Introduction

In freshwater ecology, particularly in studies of running waters, the consideration of feeding relationships of benthic macroinvertebrates has been crucial to conceptualizing community dynamics and predicting ecological relationships (Vannote et al. 1980). Cummins (1973, 1974) concluded that mouthpart morphology was a good reflection of feeding mechanism, as animals can only be opportunistic within the limitation of their feeding morphology and the behavior that drives it.

McShaffrey & McCafferty (1986, 1988) have demonstrated the advantages of using combined methods to assign macroinvertebrates to Functional Feeding Group (FFG) categories, including mouthpart morphology and behaviour. According to Arens (1989), insect mouthparts morphologic pre-adaptations allow only some behavioural arrangements for the obtainment of specific feeding resources. In insects, basal parts of the labium (post and prementum) are always fused medially and its distal appendages have low mobility (glossae and paraglossae). Generally, only the labial palps have enough mobility to manipulate food, and the more robust distal parts of the maxillae (laciniae and galeae) are theoretically suited for scraping hard surfaces. Also, mandibles are able to move freely, being hard enough to scrap and/or triturate food.

Functional feeding groups of Brazilian Ephemeroptera nymphs: ultrastructure of mouthparts


In order to assign 18 mayfly taxa found in streams in the Macaé River basin into Functional Feeding Groups, the anatomy of their feeding apparatus was examined through scanning electron microscopy. Also, habitat preference and field observations of feeding behaviour were made to assure FFG assignment. Ephemeropteran taxa were classified into five FFGs: Passive Filterers - *Hylister plaumanni*; Active Filterer - *Lachlania boanovae* and *Campylocia* sp.; Brushers - *Askola froehlichi*, *Farrodes carioca*, *Hugenalogesis* spp., *Massartela brieni*, *Miroculis froehlich*, *Miroculis* sp., and *Thraulodes* spp.; Grazers - *Cloeodes* spp., *Americabaetis* spp., *Camelobaetidius* spp. and *Baetodes* spp.; Scrapers - *Leptohyphes pereirae*, *Leptohyphes* spp., *Tricorythodes* spp. and *Tricorythopsis* spp. Species of the three best represented mayfly families in south-east Brazil were assigned to different FFGs (Leptophlebiidae - Brushers; Baetidae - Grazers and Leptohyphidae - Scrapers), with one exception, *Hylister plaumanni* (Leptophlebiidae; Active filterers). This information is useful to understand the role of mayflies in stream ecosystems, and to help the development of ecological theories for tropical streams.

Keywords: Ephemeroptera, FFG, neotropical streams, scanning electron microscopy, ultrastructure of mouthparts.

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Some studies on mayflies have combined observation and morphological analyses (Brown 1961, Froehlich 1964, Palmer et al. 1993). Based on morphological ancestral adaptations, Ephemeroptera nymphs are more often assigned to two basic FFGs, filterers and collectors (Arens 1989, Arens 1990, Elpers & Tomka 1994), although some are predators and only a few are shredders (Edmunds et al. 1976, Merritt & Cummins 1996).

Since most studies dealing with aquatic insect FFGs were conducted in temperate ecosystems, with only a few studies conducted in other regions (Palmer et al. 1993, Bello & Cabreras 2001, Cummins et al. 2005), the aim of this study was to analyze the anatomy of the feeding apparatus of 18 mayfly taxa found in streams in south-east Brazil through scanning electron microscopy in order to assign these taxa to FFGs. Also, we described habitat preference and in-field observations of feeding behaviour to aid in the assignment of species to FFGs.

Methods

Sampling and FFG classification

Mayfly nymphs were sampled in Macaé River basin, Rio de Janeiro State, Brazil, a 6th order river, located in the Serra do Mar mountains. Sites sampled were in 1st, 2nd, 4th, 5th, and 6th order stream reaches. Sampling sites were at altitudes between 60 and 1100 m a.s.l. Two distinct macroinvertebrate assemblages were found in this basin: one from first to fourth order stream reaches (upper reaches), and other comprising fifth and sixth order stream reaches (lower reaches) (Baptista et al. 2001). Specimens were collected from four substrate types (fine sediment and litter in pool areas; stones and litter in riffle areas) using a Surber sampler (900 cm², mesh size 125 µm), and preserved in 80% ethanol for further examination of mouthparts.

