

Resource partitioning in a grazer guild feeding on a multilayer diatom mat

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Abstract. The gut contents of a guild of invertebrate grazers inhabiting the moss *Fontinalis* and feeding on epiphytic diatoms in a small Québec stream were analyzed to characterize resource partitioning and food selection. A multivariate approach (RLQ analysis coupled with a revised version of 4th-corner analysis) identified distinct diet patterns among co-occurring grazers. These patterns were mainly explained by differential ingestion of diatoms that differed in their spatial positions within the multilayered periphyton mat. When the size range of available diatoms was large, diet differences were partly explained by diatom size. Comparison of diatoms in grazer guts with diatoms available in the environment indicated selective feeding in different levels of the periphyton mat by grazers. Some grazers (scrapers) fed preferentially on tightly attached diatoms, whereas others (surfers) favored overstory diatoms. Spatial segregation of feeding within the periphyton mat by members of the grazer guild was more evident in a period of potential resource limitation (July) than when food was abundant (May). Our results suggest that all layers/growth forms in the diatom mat are used, resulting in spatial partitioning of the resource when considering the entire grazer community. Therefore, foraging theories already established for other ecosystems are confirmed in the unique context of stream benthos.

Key words: diatoms, grazing, multivariate analysis, resource partitioning, stream invertebrates.

Resource partitioning is a central concept in community ecology and is often invoked to explain the coexistence of species using the same limiting resource (Schoener 1974). Resource partitioning has been confirmed mostly for terrestrial vertebrates (MacArthur 1958, review by Schoener 1974). Despite the typically large number of grazer taxa that co-occur in the benthic communities of streams and lakes, attempts to demonstrate spatial or food segregation in these guilds have largely failed (e.g., Tokeshi 1986, Tavares-Cromar and Williams 1997). Most benthic grazers are considered generalist feeders (Cummins 1973, Mihuc 1997), so large overlaps in their diets are not surprising. However, some evidence suggests that grazers may select food on the basis of algal size, growth form, or chemical composition (Baker and McLachlan 1979, Peterson 1987, Steinman 1996). Thus,

it is possible that resource partitioning does occur but is detectable only at appropriate scales of grazer spatial distribution and diet description.

Periphyton, a complex matrix of algae, bacteria, and fungi, grows on a substratum. Therefore, it has a multilayered structure resembling, at a microscopic scale, the vertical stratification of terrestrial forests. Periphytic algae, the main resource of benthic grazers, occupy different spatial levels in the periphyton mat according to their degree of attachment to the substratum. Adnate forms are in contact with the substratum along their entire cell wall, in contrast with erect or pedunculated forms, which are attached to the substratum only through a basal cell or through mucilage. An overstory is formed by algae with no direct connection with the substratum. Algae in the upper layer of the mat should be easily accessible to most grazers, whereas prostrate forms in the understory can be ingested only by grazers possessing mouthparts specialized for detaching these algae from the substratum (Steinman 1996).

We tested the hypothesis that resource partitioning based on preferential feeding at different levels of a

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multilayer periphyton mat occurs in a grazer guild. The gut contents of invertebrates inhabiting the moss *Fontinalis* and feeding on its algal epiphytes in a small Québec stream were analyzed. Our study focused on ingestion of diatoms, the dominant algal group. Sampling was done on 2 dates when diatom assemblages differed in abundance and composition because resource partitioning may depend on the quantity and quality of the resource (Schoener 1974). Our specific objectives were to: 1) determine if diet variation was explained by differential ingestion of diatoms from various spatial positions (levels) in the periphyton mat, 2) link grazer and diatom characteristics, especially size and position in the periphyton mat, and 3) determine if diet patterns reflected selective feeding on diatoms in different spatial positions in the periphyton mat.

Methods

Study area and sampling

Diatoms and grazers were collected in May and July 1998 from the moss *Fontinalis dalecarlica*, which is abundant on the side of large stones in Ruisseau Pin Rouge. This 2nd-order stream, located 80 km north of Montréal in the Laurentian Mountains (lat 45°59'N, long 74°01'W), is oligotrophic, colored, and slightly acidic (Cattaneo et al. 2004), as is typical of Canadian Shield streams. Quantitative samples of moss with their epiphytic algae and invertebrates were used to study the diet of benthic grazers. Samples were collected in May and July 1998 to capture the effects of varying discharge on stream communities (Cattaneo et al. 2004). Moss was gathered using a modified Hess sampler (surface area = 69 cm²) from various points (8 in May and 9 in July) along a 100-m run. The mosses enclosed in the sampler were detached from the substratum by hand and placed in a 100- μ m-mesh net. Material dislodged during manipulation was siphoned from the sampler before lifting the sampler from the substratum. The moss and net were rinsed in a known volume of stream water and kept cold until processing (~2 h later). In the laboratory, algae and invertebrates were separated from the moss by vigorous shaking in a jar half filled with tap water. A portion (6 mL) of the suspension was fixed with Lugol's solution for algal analysis, and the rest was concentrated through a 63- μ m sieve and preserved with 95% ethanol for invertebrate analysis.

