Feeding Habits of Plecoptera Nymphs from the Atlantic Forest, Southeast Brazil

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ABSTRACT
Plecoptera constitute a numerically and ecologically significant component in freshwater ecosystems. Feeding habits of Plecoptera nymphs from the Campos do Jordão State Park have been studied. The gut contents of 108 nymphs belonging to six genera were examined in order to ascertain their feeding preferences. Nymphs of each genus were separated into three size classes. Diet of Anacroneuria and Kempnyia was composed mainly of Simuliidae, Chironomidae, Baetidae and Hydropsychidae larvae. Diet varied in relation to different size classes, from detritus and chironomids in the earliest instars, to primarily simulids, to a broader diet in which mayflies and caddisflies increased in importance relative to other prey. The diets of Gripopteryx, Guaraniperla, Paragripopteryx and Tupiperla nymphs were similar, comprising both algae and particulate organic matter associated with the microbiota. This result emphasizes the importance of periphyton as a direct food source for these taxa. Cluster analysis based on the stomach contents grouped the nymphs into the two major feeding groups. Diet analyses show that Anacroneuria and Kempnyia are mainly predatory, while gripopterygids fed mainly on periphyton.

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INTRODUCTION

The Atlantic Forest is one of the biologically richest and most threatened ecosystems of the planet (Myers et al., 2000). Rivers that flow through this ecosystem contain high levels of endemism (Brown and Brown, 1992). The “Serra da Mantiqueira” is a mountain range covered with a mosaic of Atlantic Rain Forest, Araucaria Forest and grassland in the highest areas, with a predominance of Atlantic Rain Forest on the Paraiba River Valley side (Seibert, 1975).

Aquatic insects are important elements in the ecological dynamics of lotic environments (Hynes, 1970) playing an important role in the cycle of materials and in trophic transfers (Cummins, 1974). In particular, the analysis of the diet and the study of trophic ecology improve the comprehension of many ecological processes in lotic ecosystems (Albariño, 2001). Among aquatic insects, Plecoptera constitute a numerically and ecologically significant component in freshwater ecosystems, mainly in running waters of all sizes, all over the world (Zwick, 2004), being mainly associated with clean, cool, running waters. In the tropical area, many taxa are limited to mountainous and/or forested areas.

Within this order, only the families Perlidae and Gripopterygidae occur in Brazil (Froehlich, 1981). Perlidae nymphs represent one of the most important groups of invertebrate predators within the benthic community that live on or in stream substrates (Gamboa et al., 2009). On the other hand, most of the Gripopterygidae nymphs are primarily grazers, some are shredders or collector-gatherers (Froehlich, 2009).

The aim of this study was to investigate the diet of the nymphs of the eight Plecoptera genus of Campos do Jordão State Park, São Paulo State.
MATERIALS AND METHODS

Study area:
This study was carried out in a stream of Campos do Jordão State Park. The Park is situated in the “Serra da Mantiqueira”, Campos do Jordão municipality, São Paulo State, Brazil (22° 30’ to 22° 41’S and 45° 27’ to 45° 31’W). Its area is approximately 8,385 ha. Altitudes vary from 1,030 to 2,007 m. The average altitude is 1,650 m. This region has a subtropical of altitude climate, without dry season. In general, annual rainfall rates are above 1,800 mm, and with the average temperature in the hottest month lower than 22°C. Rainfall is distributed differently during the year; January and February were the rainiest months, and July was the driest month.

Collections and identification:
Samplings were carried out in the Galharada stream (22°41’40”S 45°27’36”W) a 3rd order stream (Figure 1), characterized by clear and well-oxygenated waters, neutral pH and low values of turbidity, electrical conductivity and water temperature (Schroeder-Araujo et al., 1986).

Fig. 1: Map showing the location of the study area: A) Brazil; B) PECJ in the northeastern of São Paulo state (SP); C) protected area by PECJ; in red, D) location of Galharada stream in PECJ.

Samplings were collected between August 2005 and February 2007. Nymphs were obtained with a Surber sampler (0.0361 m2 of area and 0.25 mm mesh), and fixed with 4% formalin. In the laboratory, nymphs were identified to genus using the keys in Froehlich (1984).

The total length of the nymphs was measured (0.1 mm accuracy), and specimens of each genus were separated into three size classes (small, medium and large), according to the range of body length of each genus (Table 1). The gut content of six individuals of each size class was analyzed; totaling 18 specimens examined per genus.

Table 1: Size classes (mm) of the six plecopteran genera sampled for analysis of stomach contents.

