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Using Baetidae Species as Biological Indicators of Environmental Degradation in a Brazilian River Basin

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Abstract The Ephemeroptera family Baetidae is one of the most specious families in the Neotropical region and a great effort to improve the taxonomy of this group has been made over the last 10 years in South America. Such studies now enable the development of biomonitoring tools at species-level in the region. A total of 2,199 baetids were collected from seven sampling sites, collected three times (autumn, winter and summer), representing an environmental gradient, draining an area of Atlantic Forest remnants. We describe the mesohabitat of nine Baetidae species and evaluate their responses to environmental degradation and water chemistry by means of biological measures (richness and abundance) and multivariate analysis (Canonical Correspondence Analysis), in order to assess their potential capacity as indicators of these impacts. Most species were found predominantly associated with stony substrates, but some were associated with pool litter, and one species was found predominantly in riffle litter substrate. Species distribution was influenced by the environmental gradient. Based on the CCA ordination, we were able to identify which species were found in pristine *versus* the most impaired areas, therefore enabling us to establish the sensitivity of each species.

Keywords Baetidae · Bioassessment · Bioindicator · Brazil · Ephemeroptera

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1 Introduction

Biological monitoring (biomonitoring) is a central component of water resource management throughout the world (Rosenberg & Resh, 1993; Barbour, Gerritsen, Snyder, & Stribling, 1999). The systematic development and testing of rapid bioassessment tools on river basins using benthic macroinvertebrates in Brazil is recent (Buss, Baptista, Silveira, Nessimian, & Dorvillé, 2002; Buss, 2001; Callisto, Esteves, Goncalves, & Fonseca, 1998; Silveira, Buss, Nessimian, Egler, & Baptista, 2005), and due to limited knowledge of the taxonomy and distribution of macroinvertebrates in Brazil, these studies have generally used supraspecific taxonomic levels.

Much effort to improve the taxonomy of some of these groups has been made in the last years in South

America, allowing new applications of biomonitoring in the region. The Ephemeroptera family Baetidae was one of the most studied of these groups (Francischetti, Salles, Lugo-Ortiz, & Da-Silva, 2003; Lugo-Ortiz & McCafferty, 1995, 1996a, 1996b, 1996c, 1997, 1998; Lugo-Ortiz, Salles, & Furieri, 2002; McCafferty & Lugo-Ortiz, 1995; Salles & Batista, 2004; Salles, Batista, & Cabette, 2004; Salles, Da-Silva, & Lugo-Ortiz, 2003; Salles & Dias, 2004; Salles & Francischetti, 2004; Salles, Francischetti, Roque, Pepinelli, & Strixino, 2003; Salles & Lugo-Ortiz, 2002a, 2002b, 2003a, 2003b; Salles, Lugo-Ortiz, & Da-Silva, 2004; Salles, Lugo-Ortiz, Da-Silva, & Francischetti, 2003), which allowed the development of taxonomic keys for use by non-specialists (Domínguez, Hubbard, Pescador, & Molineri, 2001; Salles, Da-Silva, Serrao, & Francischetti, 2004).

The family Baetidae was used in this study as bioindicator of anthropogenic impacts because: (1) it is, together with Leptophlebiidae, the most specious Ephemeroptera family, with approximately 20% of all Ephemeroptera species, increasing the spectrum of responses to different types of impact; (2) it is one of the most abundant macroinvertebrate groups in this river basin (Buss et al., 2002); (3) its nymphs are found in a wide variety of substrates, also increasing the chance of detection of impacts (Buss, Baptista, Nessimian, & Egler, 2004); (4) it is one of a very few groups which taxonomy is based on its immature form, what allows the identification without the need of association with the adult form; (5) in the last 10 years it was one of the most studied mayfly family in the neotropical region; (6) since most of these studies was concentrated in the Brazilian south-east region, Baetidae taxonomy is well known in this region.

The aim of this paper is to describe the mesohabitat of Baetidae species and to evaluate their responses to environmental degradation and water chemistry by means of biological measures (richness and abundance) and multivariate analysis (Canonical Correspondence Analysis), in order to assess their potential capacity as indicators of these impacts.

2 Materials and Methods

2.1 Study area

This study was carried out in the municipality of Guapimirim (22°32′14″ S, 42°58′55″ W), State of Rio de Janeiro, Brazil. This region is ecologically important due to its three conservation areas, Parque Nacional da Serra dos Órgãos, Estação Ecológica do Paraíso, and Área de Proteção Ambiental Guapimirim, all of which carry Atlantic Forest remnants. The main river draining this region is the Guapimirim River. This river is used as water supply and is also important due to its flow into the Guanabara Bay, which holds the largest mangrove ecosystem in the State. Accelerated deforestation and the absence of adequate wastewater treatment are the major environmental problems in the region. Most domestic wastes from the city of Guapimirim (around 43,000 inhabitants) enter untreated in the three main tributaries of the Guapimirim River.