Invertebrate preparation and analysis of gut contents

Whenever possible, the gut contents of ≥ 10 individuals from each of the main grazer taxa present in

the community were analyzed (Table 1). Individual invertebrates were macerated in concave slides with 30% H₂O₂ for 3 to 24 h (depending on size and cuticle texture), mounted in Hoyer's mounting medium, and identified to genus (Wiederholm 1983, Merritt and Cummins 1996). Head-capsule width (for insects) or body width (for other grazers) was measured to the nearest 0.01 mm using an image analysis system (Image Pro Plus, version 4.1 for Windows, Media Cybernetics, Silver Spring, Maryland) connected to a dissecting microscope. Maceration digested organic material and allowed observation of the diatoms contained in the gut. All ingested diatoms (or a subset of 100 when there were >100) were counted and identified to genus, and the Greatest Axial Length Dimension (GALD) of each diatom was measured to the nearest 1 μ m under a microscope connected to the image analysis system. Only grazers having ≥ 10 diatoms in their gut were considered in the analyses.

Available diatoms

Algae dislodged from the moss by shaking and algae remaining attached to the moss were considered when investigating diatom availability in the stream. Quantitative samples of the algal suspension and the moss (8 replicates in May and 9 in July) were treated with 30% H₂O₂ (Battarbee 1986). Permanent mounts of the cleaned diatoms were made using Naphrax (Northern Biological Supplies, Ipswich, UK). At least 100 diatoms encountered along ≥ 1 grid lines (depending on diatom density on the slide) were counted and identified to genus or species, and the GALD of each was measured with the image analysis system (magnification 400 \times). Colonial algae were measured as a single large cell because grazer ability to ingest colonial algae is likely to depend on the size of the whole colony. Diatom composition varied little among replicates within dates; therefore, samples were pooled within dates to represent the available diatom community.

Diatom spatial position in the periphyton mat was described by assigning diatoms to 1 of 3 levels in the periphyton mat based on the ease with which they were detached from the moss by vigorous shaking. Level I consisted of diatoms tightly attached to the moss (<85% detached from the moss by shaking), level II consisted of diatoms loosely attached (85–95% detached) to the moss, and level III consisted of unattached diatoms (>95% detached). Moss samples with their intact periphyton were collected in Ruisseau Pin Rouge on similar dates (May and July) during the summer of 2002 and observed with a scanning electronic microscope (SEM) to confirm that these

TABLE 1. The grazer guild studied in Ruisseau Pin Rouge showing the taxon code, number of individuals analyzed for gut content (n), the relative abundance (% of total grazer biomass), and the size range (median) measured as head-capsule width (body width for copepods and oligochaetes) of each taxon.

Taxon	Code	May			July		
		n	Relative abundance	Size range	n	Relative abundance	Size range
Chironomidae							
Chironomini	CHIR	4	1	65–375 (87)	12	5	70–401 (175)
Tanytarsini	TANT	9	8	75–370 (219)	18	8	112–341 (195)
Orthocladinae	ORTH	23	26	125–750 (309)	14	24	75–286 (138)
Tanypodinae	TANP	13	10	200–890 (323)	8	12	132–390 (285)
Coleoptera							
Elmidae	ELM	7	20	155–335 (237)	9	16	120–360 (240)
Ephemeroptera							
Baetidae	BAE	3	5	85–150 (105)	10	3	140–600 (440)
Ephemerellidae	EPH	2	10	190–265 (228)	6	4	360–800 (560)
Trichoptera							
Hydroptilidae	HYD	3	10	205–280 (270)	6	27	200–320 (300)
Copepoda	COP	5	2	80–85 (82)	–	<1	–
Oligochaeta	OLI	8	7	70–340 (115)	–	1	–
Total		77	99		83	100	

levels of substratum attachment corresponded to diatom spatial positions in the periphyton mat. For SEM observation, moss and periphyton were freeze-dried and small sections were attached to aluminum stubs and coated with a thin layer of Au/Pd alloy to prevent the buildup of high-voltage charges on the specimens. Based on SEM observation, diatoms were assigned to level I when their entire surface was attached to the moss, level II when contact was limited to a stalk or a small portion of the frustule, and level III when no contact with the moss was observed. In general, the 2 classification systems (% of detachment during shaking and SEM observation) agreed for most diatoms except *Cyclotella*, *Cymbella*, and *Meridion*, which were assigned to level II or III depending on the classification system. In controversial cases and for rare diatoms not observed in the SEM samples, we used the classification based on % of detachment during shaking.

Statistical analyses

Data tables.—The invertebrate and diatom data were organized into 3 tables. The 1st table consisted of the diet composition of each invertebrate expressed as the % of each diatom taxon ingested. The 2nd table consisted of grazer taxonomic information (family and genus) and size. Statistical analyses were based on classification at the tribe level for chironomids and at the family level for all the other invertebrates because of the small number of individuals examined in certain genera. The 3rd table consisted of variables related to

diatom spatial position in the periphyton mat (level I, II, or III) and size (GALD).

