Algal ingestion and digestion by two Ephemeropteran larvae from a Patagonian Andean stream

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Abstract

The ingestion and digestion of two co-existing Ephemeropteran larvae, *Meridialaris diguillina* (Leptophlebiidae) and *Baetis* sp. (Baetidae) were examined and their mouthpart morphologies were compared. *M. diguillina* bears conspicuous brushing-like maxillae and *Baetis* sp. has toothed, scope-like lacinia-galeae and mandibles. Larvae were fed on periphyton and faeces were collected for examination. The proportions of dead, dying and live cells in faeces were compared between grazers and with periphyton. The two species showed differences in grazing efficiency. *Baetis* sp did not feed on prostrated diatoms while *M. diguillina* fed on the whole periphyton assemblage. In terms of digestion, *M. diguillina* presented apparent higher digestion ability than *Baetis* sp. These differences showed dissimilar resource exploitation and therefore the two species might influence the structure and dynamic of the algal assemblage in different ways.

Keywords: grazing, mouthpart morphology, diatoms, *Meridialaris*, *Baetis*.

Research Update on Ephemeroptera & Plecoptera
2003, E. Gaino (Ed.), University of Perugia, Perugia, Italy.

Introduction

Most mayfly larvae are common stream dwellers, adapted to consume the biofilm growing on stones (Edmunds and Waltz, 1996). Their ability of accessing to different algal habits depends on larval mouthpart morphology (Karouna and Fuller, 1992). Collector-gatherers are provided with several sets of setae that allow them to access to loose material while scrapers present some sharp structures that enable them to dislodge highly attached algae (McShaffrey and McCafferty, 1988). Therefore, herbivory usually induces changes in the periphytic assemblage related to the differential susceptibility of algal species to grazing (Sumner and McIntire, 1982; Colletti *et al.*, 1987; Pringle, 1996).

Grazers may depress benthic algal quality since living food sources generally have a higher nutritive and caloric content than the same but dead materials (Lamberti and Moore, 1984). A decrease in food quality, caused by repeated ingestion and egestion of algae, may induce a depletion of the grazer population (Grimm and Fisher, 1989). Depending on the herbivore´s capability of digesting of ingested food, the reduction of food quality will occur at different velocities (Peterson *et al.*, 1998). This variation in digestion efficiencies among herbivores may produce different effects on grazer populations.

In South Andean streams, the ephemeropteran *Meridialaris diguillina* (DEMOULIN, 1955) (Leptophlebiidae) and *Baetis* sp. (Baetidae) are widely distributed and abundant inhabitants of stony riffle areas (Pescador and Peters, 1987). As Leptophlebiids and Baetids have contrasting mouthpart morphologies (Palmer *et al.*, 1993), we hypothesised that these herbivores would display different capabilities of accessing to algae. The aims of this study were to analyse their capability of ingesting different algal species and to determine the digestibility of the consumed algae through the analysis of faeces content.

Material and Methods

Larvae were collected from Gutiérrez stream (41º 07' S; 71º 25' W) in February 2001 with a Surber sampler (0.09 m^2 and 200 μm mesh size). They were carried immediately to the laboratory, where twenty individuals of each species were put in containers with 250 ml of filtrated stream water (3 replicates for each species). Body length (not including cerci) of *Baetis* sp. and *M. diguillina* larvae were measured under a stereomicroscope. *Baetis* sp. larvae averaged 4.78 μm (±0.16 s.e.,

n=20) and *M. diguillina* larvae averaged 8.82 µm (±0.03 s.e., n=20).

