents of *Baetis* and *Rhithrogena* show some distinctness, except for the most dominant *Achnanthidium/Achnanthes*-type diatoms. Interestingly, only 8 species of 52 taxa of diatoms in this

study were dominant in the gut contents, and they were mainly small diatoms. These small cells are monoraphid heterovalves, except the stalked *Gomphonema* (asymmetrical biraphid). *Baetis* preferred the stalked *Gomphonema*, but not *Rhithrogena*. Different types of grazers occupied different feeding niches in relation to the key growth forms in the benthic algal mat (Steinman 1996). Scraping and gathering grazers feed on stalked or short filament algal species (e.g., genus *Gomphonema*) and tend to feed on the mid-layers of algal communities. Rasping and scraping grazers feed on prostrate and adnate algal species such as genus *Cocconeis* and small *Achnanthidium/Achnanthes* species. In general, the biovolume of *Gomphonema* was greater than that of small *Achnanthidium/Achnanthes*-type diatoms. The preference for *Gomphonema* by *Baetis* in the dry season might not be caused by the size or characteristics of the mouthpart but instead be a result of the hydrological condition in the stream. *Baetis* has a fusiform body and prefers a near-bed current velocity of $<80 \text{ cm s}^{-1}$, whereas *Rhithrogena* has a flattened body and prefers a current velocity of $>80 \text{ cm s}^{-1}$ (S. F. Yu, personal observation). High velocity scoured off the stalked or large-sized algae, especially the overstory and mid-layer algal species. Therefore, *Baetis* had more opportunity to feed on larger diatom species than *Rhithrogena*. A similar study conducted in northeastern Taiwan also indicated that *Rhithrogena* preferred riffles with faster current velocity, though not so fast as that in the Chichiawan Stream (Yang 2012).

Seasonal variation in algal composition

The difference in diatom composition on the insect-excluded bricks in the Chichiawan Stream between the dry and wet seasons was contributed mostly from genera *Cocconeis*, which adnates to the understory of algal mat or to the surface of filamentous algae and was more abundant in the wet season. However, such diatoms were not the preferred species for the dominant mayfly grazers in the stream. Two possible explanations must be considered for why *Rhithrogena* and *Baetis* did not prefer *Cocconeis* spp. in the stream. First, genus *Cocconeis* was attached directly via the raphid valve by mucilage, but *Achnanthidium* and *Achnanthes* were attached to rock using a mucilage stalk secreted from the end of the raphid valve. Therefore, the short-stalked *Achnanthidium* and *Achnanthes* were relatively easier for mayfly to graze than the prostrate and tightly attached *Cocconeis*. The second reason might be that *Cocconeis* usually attach tightly to the surface of filamentous green algae, such as *Cladophora* and *Spirogyra*. However, filamentous algae are usually difficult for grazing insects to harvest or digest (Lamberti and Resh 1983). Overgrowth of the filaments of *Cladophora* interfered with the feeding of *Rhithrogena* that requires a relatively flat substrate free of obstructions to the movements of the

maxillary palps (McShaffrey and McCafferty 1988). Moreover, small and stalked *Achnanthyidium*/*Achnanthes*-type diatoms with monoraphid were always attaching to the rocks in the rapid riffles with less filamentous algae, and they became the most important food source for *Baetis* and *Rhithrogena* in the stream.

Regardless of dry or wet season or changing flow regime, the two mayfly grazers preferred small adnate diatoms species in the Chichiawan Stream. The Chesson's α values were much higher for adnate diatoms, *Achnanthes* spp. and *A. pyrenaicum*, than the values for the other species, indicating that they were the most preferred diatom species for mayfly grazers, *Baetis* and *Rhithrogena*, in both seasons. These small adnate diatom species were also the most abundant algal species in the Chichiawan Stream (Yu and Lin 2009) and were usually found in riffles with high current velocity (Su et al. 2009).