RLQ analysis.—RLQ analysis (Dolédec et al. 1996) was used to identify feeding patterns in the grazer guild. In particular, the relationship between grazer taxon and diatom spatial position in the periphyton mat was investigated. Diatom size also was considered because of its influence on grazer selection (Tall et al. 2006). RLQ analysis is an extension of coinertia analysis (Dray et al. 2003) that does simultaneous ordination of 3 tables of interest. This method was developed to study the relationships between species traits and environmental variables through the link expressed by a species \times site abundance table. This approach was used in a new context in our study: table **R** ($k \times n$) contained information on n attributes related to k grazers, table **L** ($k \times m$) contained data on the diatom composition (m diatom taxa) in the diets of k grazers, and table **Q** ($p \times m$) described p attributes of the m diatom taxa (Dolédec et al. 1996). RLQ analysis maximizes the relationship between 2 sets of scores, which are linear combinations of diatom (p) and grazer (n) attributes. The link that maximizes the relationship is a compromise between the correlation between tables **R** and **Q** (weighted by diatom composition, table **L**) and the variation explained in each table. Based on RLQ scores, we used K -means (Fisher 1958, MacQueen 1967) analysis to identify groups of grazers with similar diets.

Fourth-corner analysis.—Fourth-corner analysis (Legendre et al. 1997) was used to test quantitatively the association between grazer and diatom attributes based on grazer diet. In addition to the 3 tables used in

the RLQ analysis, a 4th table **D** ($p \times n$) was computed as the matrix product $\mathbf{QL}^t\mathbf{R}$. Bivariate associations between diatom and grazer attributes are estimated by the values in table **D** and are tested using a permutation procedure. When 2 qualitative variables were involved (e.g., taxonomy of grazers and diatom levels), table **D** was a contingency table, which contained the number of occurrences of each association between grazer and diatom attributes. A global χ^2 or *G*-statistic measured the association between the 2 variables. The association between particular levels of 2 qualitative variables (e.g., taxon *j* and diatom level *i*) was measured by the number of occurrences having these particular attributes in table **D**. The global association between a quantitative variable and a multistate qualitative variable was measured by an *F*-statistic. The detailed association between the quantitative variable and a particular level of the qualitative variable was measured by computing the correlation between a binary variable coding for this level and the original quantitative variable.

The original 4th-corner method was defined to deal only with presence-absence data in table **L**, but a modified version of the original approach was used in our study. Abundance values contained in table **L** were used as weights in the computation of the association measurements (SD, unpublished data). The significance of these results was tested by 9999 permutations based on model 1 of Legendre et al. (1997) in which the identity of the diatom taxa was permuted at random within each grazer gut content. This model seemed the most appropriate for our study because the permutations produced realizations of the null hypothesis that grazers selected diatoms at random. The alternative hypothesis was that diatoms were not grazed randomly; i.e., they were selected according to their attributes. The significance level was fixed at $p = 0.05$ for global and particular associations. Holm's correction procedure was used to test particular associations to account for multiple tests.

Electivity index.—We compared grazer diet composition to composition of diatoms in the environment to determine if selective feeding at different levels in the mat occurred in our benthic community. Selection based on diatom levels was estimated for 3 groups of grazers (A, B, and C; determined by 1st-axis ordination scores, see below) using the *E** electivity index (Vanderploeg and Scavia 1979). This index, which ranges between +1 and -1, is calculated as:

$$E^*_i = [W_i - (1/n)]/[W_i + (1/n)]$$

where n = number of available levels, r_i = proportion of diatoms of level *i* in the diet, p_i = proportion of diatoms of level *i* in the environment, and $W_i = (r_i/p_i)/\sum_i(r_i/p_i)$.

Positive values indicate positive selection, whereas negative values indicate avoidance; 0 indicates random feeding.

Results

Grazer and diatom communities

Chironomids, which were highly diversified taxonomically, dominated the invertebrate community on *F. dalecarlica* in May and July (Appendix; Cattaneo et al. 2004). Other insect larvae (Elmidae, Baetidae, Ephemerellidae, and Hydroptilidae), Oligochaeta, and cyclopoid copepods also were consistently present in our samples (Table 1). Grazer biomass was $\sim 2\times$ greater in July (1.1 g/m²) than in May (0.5 g/m²).

The algal community on moss was dominated by diatoms, which made up 95% and 55% of the total algal biovolume in May and July, respectively (T. Zakhodnova, Botanical Institute, Russian Academy of Science, personal communication). Diatom communities differed in abundance and in spatial structure between dates. Diatom density was almost 6 \times higher in May (16.3×10^5 diatoms/mg moss) than in July (2.8×10^5 diatoms/mg moss). Only a small fraction of the diatoms (<20%) was tightly attached (level I) to the moss in May when the community was dominated by *Tabellaria flocculosa*, *Synedra ulna*, and *Fragilaria* spp. (levels II and III) (Table 2). The adnate *Eunotia* spp. and *Achnanthes* spp. were the most abundant diatoms in July and level I diatoms made up almost 50% of total diatom density (Table 2, Fig. 1A). The range of GALD values was wider in May (10–320 μm) than in July (10–120 μm) when the larger size classes (diatoms >120 μm) were completely absent (Fig. 1B).

Diet patterns

RLQ scores were represented along only the 1st axis because this axis accounted for most of the costructure in the analyses (70% in May and 67% in July). Based on the 1st-axis scores, 3 groups of grazers (A, B, and C) were obtained by *K*-means partitioning according to the similarity of their diet. The 4th-corner method was used to test if the diet of each grazer taxon (tribe or family) was statistically associated with diatom attributes (spatial position and size).