The larvae were acclimated for 24 h at stream temperature (15ºC), with a photoperiod of 12:12 hours (light:dark) and gently bubbling. During this period, they were supplied with a ceramic tile (8x8 cm), placed almost vertically in the container, which had been placed for two weeks in the Gutiérrez stream to be colonized by periphyton (Fig. 1A). Afterwards, larvae were starved for 24 h on a clean ceramic tile to allow their guts to clear (Fig. 1B). To reduce coprophagy, containers were covered laterally in the upper section and they were illuminated for 24 h. Faeces were observed to sink and larvae to avoid the highly lighted bottom. Then, we substituted the clean tile with a colonized one and larvae fed during 4 h in darkness (Fig. 1C). Grazing activity was checked every 20 minutes with a red light to diminish disturbance of larvae while feeding. Later, this tile was removed and a clean one was introduced to allow gut clearance during 4 h. We prevented faeces ingestion in the same way as described above (Fig. 1D). Finally, faeces were carefully collected (Fig. 1E). To compare algal content in faeces with natural periphyton, a quarter of the colonized tile was scraped with a razor blade before offered to the herbivores. Samples were preserved using 4% formalin. Larval mouthparts were dissected, and cleared with hot KOH 5%. Afterwards they were placed on a slide in Euparal and observed under direct microscope and stereomicroscope.

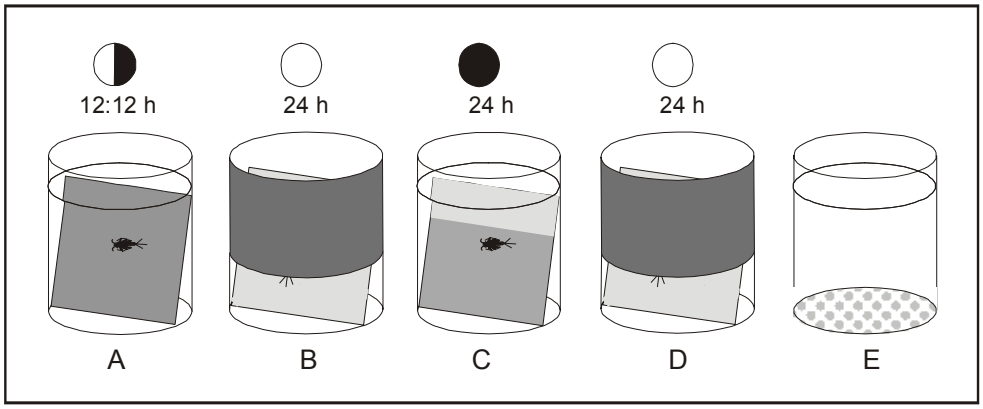

Fig. 1 - Experimental design showing sequence of the paths followed to obtain larval faeces; A) acclimation; B) starvation for gut clearance; C) food supply; D) starvation for faeces production; E) decantation of faeces. Dark grey tiles are colonised with periphyton, light grey tiles are clean. Size of the tile: 8x8 cm. Number of individuals per container: 20.

Periphyton samples and faeces were homogenised separately and then observed under direct microscope at 400X. Diatom cells were counted and differentiation between live cells (with full chloroplasts), dying cells (with fragmented chloroplasts) and dead cells (empty frustules) was done (Fig. 2).

Differences between relative abundance of the most representative algal species in periphyton and in faeces content were tested using one-way ANOVA. Percentages of dead, dying and live algal cells in periphyton and in faeces were also compared using one-way ANOVA. When significant differences were found, a multiple comparison *a posteriori* Student-Newman-Keuls test (S-N-K) was performed (Sokal and Rohlf, 1981).

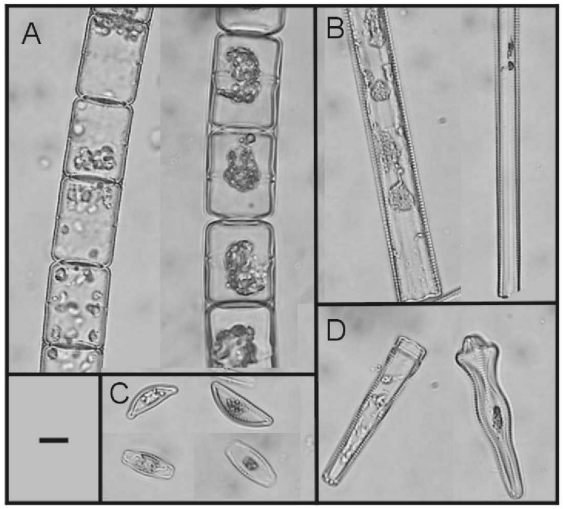

Fig. 2 - Light microscopic photographs showing the difference in the state of the chloroplast in live (left) and dying (right) diatom cells. A) *Melosira varians*; B) *Synedra ulna*; C) *Cymbella silesiaca*; D) *Gomphonema acuminatum*. Scale bar: 10 µm.