The basic system for FFG assignment was the classification scheme of Cummins (1973, 1974), based on the resource partitioning. In this study, we also considered the classification scheme described in Palmer et al. (1993), based in part on McShaffrey & McCafferty (1988), because it allows the inclusion of additional ecological information on how macroinvertebrates acquire these resources. In this respect, we included the Grazer FFG, as follows:

(a) Filterers

(i) Passive - feed on seston, which is moved by a current, using body parts

(ii) Active - resuspend deposits which are filtered using body parts

(b) Collectors

(i) Gatherers - use structures other than setae to remove lightly attached or loosely deposited organic material

(ii) Brushers - use setae to remove lightly attached or loosely deposited organic material

(iii) Scrapers - have structural adaptations which allow to feed from tightly accreted material

(iv) Grazers - use mouthparts to feed on algae, by reaping off parts of living material

Ultrastructure of mouthparts

For scanning electron microscopy, mouthparts were fixed in 70% ethanol, washed with cacodylate buffer, post fixed in 1 % OsO₄ in 0.1 M cacodylate buffer containing 0.8 % potassium ferrocyanide and 5 mM CaCl₂, washed with cacodylate buffer, dehydrated in a graded ethanol series, critical point dried, sputter coated with gold, and examined in a Jeol JSM 5310 scanning electron microscope operating at 15 kV.

Results

Generally, labia and maxilla provided the most useful information on feeding strategy used by Ephemeroptera nymphs. Taxa were assigned to five FFGs: Passive Filterers, Active Filterers, Brushers, Grazers or Scrapers.

The species Askola froehlichi (Peter 1969), Farrodes carioca (Dominguez et al. 1996), Hagenulopsis sp., Massartela brieni (Lestage 1924), Miroculis froehlichi (Savage & Peter 1983), Miroculis sp., and Thraulodes spp (Leptophlebiidae) were assigned to the Brusher FFG category. In these species, the distal part of the maxilla ends in a tuft of brush-shaped setae (Figs 1a, b, c). The labrum is covered by few short setae, except the distal part where a fringe of bipectinate setae is present (Fig. 1d; Table 1). Such features are ideal for brushing lightly attached or loosely deposited organic material. A. froehlichi was associated exclusively with slow-flowing depositional areas in the upper reaches. M. froehlichi was restricted to the lower reaches, while Miroculis sp. occurred only in the upper reaches. F. carioca, Hagenulopsis sp., and Thraulodes spp. occurred all along the longitudinal gradient, the latter restricted to high-current areas.

The species Hylister plaumanni (Dominguez & Flower 1989) was an exception among the leptophlebiids.
It was assigned to the Passive Filterer FFG. The species have reduced glossa and paraglossa, and have long fringes of setae, designed to filter, with the labial palps and maxilla (Figs 2a, b, c; Table 1), suspended organic material carried by water current, and the most prominent structures used for filtering were the maxillary palps (Fig. 2d). In this study, H. plaumanni nymphs occurred exclusively in riffle biotopes, associated to litter substrates, in the lower reaches of the Macaé River.

In the species Lachlania boanovae (da Silva 1992) (Oligoneuriidae), the maxillae and labium are covered by dense fringes of setae (Fig. 3a), suspended organic material carried by water current, and the most prominent structures used for filtering were the maxillary palps (Fig. 3c). This species has long mandibular tusks covered with setae and forelegs with a dense fringe of setae (Fig. 3f). Based on the morphology of mouthparts and legs, these species could be assigned to the Passive Filterer FFG category. However, they were classified as Active Filterers because leg setae are without microtrichia, therefore not structurally adapted for passive filtering (according to Palmer et al. 1993). By way of corroborating this FFG assignment, they were associated with areas of low water current (Campylocia sp. in the upper reaches and L. boanovae in the lower stream reaches), and behavioural observations revealed that specimens used the internal margins of the anterior tibia (and femur in L. boanovae), which are covered with long setae (Fig. 3f), to resuspend organic particles (Campylocia sp. also uses its mandible tusks with this purpose; Table 1). In-field observations revealed that Campylocia sp. also used forelegs for cleaning mouthparts and/or sweeping organic particles to the pre-oral cavity.

Based solely on the ultrastructure of mouthparts, the species Americabaetis spp., Baetodes spp., Camelo- baetidius spp., and Cloeodes spp. (Baetidae) would be classified as Collector-gatherers. However, they were classified as Grazers. They have complex mouth apparatus, with labial palps and articulated maxillary palps specialized to manipulate detritus. The paraglossae and glossae have few short setae (Figs 4a, b, c, d). On the tip of maxilla, there is a crown of chitinous teeth (Fig. 4b). The tip of the maxillae are covered by fringed curved bipectinate setae (Fig. 4e). These species probably use the long paraglossae to remove deposited particles, while labial and maxillary palps manipulate detritus. These genera were found in all biotopes, but occurred mainly on stony substrates. The design of their tarsal claws may be an adaptation to live under hydraulic stress and, in some species (like genus Camelo- baetidius), tarsal claws could be used for scraping (Fig. 4f).
The species *Leptohyphes pereirae* (Pereira 1993), *Leptohyphes* spp., *Tricorythodes* sp. and *Tricorythopsis* sp. were classified as Scrapers. On all mouth structures there are few small setae, clearly not suited for brushing or filtering activities (Figs 5a, b). In the distal part of the mandibles, there are two chitinous wedge-shaped teeth and the molar part is covered by robust spiculae, characterizing structures specialized for scraping periphyton (Figs 5c, d). These species were found predominantly in stream reaches with little canopy cover (4th and higher stream orders in stony and litter in riffle substrates).