May.—Chironomini and Copepoda (group A) were isolated on the left end of the RLQ ordination axis (Fig. 2A). These invertebrates were small (head width <100 μm) and fed predominantly on diatoms in level I (Fig. 2B). Diatoms in level I consisted mostly of *Achnanthes*, the smallest taxon in our study (Fig. 2C). Baetidae, Oligochaeta, Elmidae, and Tanypodinae were clustered close to the origin of the RLQ ordination axis,

TABLE 2. Composition and taxon codes of the diatom community growing on the moss *Fontinalis dalecarlica* in Ruisseau Pin Rouge in May and July. Level indicates the degree of attachment of the diatom to the moss: I = tightly attached, II = loosely attached, III = detached. Size is the mean Greatest Axial Length Dimension. The abundance of each taxon is expressed as a % of the total diatom density on the moss.

May				July			
Genus/species	Code	Size (µm)	Relative abundance (%)	Genus/species	Code	Size (µm)	Relative abundance (%)
Level I				Level I			
<i>Achnanthes</i> spp.	ACHN	11	12	<i>Achnanthes</i> spp.	ACHN	13	17
<i>Eunotia</i> spp.	EUNO	30	4	<i>Cocconeis placentula</i>	COCC	18	4
<i>Gomphonema</i> spp.	GOMP	31	2	<i>Cymbella</i> spp.	CYMB	28	1
				<i>Eunotia</i> spp.	EUNO	34	21
				<i>Frustulia</i> spp.	FRUS	47	2
				<i>Gomphonema</i> spp.	GOMP	33	3
				<i>Meridion circulare</i>	MERI	29	<1
				<i>Pinnularia</i> spp.	PINN	63	<1
Level II				Level II			
<i>Cyclotella</i> spp.	CYCL	11	6	<i>Fragilaria capucina</i>	FCAP	24	4
<i>Navicula</i> spp.	NAVI	22	1	<i>Fragilaria construens</i>	FCON	12	10
<i>Tabellaria fenestrata</i>	TFEN	44	11	<i>Navicula</i> spp.	NAVI	34	5
<i>Tabellaria flocculosa</i>	TFLO	21	26				
Level III				Level III			
<i>Cymbella</i> spp.	CYMB	30	3	<i>Cyclotella</i> spp.	CYCL	10	6
<i>Diatoma</i> spp.	DIAT	14	<1	<i>Nitzschia</i> spp.	NITZ	36	4
<i>Fragilaria</i> spp.	FRAG	22	14	<i>Surirella</i> spp.	SURI	92	<1
<i>Meridion circulare</i>	MERI	37	1	<i>Synedra ulna</i>	SULN	90	2
<i>Nitzschia</i> spp.	NITZ	32	5	<i>Tabellaria fenestrata</i>	TFEN	54	14
<i>Pinnularia</i> spp.	PINN	31	1	<i>Tabellaria flocculosa</i>	TFLO	21	5
<i>Surirella</i> spp.	SURI	38	<1				
<i>Synedra ulna</i>	SULN	141	14				

forming group B (Fig. 2A). Their size was intermediate (head width 100–400 µm), and they fed on diatoms in levels II and III (Fig. 2B). Tanytarsini, Orthoclaadiinae, Ephemerellidae, and Hydroptilidae (Group C) included the largest grazers (head width up to 800 µm) (Fig. 2A), with a diet mainly composed of diatoms in levels II and III (Fig. 2B). In particular, the diet of the Orthoclaadiinae was characterized by the presence of the level-III diatom *S. ulna* (SULN in Fig. 2C), which was the longest diatom in the community (mean GALD = 141 µm in May; Table 2). Diatom size was correlated with RLQ scores ($r = 0.78$, not presented) and, thus, with diatom level.

Global relationships between grazer taxa and diatom level and size were highly significant (4th-corner analysis; Table 3). No significant relationships were observed between group A grazers and diatom level but the Chironomini were negatively associated with diatom size. No significant relationships were observed between group B grazers and with diatom level or size except for the negative association of the Elmidae with diatoms in level II. The Orthoclaadiinae showed a positive association with diatom size, a positive association with diatoms in level III, and a negative association with diatoms in level I; these

relationships were not significant for other grazers in group C.

July.—The grazer community was more clearly separated into 3 groups (A, B, and C) in July than it was in May (Fig. 2D). Hydroptilidae, Ephemerellidae, and Elmidae (Group A) were positioned to the left of the RLQ ordination axis (Fig. 2D) and were related to diatoms in level I (Fig. 2E). Tanytarsini and Baetidae (group B) were in the center of the ordination axis (Fig. 2D) and were related to diatoms in level II (Fig. 2E). Group C, to the right of the ordination, contained only chironomids (Tanytarsini, Orthoclaadiinae, and Chironomini; Fig. 2D) and was related to diatoms in level III (Fig. 2E). Diatom size and RLQ scores were not correlated in July ($r = 0.03$), indicating that diatom size and level were not related. For example, the medium-sized *Eunotia* and *Cocconeis* were in level I with the small *Achnanthes* (Fig. 2F).