Results

Mouthpart morphology

Mouthpart morphologies of *Meridialaris diguillina* and *Baetis* sp. were highly contrasting. Mouthparts of *M. diguillina* are mainly characterised by bearing a large number of bristles. Maxillae are the most complex structures with fused lacinia and galea (Fig. 3A). The apex of the lacinia-galea is wide, straight and very sclerotized, and it is provided with a dense brush of stout bristles. Maxillary palps are long and stout and exhibit an apical brush of long bristles (Fig. 3B). Labium is constituted by very small glossae and wide and flattened paraglossae densely covered by bristles upwardly directed. Labial palps are three-segmented and the apical one bears few short hairs. Mandibles are large, flattened and sclerotized structures with slender incisors and a reduced inner molar area (Fig. 3B).

Maxillae of *Baetis* sp. are also composed by fused lacinia and galea. They are conical structures bearing a row of large hairs at the inner margin and the apex ends in four sharp teeth (Fig. 3C). Maxillary palps are membranous and bear short hairs at the apex. Labium is little sclerotized with small glossae and paraglossae and short palps that bear sparsely and short hairs. Mandibles are stout and have an apical blade ending in a blunt-toothed edge (Fig. 3D). They bear moderately large inner grinding areas.

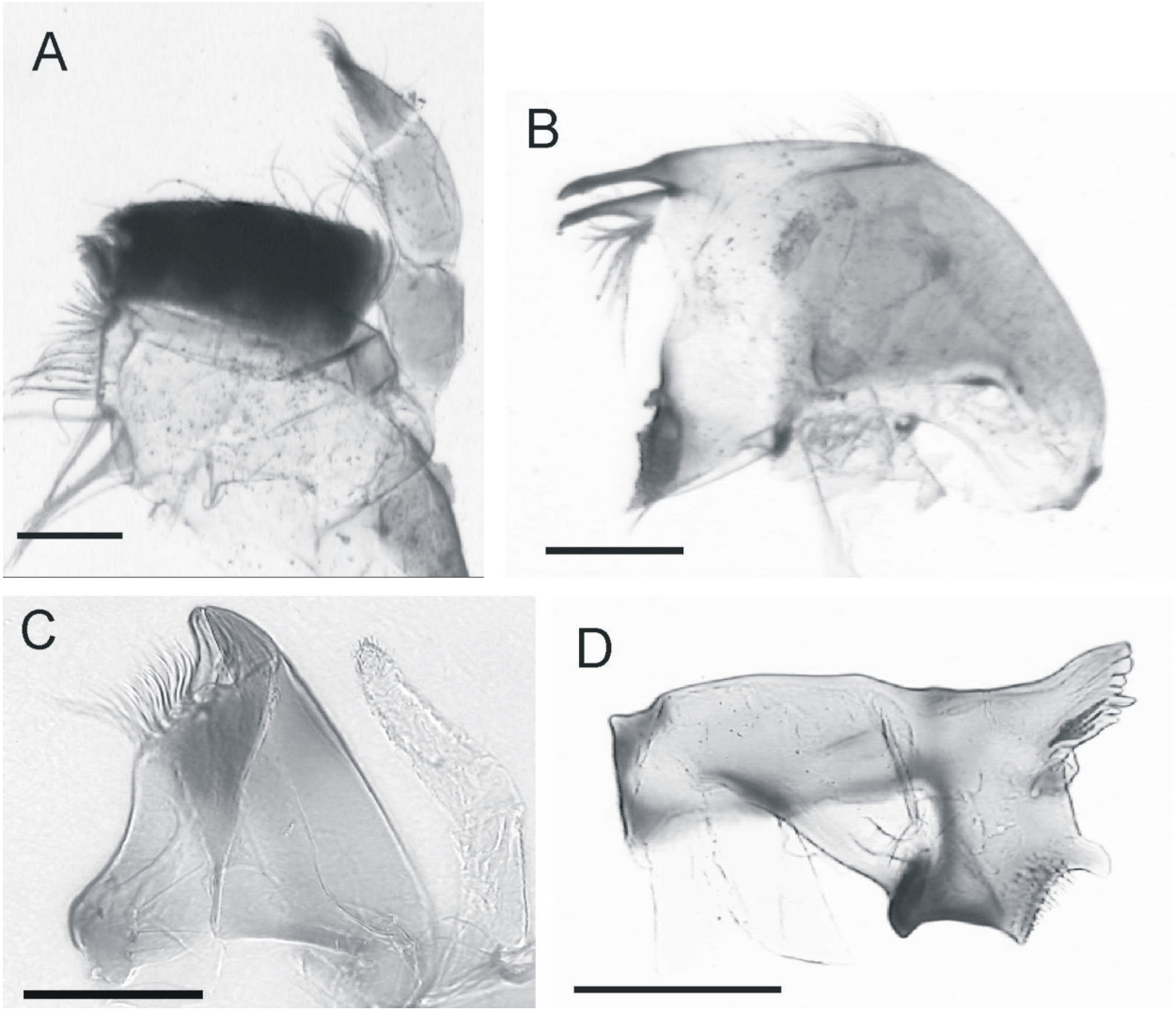

Fig. 3 - Mouthparts of *Meridialaris diguillina* and *Baetis* sp. A) Ventral view of the left maxilla of *M. diguillina* (scale bar: 100 µm); B) dorsal view of the right mandible of *M. diguillina* (scale bar: 100 µm); C) ventral view of the left maxilla of *Baetis* sp. (scale bar: 50 µm); D) dorsal view of the left mandible of *Baetis* sp. (scale bar: 100 µm).

Periphyton-faeces comparisons