Baetidae species, as well as physical, chemical and environmental variables were sampled at seven sites on three tributaries of the Guapimirim River. Six sites were third order streams and one a fourth order, and all sites were at altitudes between 40 and 100 m a.s.l. The furthest distance between sites was 4.5 km to avoid differences in climate and rainfall. Sites were chosen to represent an environmental gradient from a pristine area (site A) to most impaired (site G). In a previous work, Buss et al. (2002) showed that these sites were divided among four classes, and concentrations of dissolved oxygen, chloride, and the environmental degradation, measured by the Riparian Channel Environment index (Petersen, 1992), exhibited the strongest relationship to macroinvertebrate assemblages. The four classes were: site A (Class 1), sites B, C, D (Class 2), sites E, F (Class 4), and site G (Class 5). Site A was located at Parque Nacional da Serra dos Órgãos, a National Park, upstream from the city of Guapimirim. This site was chosen as a reference because of its dense riparian vegetation (75% stream cover) and the absence of anthropogenic stressors upstream. Despite natural retention mechanisms like logs and boulders and high availability of leaf and wood from marginal vegetation, the frequent spates made litter substrates hard to find at this site at all sampling periods. Class 2 sites had riparian forest (around 15 m wide) and natural mechanisms of retention such as stones and logs. Class 4 sites were located in the urban area. At these sites the river was artificially channeled, silt was common, and the riparian vegetation was scarce, composed mainly of pioneer trees and shrubs. Class 5 site was located downstream from the city, received gross pollution,

was channeled, had no riparian vegetation, and the river bed was dominated by silt and few stones partially covered with fine sediments. Another characteristic of all the sites was the occurrence of spates, mostly during summer – from December to April – because headwaters are located at about 2,000 m a.s.l. at a high slope.

2.2 Field and laboratory procedures

A Surber sampler (mesh size 125 mm, 0.09 m^2 area) was used to collect Baetidae species. On each sampling occasion, three samples of each substrate were collected (stones and riffle litter in riffle areas, sediment and pool litter in pool areas) at each site, in order to represent distinct patches of each substrate. At site G, litter substrates were absent at all sampling periods. The three samples of each substrate were then combined, forming four composite samples per site per sampling occasion. Samples were preserved in 80% ethanol and packed for examination in the laboratory. Sampling was performed on three occasions: at the end of the wet season (May 1998), during the dry season (August 1998) and during the wet season (January 1999).

Each time a biological sample was taken, water was sampled in order to measure further physical and chemical variables in laboratory: conductivity, alkalinity, hardness, NH_4-N, NO_3-N, PO_4-P, Cl^-, Dissolved Oxygen, and Biochemical Oxygen Demand. The habitat of each site was assessed at each sampling period, based on the Riparian Channel Environment index (RCE; Petersen, 1992).

The baetid genera were identified using a taxonomic key for Southeastern Brazil (Salles et al., 2004), while the species were identified using the following papers: Traver and Edmunds (1968); Waltz and McCafferty (1987); Lugo-Ortiz and McCafferty (1996b, 1996c, 1998), and Salles and Francischetti (2004).

2.3 Data analysis

The structure of the Baetidae assemblage was evaluated by species richness and abundance. A Canonical Correspondence Analysis (CCA) was performed to determine relationships between environmental variables and the respective biotic components. In the CCA, we included the environmental and water chemical parameters considered significantly correlated to the macroinvertebrate community in this river basin (Buss et al., 2002). Environmental data were standardized, therefore reducing the effects of different scales of measurement in different characters (Manly, 1986). Biological data were tested for normality (Kolmogorov-Smirnov test) and $\log_{10}(x+1)$ transformed to achieve the assumed conditions of normality and homocedasticity of the data (Sokal & Rohlf, 1995).

3 Results

A total of 2,199 individuals corresponding to nine baetid species were recorded in this study: *Americabaetis alphus* Lugo-Ortiz and McCafferty, 1996c, *Americabaetis labiosus* Lugo-Ortiz and McCafferty, 1996c, *Baetodes* sp., *Camelobaetidius anubis* Traver and Edmunds, 1968, *Camelobaetidius* sp., *Cloeodes irvingi* Waltz and McCafferty, 1987, *Cryptonympha dasilvai* Salles and Francischetti, 2004, *Paracloeodes eurybranchus* Lugo-Ortiz and McCafferty, 1996b and *Zelusia principalis* Lugo-Ortiz and McCafferty, 1998.