Global relationships between grazer taxa and diatom level were significant, but relationships between grazer taxa and diatom size were not significant (4th-corner analysis; Table 3). All taxa in group A had a strong negative association with diatoms in level III and a positive association with diatoms in level I. In contrast, grazers in group C were positively associated

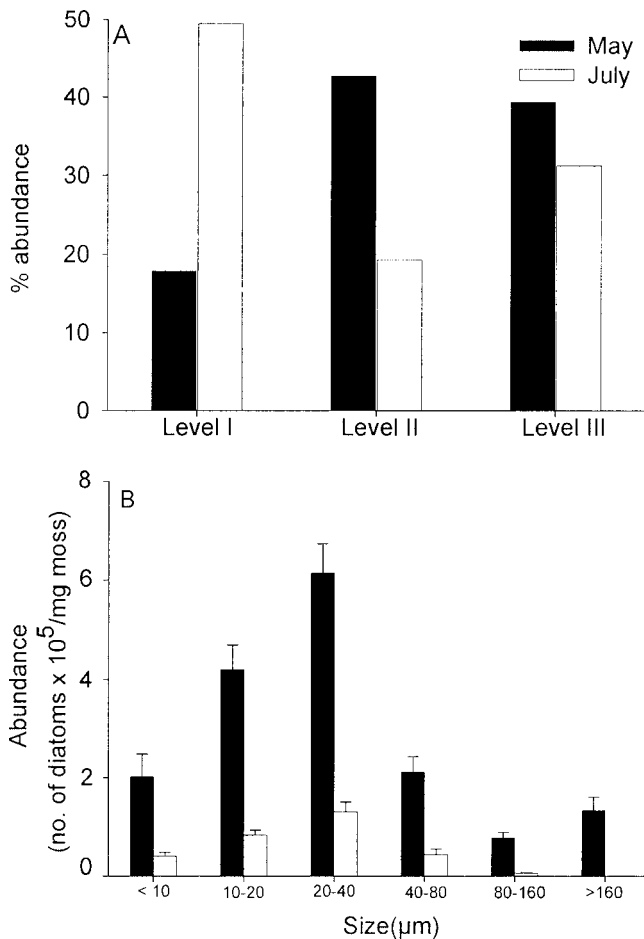


FIG. 1. Attributes of the epiphytic diatom community in Ruisseau Pin Rouge. A.—Percentage of total number of diatoms in each level (I = tightly attached, II = loosely attached, III = detached) of the periphyton mat. B.—Mean (+1 SE) abundance of diatoms in each size class (assessed as the Greatest Axial Length Dimension) in May ($n = 8$) and July ($n = 9$).

with diatoms in levels II and III (Orthocladinae with diatoms in level II, Chironomini and Tanytarsini with diatoms in level III). The Tanytarsini also had a strong negative association with diatoms in level I. Grazers in group B were not significantly linked with any diatom level.

Electivity indices

Comparison of diatom composition in grazer gut contents with ambient composition showed that the grazer groups identified by the multivariate analyses differed in electivity for the 3 levels in the diatom mat (Fig. 3A, B, C). Grazers in group A had strong positive electivity for level I diatoms coupled with clear avoidance of level III diatoms in May and July (Fig. 3A, B). An opposite, but less pronounced, trend was

observed for grazers in group C, which were the only grazers with positive electivity for level III diatoms (Fig. 3A, B). Electivity patterns for group B were less distinct and less consistent than electivity patterns for groups A and C (Fig. 3A, B).

Discussion

Resource partitioning by stream grazers

Our results provide evidence that, at least during periods when available food is reduced (July, in our community), a guild of stream grazers partitions its food resources (diatoms) on the basis of the spatial position of the diatoms in the periphyton mat. In May, when diatom size and level were correlated, the separate associations of these attributes with grazer diet could not be distinguished (although grazer size and maximum size of ingested diatoms were strongly related; Tall et al. 2006), and only 3% of the total grazer biomass (group A) showed clear selectivity. Our failure to detect associations between grazer diets and diatom attributes may have been related partly to low power of the statistical tests for Ephemeroptera and Trichoptera, which were underrepresented in our gut analyses.

In contrast, in July, when the range of diatom sizes was smaller than in May and when diatom size was not correlated with level, grazer diet was unequivocally associated with diatom spatial position in the periphyton mat, and 3 distinct feeding patterns were observed. A group of *true scrapers* (group A, 47% of the total biomass), mainly composed of the coleopteran *Promoresia* and the caddisfly *Hydroptila*, fed selectively on the adnate algae and avoided the overstorey algae. A group of *generalists* (group B, 15% of the biomass), composed of the mayfly *Baetis* and Tanytarsini chironomids, did not appear to select diatoms in any particular spatial position in the periphyton mat. Last, a group of *surfers* (group C, 37% of the biomass) that included only chironomids avoided the adnate algae and fed preferentially on detached overstorey diatoms.