Comparison of the periphytic assemblage and faeces accounted for differences in algal taxa susceptibility to each herbivore (ANOVA, $P<0.05$). *M. diguillina* ingested the diatoms *Cyclotella stelligera* (a prostrate species) and *Gomphonema angustum* (a short pedunculate species) in a higher proportion than they were in periphyton (Student-Newman-Keuls, $P<0.05$). In contrast, the filamentous *Fragilaria pinnata* and *Melosira varians* were found in a lower proportion than they were in periphyton ($P<0.05$) (Fig. 4). On the other hand, *Baetis* sp. consumed a great proportion of the pedunculate *Cymbella silesiaca* (>50% of total diatom cells) ($P<0.05$), a species with a long path that grows overstory in the periphyton, but it ingested the other species in lower proportions than they were in periphyton (Fig. 4).

The physiological condition of cells (live, dying and dead) also differed between faeces of both species and periphyton (ANOVA, $P<0.05$). *M. diguillina* faeces contained lower proportion of live cells than periphyton and higher proportion of dying and dead cells (S-N-K, $P<0.05$) (Fig. 5). Dead cells represented 62% of total cells in faeces while only 10% survived passage through gut. In contrast, *Baetis* faeces contained the same proportion of live cells than periphyton (S-N-K, $P>0.05$) and a higher proportion of dying cells ($p<0.05$) (Fig. 5).

Diatom species showed differences in their susceptibility to digestion (Fig. 6). *F. pinnata* and *M. varians*, both non-preferred taxa, were highly digested, especially by *M. diguillina* (Fig. 6A, B). However, dead cells of *C. silesiaca*, a highly consumed species by *Baetis* sp., were relatively more abundant in periphyton than in faeces, suggesting that both herbivores ingested live cells in a higher proportion than they were in periphyton (Fig. 6C). Besides, the low proportion of dead cells in faeces of both grazers revealed a low digestibility of this diatom. A similar grazer preference for live cells was apparent on *G. angustum*. This species appeared as empty frustules in periphyton but it presented 20% of dying cells in *M. diguillina* faeces and 10% in *Baetis* sp. faeces (Fig. 6D). On the contrary, *C. stelligera* was always found as empty frustules in the periphyton and in faeces indicating no selectivity for live cells (Fig. 6E).