The temporal structure of the baetid assemblage was similar among the three sampling periods: at the end of wet season, baetids were found in 17 composite samples (of a maximum of 26), in 18 at the dry season, and in 15 at the wet season. The total number of individuals was highest at the dry season (826 individuals), intermediate at the end of the wet season (734 individuals) and lowest at the wet season (639 individuals). The most abundant species was *Baetodes* sp. with 56% of the individuals collected.

3.1 Substrate preference of Baetidae species

Most species were found predominantly associated with stony substrates (*Americabaetis labiosus*, *Baetodes* sp., *Camelobaetidius anubis*, *Camelobaetidius* sp., *Cloeodes irvingi* and *Cryptonympha dasilvai*), but some were predominantly associated with pool litter (*Paracloeodes eurybranchus* and *Zelusia principalis*), and one species was found predominantly in riffle litter substrate (*Americabaetis alphus*). No species were abundant in fine sediment, and most species were not found in this substrate (Table I).

Table I Baetid species percent per substrate and total number of individuals collected in the seven sampling sites in the Guapimirim river basin

	Riffle litter (%)	Stones (%)	Pool litter (%)	Sediment (%)	No. of individuals
Americabaetis alphus	71.1	27.76	0.28	0.85	353
Americabaetis labiosus	5.00	95.00	0	0	20
Baetodes sp.	12.81	86.94	0.08	0.16	1,233
Camelobaetidius anubis	4.63	94.04	1.32	0	151
Camelobaetidius sp.	2.40	97.60	0	0	208
Cloeodes irvingi	3.77	66.04	30.19	0	53
Cryptonympha dasilvai	3.63	94.55	0.91	0.91	110
Paracloeodes eurybranchus	14.52	17.74	66.13	1.61	62
Zelusia principalis	33.33	11.11	55.56	0	9
Total no. of individuals					2,199

3.2 Site preference of Baetidae species

Baetid species richness followed the gradient of environment and water chemistry degradation in the Guapimirim river basin. All nine baetid species were associated to sites of classes 1 and 2, and two species (18 individuals of *Americabaetis alphus* and one individual of *Paracloeodes eurybranchus*) were found in the most impaired site. Class 4 sites had species richness within this range (four to six species). Species abundance were higher in sites of intermediate integrity, at site C (28.38% of the total abundance), site F (24.47%), and site E (19.51%) (Table II).

In order to determine which environmental parameters were associated with baetid species distribution, a Canonical Correspondence Analysis (CCA) was performed. CCA first and second axes were significant according to the broken-stick model and accounted for 23% and 16.3% of the total variance explained, respectively, and correlation between matrices were 0.910 with first axis and 0.943 with second axis. According to the CCA ordination (Figure 1), the species *Americabaetis labiosus*, *Zelusia principalis*, and to a lesser extent the species *Cloeodes irvingi* and *Paracloeodes eurybranchus*, were associated to class 1 site samples (A1-3) and were positively correlated to the RCE index. The upper portion of the ordination represented sites with high biochemical oxygen demand and high concentration of hardness, conductivity, alkalinity and low concentration of dissolved oxygen. The species *Americabaetis alphus* was the

Table II Percent of occurrence of each baetid species at each sampling site, total number of individuals, and richness numbers collected at each sampling site in the Guapimirim river basin

	Class 1	Class 2			Class 4		Class 5	
	A	B	C	D	E	F	G	Total
Americabaetis alphus	1.70	13.03	4.53	10.20	8.50	56.94	5.10	353
Americabaetis labiosus	65.00	25.00	5.00	5.00	0	0	0	20
Baetodes sp.	9.16	5.11	33.74	4.70	29.36	17.92	0	1,233
Camelobaetidius anubis	49.67	1.32	6.62	13.25	21.85	7.28	0	151
Camelobaetidius sp.	8.17	2.40	67.31	0.48	1.92	19.71	0	208
Cloeodes irvingi	22.64	9.43	20.75	45.28	0	1.89	0	53
Cryptonympha dasilvai	0.91	19.09	21.82	0.91	0	57.27	0	110
Paracloeodes eurybranchus	16.13	11.29	8.06	62.90	0	0	1.62	62
Zelusia principalis	55.56	22.22	11.11	11.11	0	0	0	9
Total no. of individuals	252	156	624	181	429	538	19	
Taxa richness	9	9	9	9	4	6	2	