Resource partitioning has been reported in bird (Lack 1971), fish (Carrassón and Cartes 2002), and mammal communities (Emmons 1980). However, before our study, resource partitioning had not been confirmed for freshwater benthic grazers although specialization on different resources has been sometimes reported in co-occurring stream consumers (Steinman 1996, Evans-White et al. 2003). No evidence of microhabitat partitioning was found among sympatric dipteran (*Blepharicera*) larvae (Alverson et al. 2001) or in a community of epilithic stream invertebrates consisting of several insect orders and mollusks (Death 2004). Community studies of chironomids on aquatic plants (Tokeshi 1986) and of chironomids and

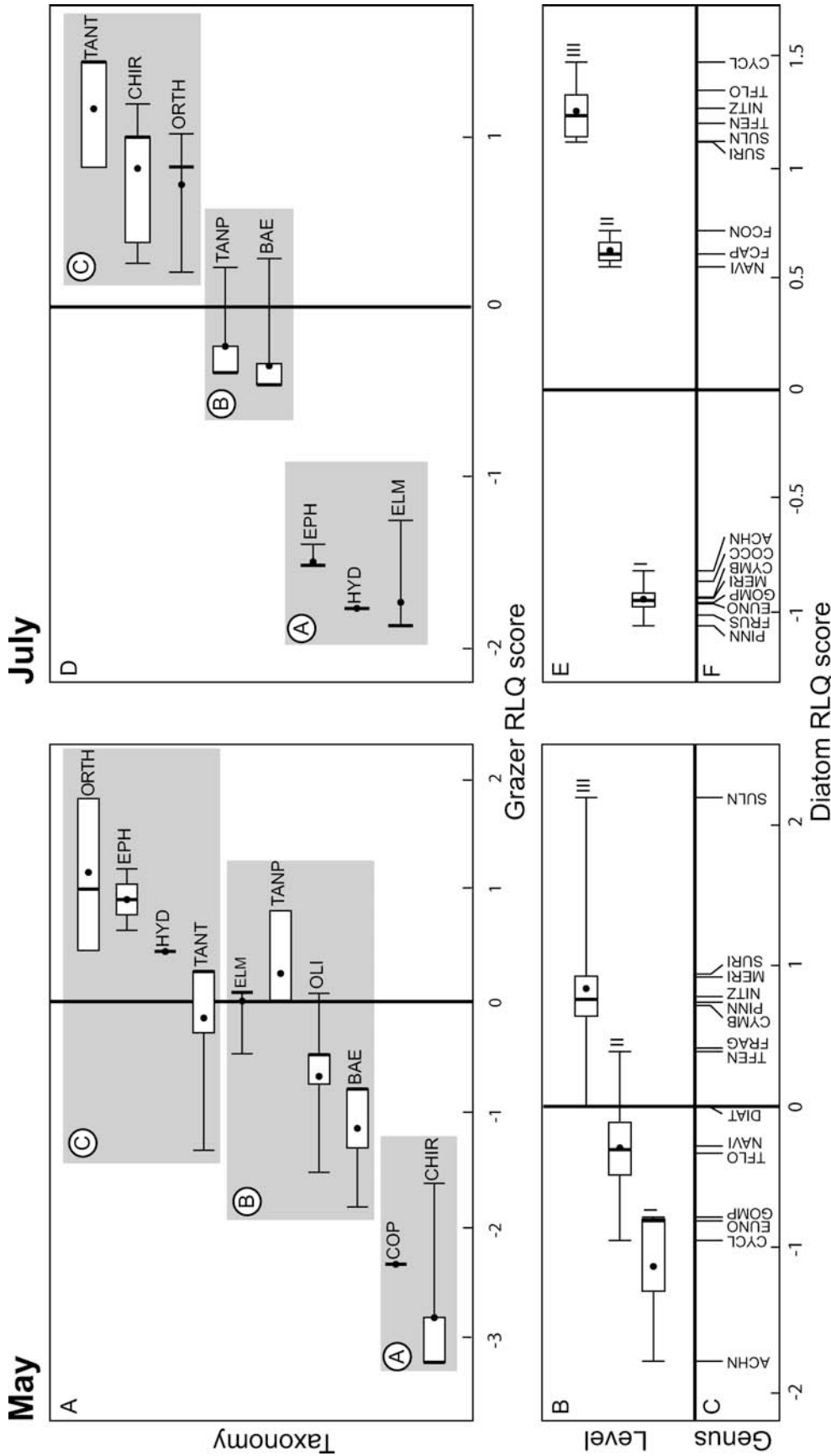


FIG. 2. Box plots showing ordination of the RLQ scores along the first RLQ axis of invertebrates differentiated by taxonomy (A, D), diatoms grouped by level in the periphyton mat (B, E), and diatoms differentiated by taxonomy (C, F) in May and July, respectively. Boxes indicate the quartiles, dots inside boxes indicate means, lines inside boxes indicate medians, whiskers indicate the 95th percentile, and vertical bars outside the whiskers indicate extreme outliers. Codes for invertebrates and diatoms are as in Tables 1 and 2, respectively. Gray boxes indicate invertebrate grazer groups (A, B, and C) determined by K-means partitioning of RLQ scores.

TABLE 3. Results of 4th-corner analyses of associations between grazer taxa and diatom attributes (spatial position in the periphyton mat [level] and size) in May and July. Level indicates the degree of attachment of the diatom to the moss: I = tightly attached, II = loosely attached, III = detached. Global tests of significance (χ^2 , F) between variables are shown with probabilities adjusted using Holm's procedures. Sign indicates whether relationship between attributes is positive (+) or negative (-). * = $p < 0.05$, ** = $p < 0.001$, *** = $p < 0.0001$.