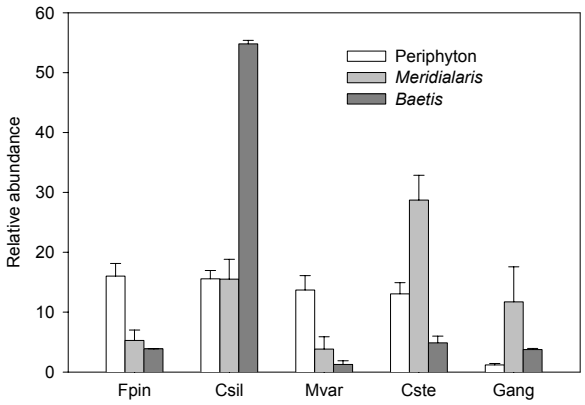

Fig. 4 - Relative abundance of the most representative diatom species in periphyton and in grazers faeces. Fpin: *Fragilaria pinnata*; Csil: *Cymbella silesiaca*; Mvar: *Melosira varians*; Cste: *Cyclotella stelligera*; Gang: *Gomphonema angustum*. Error bars are standard errors

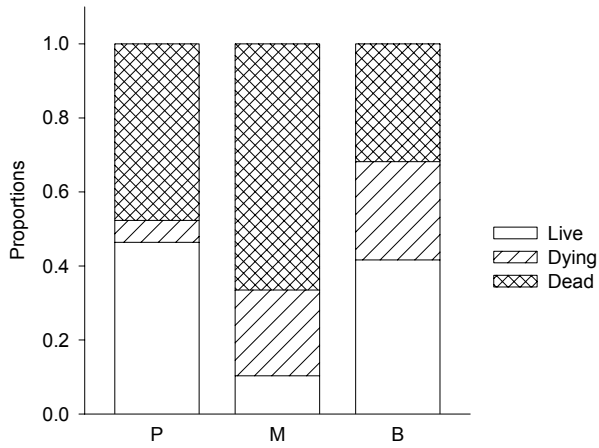

Fig. 5 - Proportion of live, dying and dead cells in periphyton (P) and in faeces content of *Meridialaris diguillina* (M) and *Baetis* sp. (B).

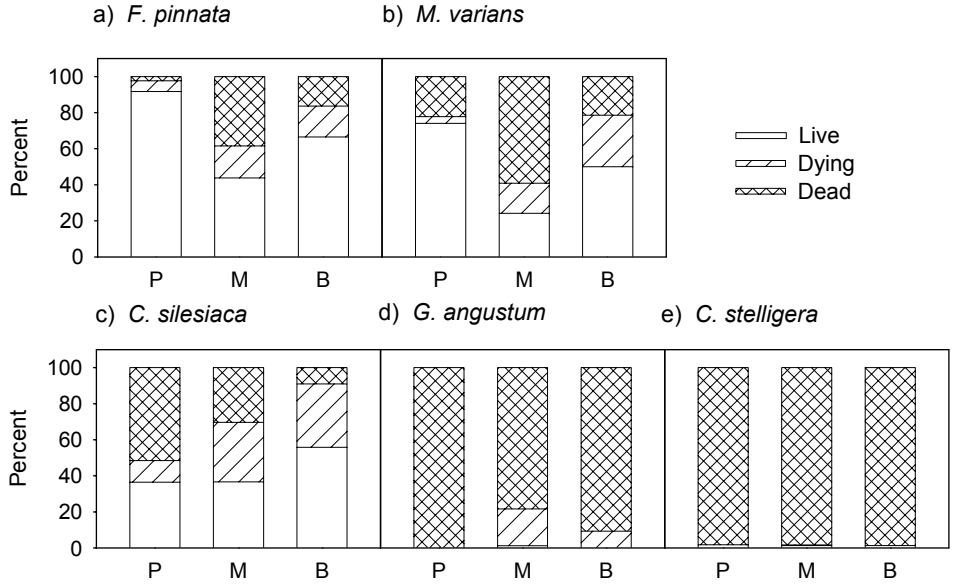

Fig. 6 - Proportion of live, dying and dead cells of the most representative species in periphyton (P) and in faeces content of *Meridialaris diguillina* (M) and *Baetis* sp. (B).