<table>
<thead>
<tr>
<th>Total range</th>
<th>Small</th>
<th>Medium</th>
<th>Large</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gripopteryx</td>
<td>0.6 – 13.0</td>
<td>0.6 – 4.6</td>
<td>4.7 – 8.8</td>
</tr>
<tr>
<td>Guaranyperla</td>
<td>1.0 – 11.0</td>
<td>1.0 – 3.7</td>
<td>3.8 – 7.5</td>
</tr>
<tr>
<td>Paragripopteryx</td>
<td>1.0 – 6.0</td>
<td>1.0 – 2.6</td>
<td>2.7 – 4.3</td>
</tr>
<tr>
<td>Tupiperla</td>
<td>1.0 – 9.4</td>
<td>1.0 – 3.8</td>
<td>3.9 – 6.7</td>
</tr>
<tr>
<td>Anacroneuria</td>
<td>1.0 – 16.0</td>
<td>1.0 – 6.0</td>
<td>6.1 – 11.0</td>
</tr>
<tr>
<td>Kempnyia</td>
<td>1.0 – 21.0</td>
<td>1.0 – 7.6</td>
<td>7.7 – 14.3</td>
</tr>
</tbody>
</table>

The foreguts were removed and the contents squashed onto a microscope slide. They were then mounted in glycerin and examined at magnifications of 200 x and 400 x. Six major diet categories were recognized: particulate organic matter, filamentous algae, unicellular algae, terrestrial animals, aquatic animals, and vascular plants.

For each specimen, the gut content was measured qualitatively according to the occupied area by this item in the gut (e.g. Froehlich and Oliveira, 1997; Tamaris-Turizo et al., 2007). Therefore, the food items were grouped into three categories: occasional (less than 1/3 of the content), frequent (between 1/3 and 1/2 content) and very frequent (more than half the content), following Tamaris-Turizo et al., (2007).
The feeding similarity between the Plecoptera genera was determined by cluster analysis, using the UPGMA method and Jaccard index (NTSYS 2.1, Rohlf, 2000). The dendrogram distortion was evaluated by the cophenetic correlation coefficient (Romesburg, 1984). That coefficient was obtained correlating the original similarity matrix with the matrix obtained from the dendrogram; \( r \geq 0.8 \) is considered a good value (Rohlf, 2000).

**Results:**

The gut contents of nymphs of six Plecoptera genera were examined. The diets of the gripopterygid genera were similar, comprising detritus and algae as important components, followed by plant material and fungi (Table 2, Figure 2). The diets of perlid nymphs although similar, were more varied and consisted mainly of larvae of Diptera and Trichoptera (Figure 3). Plant material, fungi, algae and detritus were poorly represented in these specimens (Table 3, Figure 3).

![Fig. 2: Some items food found in the stomach of Gripopterygidae nymphs. A. insect remain (10X); B. vegetable (10X); C. detritus (10X); D. algal fragments (10X).](image)

The diets among nymphs of different size were similar in each Gripopterygidae and Perlidae. In Gripopterygidae no major differences were noticed, so all size classes were pooled. However, in Perlidae, larger nymphs included larvae of Leptophlebiidae and Baetidae (Ephemeroptera) and Hydropsychidae (Trichoptera) in their diets, and reduced occurrences of the larvae of Diptera, especially Chironomidae and Simuliidae.

![Fig. 3: Some items food found in the stomach of Perlidae nymphs. A. stonefly (10X); B. chironomids (10X); C-D. caddisflies (10X); E-F. simuliids (10X).](image)
The cluster analysis dendrogram, based on composition percentage of the food items defined two groups (Figure 4) with a good data adjustment, represented by the cophenetic correlation value, r = 0.96. The first group, predators, was represented by *Anacroneuria* and *Kempnypia*. The second one, scrapers, mainly periphyton feeders, included the *Gripopteryxidae*. However, there was no clear separation among the different size classes or genera analyzed.

**Table 2:** Stomach contents of the *Gripopteryxidae* nymphs collected in stream Galharada between August 2005 and February 2007. (+ = occasional, ++ = common, +++ = very common).

<table>
<thead>
<tr>
<th>Size (mm)</th>
<th>Small</th>
<th>Medium</th>
<th>Large</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vegetable</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Algal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fungi</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Detritus</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Mineral</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
</tbody>
</table>

**Table 3:** Stomach contents of the *Perlidae* nymphs collected in stream Galharada between August 2005 and February 2007. (+ = occasional, ++ = common, +++ = very common).