	Diatom level			Diatom size
	I	II	III	
May				
Global tests:	$\chi^2 = 1078.60$ $p = 0.0001^{***}$			$F = 78.9$ $p = 0.0001^{***}$
Group A				
Copepoda	+	-	-	-
Chironomini	+	-	-	-**
Group B				
Elmidae	+	-*	-	+
Tanypodinae	+	+	-	-
Oligochaeta	+	+	-	-
Baetidae	-	+	-	-
Group C				
Orthoclaadiinae	-*	-	+	+
Ephemerellidae	-	-	+	+
Hydroptilidae	-	+	-	+
Tanytarsini	-	+	-	-
July				
Global tests:	$\chi^2 = 2146.82$ $p = 0.0001^{***}$			$F = 10.70$ $p = 0.630$
Group A				
Ephemerellidae	+	+	-**	+
Hydroptilidae	+	-	-**	+
Elmidae	+	-**	-**	+
Group B				
Tanypodinae	+	+	-	-
Baetidae	+	-	-	+
Group C				
Tanytarsini	-**	+	+	+
Chironomini	-	-	+	+
Orthoclaadiinae	-	+	-	-

Elmidae in a stream (Tavares and Williams 1990, Tavares-Cromar and Williams 1997) indicated almost complete dietary overlap.

Several factors related to the scale at which previous studies have been conducted may have prevented observation of resource partitioning. First, studies of resource partitioning in benthic communities often have focused on closely related taxa within a family or genus, but taxonomically related grazers usually have similar mouthpart morphology and probably have limited possibilities for diversifying their feeding strategies. Thus, resource partitioning may be more likely between grazers that are not closely related.

(However, we observed resource partitioning despite the presence of many chironomid taxa in our samples.) Second, diet differences among grazers may be overlooked when food is classified into large categories (e.g., algae, detritus), as is the case in most studies of invertebrate gut contents. Third, the choice of spatial scale for a study of resource partitioning is crucial. A pattern of broad overlap has been observed in studies that considered spatial segregation among patches of the same microhabitat type (e.g., stones: Death 2004, macrophyte branches: Tokeshi and Townsend 1987). In our study, grazer distributions overlapped among moss patches, and grazer diets did not vary among individuals of the same taxon collected from different patches of moss. However, we did observe resource partitioning *within* moss patches. Therefore, a multi-layer periphyton mat probably provides a realistic scale at which to study of a guild of invertebrate grazers, considering their size and poor swimming ability.

Grazer efficiency and removal of algae

In the multilayered periphyton community, grazer efficiency on diatom taxa is thought to depend on the strength of diatom adhesion to the substratum and, thus, on the spatial position of the taxon in the periphyton mat (Gregory 1983, Hudon 1983, Steinman 1996). This hypothesis has been supported by numerous observations that overstory algae are more vulnerable than understory algae to grazers (Marker et al. 1986, Hill and Knight 1988, Silver Botts and Cowell 1992). Grazers showed a preference for overstory diatoms in 77% of the studies examined in an extensive literature analysis (Steinman 1996). However, the opposite pattern also has been reported. For instance, limpets (Blinn et al. 1989) and several species of *Blepharicera* larvae (Alverson et al. 2001) preferentially ingest adnate diatoms. Our results reconcile these conflicting observations. All levels of the algal mat are used when the entire grazer guild is considered.

Tightly attached algae are less protected from grazing than is generally thought. We found that almost 1/2 of the total grazer biomass preferentially used attached algae in July. These grazers (Elmidae, Hydroptilidae, and Ephemerellidae), which usually are described as collector-gatherers (Merritt and Cummins 1996), have mouthpart morphologies that allow them to detach algae from the substratum. Except for the Elmidae, these scrapers did not select attached algae in May when they were young instars and when the number of diatoms per gram of grazers was 10 \times higher than in July. The Chironomidae, also