Discussion

Differences in mouthparts morphology between *M. diguillina* and *Baetis* sp. were reflected in different access to algae. On one hand, *M. diguillina´*s capability of ingesting prostrate species, such as *Cyclotella stelligera*, indicated the high efficiency of their brushes in accessing to species that grow close to the substrate. Similar results were obtained for *Meridialaris chiloeensis*, which fed on the whole periphyton assemblage in streamside channel experiments (Díaz Villanueva *et al.*, unpublished). However, *M. diguillina* consumed the filamentous *Fragilaria pinnata* and *Melosira varians* in a lower proportion than these taxa were in periphyton. Similarly, McShaffrey and McCafferty (1988) found that the mayfly *Rithrogena pellucida*, whose mouthparts resemble very much those of *Meridialaris*, was not able to consume filamentous algae. The authors suggested that this could be the result of a mismatch between mouthpart morphology and the conspicuous filamentous habit.

On the other hand, *Baetis* sp had unspecialized mouthparts. Palmer *et al.* (1993) assigned four Baetidae species to the collector-gatherer functional group because their mouthparts beard different bipectinate setae. The scarcity of setae found in *Baetis* sp. mouthparts would indicate that the main structures that larvae used to get and handle periphyton were the mandibles and maxillae. In our experiment, *Baetis* sp. ingested substantially more of the pedunculate *Cymbella silesiaca* than of any other species, suggesting that the toothed-tips of both mandible and lacinia+galea acted as reaper more than as scrapping devices. Species of the genus *Baetis* are consistently found to have slight effects in periphyton biomass and to access to the erect growing algal species (Feminella and Hawkins, 1995). Dead cells in the faeces of *Baetis* sp. were proportionally lower than in periphyton and this result is only possible if live cells were ingested in a higher proportion than they were in periphyton. Since the proportion of live algal cells is higher in the upper layer than in the inner zone (McIntire, 1973), this result provided further proofs confirming that *Baetis* sp. harvested the upper layer of periphyton.

The presence of prostrate diatom cells in the faeces of *M. diguillina* suggested its ability to access to the inner matrix of the periphyton. Therefore, it could mean that this species ingested dead cells in a high proportion. However, the inner periphytic layer is not a dead zone but a more heterotrophic one, so *M. diguillina* could have made use of bacteria and fungi as an additional carbon source. The importance of these microorganisms in the diet of grazers was demonstrated for the mayfly *Delatidium* (Winterbourn *et al.*, 1984) and also for snails (Morales and Ward, 2000). Besides, the proportion of dead cells in *M. diguillina* faeces

(70%) showed that this mayfly has a high digestion efficiency. It was higher than the value registered in other studies of diatom digestibility, such as in the mayfly *Ameletus* sp. (60%) (Peterson *et al.*, 1998) and in some caddisflies (58%) (Peterson, 1987) and gastropods (45%) (Nicotri, 1977). In view of this high digestibility, diatoms might be an important high-quality food resource.

Underwood and Thomas (1990) and Peterson *et al.* (1998) accounted for differences in algal taxa resistance to digestion. But yet little is known about the defensive strategies which enable certain species to survive gut passage. They proposed small size as an advantage because it would reduce the probability of being damaged. However, our results showed that some small diatoms, such as *Fragilaria pinnata*, might also be efficiently digested. An observed pattern was that the most susceptible species to be ingested (e.g. *Cymbella silesiaca*) were also the most resistant to be digested and viceversa (e.g. *Fragilaria pinnata*, *Melosira varians*). Peterson *et al.* (1998) found the same relation and they hypothesised that this pattern would indicate that natural selection would favour the resistance to digestion in those grazing susceptible taxa. Underwood and Thomas (1990) suggested that DOM released in the gut by algae could enhance algal survivor as it could reduce the selective pressure on grazers to evolve more efficient digestive enzymes.

Differences in algal survivorship through gut passage between *M. diguillina* and *Baetis* sp. (10% and 40% respectively) would indicate that algal mat quality diminishes more rapidly upon exposure to *M. diguillina* than to *Baetis* sp. Our results imply that the structure of algal assemblage would be not equally influenced by both grazers, leading to dissimilar periphyton quality as food resource. While studies on algal digestibility had proved to enhance algal fitness of those species that can resist digestion in the plankton (Porter, 1975, 1976), nothing is known of what happens in periphyton. So, further experimental studies are needed to understand the role of aquatic insects in the dynamics of periphytic algal assemblage.