**Discussion**

In this study, species of the family Leptophlebiidae were assigned to the Brusher FFG (Collector-gatherer FFG, according to the Merritt & Cummins (1996) classification scheme), with one exception, *Hylister plaumanni*. According to Polegatto & Froehlich (2001), *Farrodes* sp. and other brusher leptophlebiids use the brushes of setae on the distal border of the maxillae to obtain food. The maxillary palps remove food particles from the brushes taking them towards the mandibles and hypopharynx, while the labrum and labium assist in retaining food. The labial palps are important in producing a water current towards the prebuccal cavity. In our study, all brusher leptophlebiid species had similar feeding apparatus to that of *Farrodes* sp. However, since these species had a broad occurrence in all substrates and along the longitudinal gradient, we speculate that the complex structure and function of mouthparts may allow brushing, filtering and gathering feeding cycles. McShaffrey (1988) ob-
served that two species of Heptageniidae and one species of Ephemerellidae used multiple feeding cycles, but assigned them to one FFG based on their predominant activity. Based on the morphological structure of their mouthparts, these leptophlebiid species were then classified as Brushers.

Morphology of the mouthparts of *H. plaumanni* indicates that it may feed on fine detritus in a different way from other leptophlebiid species. This species belong to the generic complex Hermanella (*sensu* Dominguez & Flowers 1989), a distinct group in the subfamily Atalophlebiinae (Ephemeroptera: Leptophlebiidae). The six genera of this generic complex (*Hermanella, Hylister, Leentvaria, Needhamella, Traverella* and *Hydrometodon*) have a wide labrum and long fringes of setae on the labrum, maxillae and labium. The occurrence of *H. plaumanni* in litter in riffle areas, in the lower reaches of the Macaé River (5th order stream, 655 m a.s.l.), where there was higher availability of suspended organic particles (Baptista et al. 2001) corroborates its assignment to the Passive Filterer FFG.

McShaffrey & McCafferty (1988) argued about the importance of combining morphological and beha-
Fig. 3. Ultrastructure of mouthparts of *Lachlania boanovae* (Oligoneuriidae) and *Campylocia* sp. (Euthyplociidae) assigned to the Active Filter FFG. (a) Frontal view of mouthparts of *L. boanovae*. La, Labrum; mp, Maxillary palp; lp, Labial palp. Scale bar 100 µm. (b) Long setae of fore leg of *L. boanovae*. fe, Femur; ti, Tibia. Scale bar 100 µm. (c) Detail of maxilla of *Campylocia* sp. mx, Maxilla; mp, Maxillary palp. Scale bar 200 µm. (d) Detail of bipectinate setae in maxillae, maxillary palps and labrum of *Campylocia* sp. Scale bar 5 µm. (e) Detail of Mandible tusk (tus) of *Campylocia* sp. Scale bar 2 mm. (f) Long setae of fore leg of *Campylocia* sp. fe, Femur; ti, Tibia. Scale bar 2 mm.
Fig. 4. Ultrastructure of mouthparts of *Americabaetis* spp., *Baetodes* sp. and *Camelobaetidius* spp. (Baetidae) assigned to the Collector-gatherer FFG. (a) Ventral view of mouthparts of *Americabaetis* spp. pg, Paraglossae; lp, Labial palp; mp, Maxillary palp. Scale bar 100 µm. (b) Ventral view of mouthparts of *Baetodes* sp. mx, Maxillary palp; lp, Labial palp. Scale bar 50 µm. (c) Ventral view of mouthparts of *Camelobaetidius* sp. mn, Mandible; lp, Labial palp. Scale bar 100 µm. (d) Detail of labium of *Baetodes* sp. lp, Labial palp. Scale bar 200 µm. (e) Detail of maxillary setae of *Camelobaetidius* spp. Scale bar 5 µm. (f) Tarsal claw of foreleg of *Camelobaetidius* spp. Scale bar 10 µm.
vioural information for assignment to FFGs. In the present study, this was clear with *Lachlania boanovae* and *Campylocia* sp. which based simply on the ultra-structure of its mouthparts these species could be classified as Passive Filterer, but due to in-field observation of its behavior they were assigned to the Active Filterer FFG category. Also, the analysis of the preferred substrate was useful information for the assignment in FFGs categories in this study: both species were found predominantly in deposited litter substrates, indicating that they probably would not perform passive filtering as their predominant feeding cycle. Pereira & da Silva (1990) also found nymphs of *Campylocia* partially burrowed in soft sediments in pool areas in a...
high altitude stream reach (but not building and/or living in burrows like Ephemeridae - Merritt & Cummins (1996)).