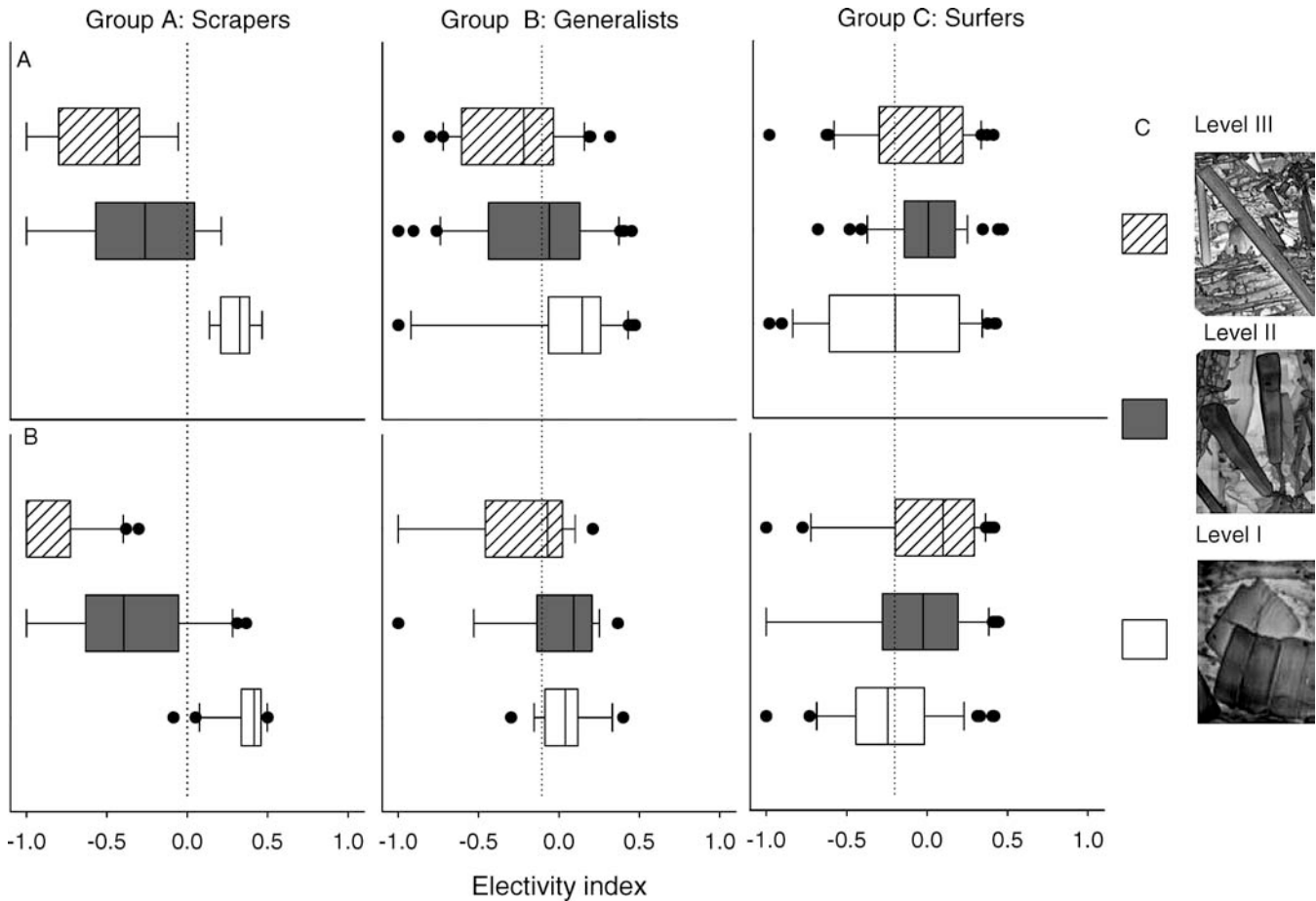


FIG. 3. Box plots of electivity indices for diatom in levels I (tightly attached), II (loosely attached), and III (detached) spatial levels in the periphyton mat calculated for the 3 groups of grazers (A = scrapers, B = generalists, C = surfers) identified by K-means partitioning of the RLQ scores in May (A) and July (B). Scanning electron micrographs in the key to symbols (C) show the appearance of the diatoms in each level of the periphyton mat. Boxes indicate the quartiles, lines inside boxes indicate medians, whiskers indicate the 95th percentile, and dots outside the whiskers indicate extreme outliers. Vertical dashed lines indicate random feeding. Positive electivity indices indicate preference, and negative indices indicate avoidance.

considered collector-gatherers, tended to be associated with overstory diatoms and to avoid adnate forms. An apparent exception was the association of Chironomini (*Paratendipes*) with adnate diatoms in May. This association probably reflected a prevalence of the small diatom *Achnanthes* in the diet of this small grazer. We could not entirely determine if the positive selection solely indicated a differential efficiency at consuming diverse algal growth forms or active selection, which can occur even in benthic systems (e.g., ciliates: Hamels et al. 2004).

In conclusion, like forest birds (MacArthur 1958, Lack 1971), different benthic grazers feed in different levels in the algal canopy, and they use algae within the algal-grazer size constraints identified in plankton ecology (Burns 1968). However, resource partitioning is detectable only at appropriate within-patch scales of grazer spatial distribution and diet description. Thus,

foraging theories established for other habitats are confirmed in the unique context of stream benthos.

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APPENDIX. Number of individuals of each grazer genus used for the gut content analyses. Grazers were grouped according to tribe (chironomids) or order in the multivariate analyses to increase statistical power.

Taxon	May	July
Chironomini		
<i>Microtendipes</i>	–	2
<i>Paratendipes</i>	3	–
<i>Polypedilum</i>	1	10
Tanytarsini		
<i>Micropsectra</i>	4	6
<i>Paratanytarsus</i>	–	8
<i>Tanytarsus</i>	5	4
Orthoclaadiinae		
<i>Corynoneura</i>	1	1
<i>Cricotopus</i>	11	–
<i>Eukiefferiella</i>	3	–
<i>Limnophyes</i>	3	–
<i>Nanocladius</i>	1	–
<i>Orthocladus</i>	3	–
<i>Parametriocnemus</i>	–	2
<i>Synorthocladus</i>	–	6
<i>Thienemaniella</i>	–	4
<i>Tvetenia</i>	1	1
Tanypodinae		
<i>Labrundinia</i>	–	2
<i>Meropelopia</i>	6	–
<i>Nilotanypus</i>	7	1
<i>Rheopelopia</i>	–	5
Elmidae		
<i>Promoesia</i>	7	9
Baetidae		
<i>Baetis</i>	3	10
Ephemerellidae		
<i>Eurylophella</i>	2	6
Hydroptilidae		
<i>Hydroptila</i>	3	6