Acknowledgements
We thank Dr. B. Modenutti for her useful comments. This work was supported by PICT 01-06035.

References

Colletti P.J., Blinn D.W., Pickart A., Wagner V.T., 1987. Influence of different densities of the mayfly grazer *Heptagenia criddlei* on lotic diatom communities. J. N. Am. Benthol. Soc. 6:270-280.

Edmunds G.F., Waltz R.D., 1996. Ephemeroptera. In: Merritt R.W., Cummins K.W., (eds). An introduction to the aquatic insects of North America. Kendall/Hunt Publishing Company, Iowa. pp 126-163.

Feminella J.W., Hawkins C.P., 1995. Interactions between stream herbivores and periphyton: a quantitative analysis of past experiments. J. N. Am. Benthol. Soc. 14:465-509.

Grimm N.B., Fisher S.G., 1989. Stability of periphyton and macroinvertebrate to disturbance and flash floods in a desert stream. J. N. Am. Benthol. Soc. 8:293-307.

Karouna N.K., Fuller R.L., 1992. Influence of four grazers on periphyton communities associated with clay tiles and leaves. Hydrobiologia 245:53-64.

Lamberti G.A., Moore J.W., 1984. Aquatic insects as primary consumers. In: Resh V.H., Rosenberg D.M., (eds). The Ecology of Aquatic Insects. New York, Praeger. pp 164-195.

McIntire C.D., 1973. Periphyton dynamics in laboratory streams: a simulation model and its implications. Ecology 43:399-420.

McShaffrey W., McCafferty P., 1988. Feeding behavior of *Rhithrogena pellucida* (Ephemeroptera: Heptageniidae). J. N. Am. Benthol. Soc. 7:87-99.

Morales J.B.T., Ward A.K., 2000. Differential incorporation of algae and bacteria by *Elimia clara* (Prosobranchia: Pleuroceridae)-a study using dual-labeled epilithon. J. N. Am. Benthol. Soc. 19:289-297.

Nicotri M.E., 1977. Grazing effects of four marine intertidal herbivores on the microflora. Ecology 58: 1020-1032.

Palmer C., O'Keeffe J., Palmer A., 1993. Macroinvertebrate functional feeding groups in the middle and lower reaches of the Buffalo River eastern Cape, South Africa. II. Functional morphology and behaviour. Freshwater Biol. 29:455-462.

Pescador M.L., Peters W.L., 1987. Revision of the genera *Meridialaris* and *Massartellopsis* (Ephemeroptera: Leptophlebidae: Atalophlebiinae) from South America. Trans. Am. Entomol. Soc. 112:147-189.

Peterson C.G., 1987. Gut passage and insect grazer selectivity of lotic diatoms. Freshwater Biol. 18:455-460.

Peterson C.G., Vormittag K.A., Valett H.M., 1998. Ingestion and digestion of epilithic algae by larval insects in a heavily grazed mountain stream. Freshwater Biol. 40:607-623.

Porter, K.G., 1975. Viable gut passage of gelatinous green algae ingested by Daphnia. Verh. Internat. Verein. Limnol. 19:2840-2850.

Porter, K.G., 1976. Enhance of algal growth and productivity by grazing zooplankton. Science 192: 1332-1334.

Pringle C.M., 1996. Atyid shrimps (Decapoda: Atyidae) influence the spatial heterogeneity of algal communities over different scales in tropical mountain streams, Puerto Rico. Freshwater Biol. 35:125-140.

Sokal R.R., Rohlf F. J., 1981. Biometry. Freeman & Co., San Francisco.

Sumner W.T., McIntire C.D., 1982. Grazer-periphyton interactions in laboratory streams. Arch. Hydrobiol. 93:135-157.

Underwood G.J.C., Thomas J.D., 1990. Grazing interactions between pulmonate snails and epiphytic algae and bacteria. Freshwater Biol. 23:505-522.

Winterbourn M.J, Cowie B., 1984. Food resources and ingestion patterns of insects along a West Coast, South Island, river system. N. Zeal. J. Mar. Freshwat. Res. 18:379-388.