Food selectivity by mayflies

The algal grazer Baetidae was observed using its gouge-shaped mandibular tips (Arens 1990). Stalked, erect, and filamentous growth forms of algae are often vulnerable to most herbivores, whereas prostrate forms are vulnerable only to rasps and scrapers (Holomuzki et al. 2010). Wellnitz and Ward (1998) indicated that adnate forms of algae such as *Achnanthes* are most vulnerable to the gouging mouthparts of Baetid mayflies. Nearly all mayflies of the family Heptageniidae can use their two-segmented strongly modified labial palps, with scrubbing brushes similar to maxillary palps, as algal scrapers (Arens 1990). These might be the reasons that understory algae rather than overstory algae were preferred by the dominant mayfly grazers in the Chichiawan Stream.

Our findings are contrary to the general impression that overstory algae are more vulnerable than understory algae to grazing insects. Steinman (1996) indicated that 37 out of 43 previous studies (or 86%) show that grazers in streams have a preference for overstory diatoms. However, these studies usually focus on the functional feeding group of gathering-collector. Relatively little attention has been paid to the feeding behavior and the gut content of Heptageniid *Rhithrogena* species with a scraping brush on the maxillary palp (e.g., McShaffrey and McCafferty 1988). Wellnitz and Ward (1998) reported that a grazer, *Ecdyonurus venosus* (Heptageniidae), uses a brushing mouthpart and other grazers, *Baetis* spp. (Baetidae), use mandibles and maxilla to scrape and gather periphyton. Research on the gut contents of *Baetis* and *Rhithrogena* in tropical and subtropical streams is still rare (Yang 2012). Our results demonstrate that *Baetis* and *Rhithrogena* preferentially feed on understory algae, which were mainly adnate form or *Achnanthes*-type algae, in the Chichiawan Stream.

The preference of small adnate diatoms species by the dominant mayfly grazers in the Chichiawan Stream year-

round is most likely a result of the high current velocity on algal physiognomy in the stream. The current velocity of the stream is the main factor affecting epilithic algal biomass and compositions in this subtropical mountain stream (Tsai et al. 2014), which can regulate the levels of subsidy and stress experienced by periphyton by simultaneously regulating nutrient uptake rates and shear forces (Biggs et al. 1998). Continental islands in the tropics/subtropics of the western Pacific are characterized by mountainous watersheds, high precipitation, and high water runoff; therefore, many mountain streams on these islands, including the Chichiawan Stream, have relatively short, straight, and steep channels in comparatively small and narrow watersheds (Smith et al. 2003). The current velocity in the Chichiawan Stream remained fast ($>0.60 \text{ m s}^{-1}$) year-round. The high gradient (130 m km^{-1}) and flow environments in the stream caused the diatom communities to shift toward more adnate forms in the stream, such as the tightly adherent diatoms *Achnanthes*, *Achnanthyidium*, and *Cocconeis*. Our results suggest that the preference by mayfly grazers in the stream is potentially influenced by algal availability.

Although a previous study found that the density of *Achnanthyidium* species decreased with increasing grazer density (Yu and Lin 2009), mayflies did not actually prefer the similar adnate form of *A. minutissimum*, which were the smallest-sized diatoms in the *Achnanthyidium* species complex in the Chichiawan stream. *A. minutissimum* can colonize rapidly in this stream after flushing floods (Lin and Lin 2009). Grazers can maintain the early successional nature of algal communities under conditions of high current velocity (Poff and Ward 1995). Small-celled unicellular algae have a low biomass, short cell cycle, and rapid growth rate. Such conditions favor organisms that follow an opportunistic or r-selection strategy (Sigg 2005). Therefore, while *Achnanthes* spp. and other *Achnanthyidium* species were preferentially grazed by the dominant mayflies, the small-sized *A. minutissimum* can grow on the lowest layer of algal mats.

Conclusions

The two dominant mayfly grazers preferred understory and small adnate diatoms species in the Chichiawan Stream in the dry and wet seasons. The algal physiognomy was most likely attributed to the impact of the high current velocity in the stream. The preference by the mayfly grazers in the stream is potentially influenced by algal availability.

Additional file

Additional file 1: Algal taxa list.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

YLC and HJL wrote the manuscript. YLC, SFY, and HJL carried out the data analysis. SFY carried out the sample collection and field studies. All authors read and approved the final manuscript.

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