About the use of the terms Active or Passive Filters, in terms of river function both groups are probably feeding on particles about the same size. However, Passive Filters would contribute to organic particle retention in one stream reach while the Active Filters (that would be assigned to the Collector-gatherer FFG in the Merritt & Cummins (1996) classification scheme), when resuspending organic particle, would contribute to mobilization of these particles. Therefore, this information could be an indicative of organic matter cycling in streams.

The species of the family Baetidae are frequently assigned to the Collector-gatherer or Scrapper FFG. According to Merritt & Cummins (1996), Collector-gatherers feed on deposited decomposing organic matter, while Scrapers feed on periphyton-attached algae and associated material. Arens (1989) stated that head posture determines to a great extent the manner in which grazing insects can use their mouthparts to harvest algal pastures. In orthognathous Ephemeroptera, like baetids, only the tips of the mouthparts come into contact with the plain surfaces of the stones. Consequently, no mouthpart is especially suited to serve as a scraping organ because of its position.

In this study, based on mouthparts, all baetid species were assigned to the Collector-gatherer FFG category due to their complex buccal apparatus and long labial palps that may be used to gather and manipulate organic matter. This general morphologic plan is found in other neotropical baetid species (Lugo-Ortiz & McCafferty 1996a, Lugo-Ortiz & McCafferty 1997, Waltz & McCafferty 1999). Some Baetidae species found have specialized labial palp segment 2 with a moderate, distomedially acute process, highly adapted to manipulate food (Lugo-Ortiz & McCafferty 1995, Lugo-Ortiz & McCafferty 1996b; Dominique et al. 2000). Therefore, based on these characteristics baetids probably would be assigned to the collector-gatherers FFG, instead of scrapers FFG.

However, studies on exclusion of baetids conducted in south-east Brazil indicates that these species have a strong negative effect on the quantity of periphyton (Moulton et al. 2004), suggesting that these species are important herbivores in this region. Corroborating this, our baetid species occurred predominantly in stony substrates.

Villanueva & Albariño (2003) studying the ingestion and digestion of one species of Baetidae and one species of Leptophlebiidae from a Patagonian Andean stream stated that since baetid nymphs had unspecialized mouthparts, the toothed-tips of both mandible and galea-lacinia acted as reaper more than as scraping devices. Once they found a high proportion of five periphyton cells ingested by the baetid nymph, and since the proportion of live algal cells is higher in the upper layer than in the inner zone, probably this species harvested only the upper layer of periphyton.

In accordance with these findings, species of Baetidae found in this study were assigned to the Grazer FFG, because they are able to use the tip of its mouthparts (similarly to the Collectors FFG as described by Arens 1989), to remove periphyton (same general food resource as the scrapers FFG, based on the resource partitioning idea of Cummins 1973).

In this study, species of Leptophyphidae were assigned to the Scraper FFG. The species Leptophyphes pereirae, Leptophyphes spp., Tricorythodes spp., Tricorythopsis spp. had mouthparts similar to those described in Palmer et al. (1993) as scrapers. According to Arens (1989), prognathous insects, like the leptohyphids in our study, press their mouthparts almost horizontally against the surface of the stones, so that larger areas of their labium and maxillae come into contact with algal pastures, therefore, prognathous insects should be able to graze down algal pastures more quickly than orthognathous species.

Scrapers are usually better at feeding on low-profile algae than those species with collector-gatherer mouthparts (Hill & Knight 1988). Therefore, although grazers and scrapers feed on periphyton, these two groups are probably not feeding exactly on the same kinds of periphyton.

In summary, our study allowed some generalization related to the assignment of mayfly species to FFGs. Mouthparts morphology may limit its function for obtaining specific food resources. Based on general morphology of mouthparts, we speculate that species of Baetidae and Leptophyphidae studied probably can not perform brushing and filtering activities, since they lacked brushing and filtering setae. Leptophlebiid species studied (except for the passive-filterer Hylister plaumanni) had complex mouthparts with setae that would allow filtering and brushing activities and labial and maxillary palps that would allow gathering activities.

As a general pattern, species of the three best represented mayfly families in south-east Brazil could be assigned to different FFGs (Baetidae - Grazers; Leptophlebiidae - Brushes (except for H. plaumanni) and...
Leptohyphidae - Scrapers). This information could be useful because although it is important to identify organisms to the genus or species taxonomic level, it is always difficult to do so with neotropical fauna.

Our study also showed the importance of recording the substrate in which specimens were collected. This information could be used when determining the roles macroinvertebrates play in the stream, especially because behavioral information is difficult to collect and is often missing.

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