<table>
<thead>
<tr>
<th>Size (mm)</th>
<th>Small</th>
<th>Medium</th>
<th>Large</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vegetable</td>
<td></td>
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</tr>
<tr>
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<td></td>
<td></td>
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<tr>
<td>Fungi</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Detritus</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Mineral</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
</tbody>
</table>

**Discussion:**

Stonelody nymphs as a group consume a wide array of food material, and individual species are somewhat eclectic in their diet (Froehlich, 2009). By their gut contents, *Gripopteryxidae* nymphs showed a preference for periphyton, a mixture of algae, other microbiod and organic matter, by all larval sizes. Similar results were obtained for Brazilian nymphs of *Gripopteryx* and *Paragripopteryx* species which fed primarily on periphyton (Froehlich and Oliveira, 1997, Motta and Uieda, 2004). Similarly, Villanueva and Albariño (1999) found diatoms to be dominant item in the gut content of *Notoperla archiplaeta* (*Gripopterygidae*). However, nymphs of *Paragripopteryx* and *Tupiperla* skeletonized leaves and also fed on moss and algae, but small nymphs had mostly diatoms in their guts (Froehlich, 1969).

Several authors (Cummins and Klug, 1979; Allan and Castello, 2007) have emphasized the importance of periphyton as a direct food source for many taxa. Mihuc and Minshall (1995) found that periphyton presents a high nutritional quality, and was used for growth by 7 of the 11 taxa experimentally studied by those authors in North America.
On the other hand, perlid nymphs showed a preference for animal items. These items are used by several insect orders and are considered the highest-quality source of calories and protein as well as having high diversity in streams (Cummins and Klug, 1979). Feeding patterns of members of the family agree with those observed by Dorvillé and Froehlich (2001), who found that *Kempnyia tijucana* had a carnivorous diet. Similarly, Froehlich and Oliveira (1997); Tomanova *et al.* (2006); Tamaris-Turizo *et al.* (2007) and Carvalho and Uieda (2009) found the diet of perlids to be mainly carnivorous.

The diet of the two perlid genera studied was dominated by larvae of Diptera (Simuliidae and Chironomidae), Ephemeroptera (Baetidae) and Trichoptera (Hydropsychidae). Similar results showing the importance of these orders in the diet of Plecoptera have been obtained in studies of different Systellognathan stonefly families in temperate zones (Fuller and Stewart, 1977; Allan, 1982; Peckarsky and McIntosh, 1998; Fenoglio, 2003). In Venezuela, Gamboa (2009) found that the diet of four *Anacroneuria* species also was dominated by these insect orders. For other species of *Anacroneuria* from southeastern Brazil, chironomids were an important component in their diets (Carvalho and Uieda, 2009).

The preference for Simuliidae and Chironomidae by perlid nymphs may be facilitated by the fact that these dipterans are both soft bodied and relatively slow movers, and are thus less likely to escape attacks (Gamboa *et al.*, 2009). This limited ability to escape together with the opportunistic strategy of the predators could make them the most vulnerable prey for active predators such as stoneflies (Resh and Rosenberg, 1984; Malmqvist, 1993; Allan and Castillo, 2007). Baetidae nymphs escape predation more easily by entering the drift, but they are usually abundant and so often captured. For the predator, they are useful prey, with their soft body and easy digestion (Allan, 1982; Allan *et al.*, 1987; Allan and Flecker, 1988; Tikkanen *et al.*, 1997).

The variation between the diet in relation to different size classes was also reported by Tamaris-Turizo *et al.* (2007), who studied the diet of juvenile and mature nymphs of four *Anacroneuria* morpho-species in Colombia. Fenoglio (2003) reported changes in the preference of food items between different instars, small nymphs classified as detritivores and larger one as carnivores. Allan (1982) reported that the diet of different species of predatory Plecoptera also changed during development, from diatoms and chironomids in the first instars, mainly chironomids, a diet in which there was an increase of the relative importance of Ephemeroptera.

The changes in herbivore-detritivores in the early stages for omnivores-carnivores in the following periods are common (Stewart and Harper, 1996; Albariño, 2001). In the early stages, many species consume fine particulate organic matter, as collectors-gatherers, because of the limitation on the size of their mouthparts (Merritt and Cummins, 1996), in the final stages, as they mature, some species of herbivore-detritivores become predators (Lamberti and Moore, 1984).

These changes are due to size, as young nymphs can only eat small food items or small prey such as chironomids and small simulids, while as they grow larger, they include larger prey as Ephemeroptera, in the diet (Tamaris-Turizo *et al.*, 2007). Because adults do not eat solid foods (Fenoglio and Tierno de Figueroa, 2003; Froehlich, 2009), they need to store their energy needs for adult life with a rich diet in the pre-imaginal stages (Fenoglio, 2003).
The diets of the nymphs of the four gripopterygid genera proved to be very similar at this level of analysis (Table 2). Despite differences observed in food composition in different size classes, the cluster analysis showed no differences among size classes or genera of the perlids (Figure 4). Further studies should be done on niche preferences of nymphs of both families.

**Conclusion:**
According to the taxa included in this analysis and results of previous authors, Perlidae can be classified as carnivores and Gripopterygidae as herbivore-detritivores. Ultimately it is possible to notice a phylogenetic segregation also in the diet of these two families according to the classification of Zwick (2000).

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