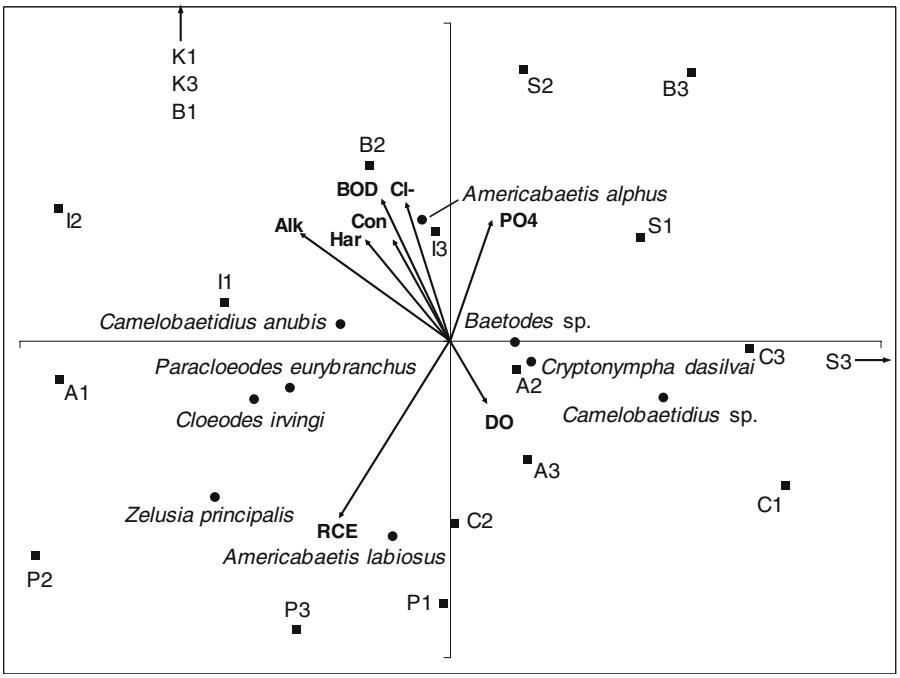

Figure 1 CCA graph of axes 1 and 2 showing sites (*A–G*), environmental variables and Baetidae species. Numbers after site codes represent sampling period (1, May; 2, August; 3, January).

only species associated with these conditions. Species associated to sites of intermediate environmental conditions (classes 2 and 4) were located on the positive portion of axis 1: *Baetodes* sp., *Cryptonympha dasilvai*, and *Camelobaetidius* sp.

4 Discussion

4.1 Substrate preference of Baetidae species

In this study, six baetid species were found predominantly in stony substrates, two in pool areas and one in litter in riffle areas. This frequent association with stony substrates may be explained by their main food source. Baptista et al. (2006) described the functional feeding of four Baetidae genera (*Americabaetis* spp., *Baetodes* spp., *Camelobaetidius* spp., and *Cloeodes* spp.). They described these species as having a complex buccal apparatus and long labial palps that may be used to gather and manipulate organic matter. Those species were assigned to the Grazer Functional Feeding Group. Studies on exclusion of baetids conducted in south-east Brazil indicate that these species have a strong negative effect on the quantity periphyton and sediment (Moulton, Souza, Silveira, & Krsulovic, 2004), suggesting that these species are important herbivores in this region.

Both species of *Camelobaetidius*, *Baetodes* sp., *Americabaetis labiosus* and *Cryptonympha dasilvai* occurred preferentially in stony substrates and exhibit morphologic adaptations to resist hydraulic stress such as the presence of tubercles in the abdomen of *Baetodes* sp., reduction of the medium filament in *Baetodes* sp. and *Camelobaetidius* sp., spatulated claws in both species of *Camelobaetidius* and robust legs and claws with numerous denticles in *A. labiosus* and *C. dasilvai*. Salles et al. (2003) found nymphs of *C. dasilvai* (as *Cryptonympha* sp.) in riffle areas, corroborating that this species probably occurs preferentially in this habitat. Unfortunately, there is no available information about the habit or habitat preference of *Cryptonympha copiosa* Lugo-Ortiz and McCafferty, 1998, the only other species of this genus.

The species *Cloeodes irvingi* was found associated to stony substrates (66%), and to a lesser extent to litter in pool areas (30%). The species *C. irvingi*, in contrast to the other species that occupied stony substrate, do not show morphologic characteristics to resist fast water current (on the contrary, nymphs have narrow legs and claws without denticles). The majority of South American *Cloeodes* species occupy slow water current or backwaters (Lugo-Ortiz et al.,

Table III Summary of the preferred substrate, morphologic adaptations and tolerance to the measured stress of the nine Baetidae species analyzed in Guapimirim river basin

Species	Preferred substrate	Habit and morphologic adaptation to live in the substrate	Tolerance to the measured stress
Americabaetis alphus	Litter in riffle areas	Clingers; live between leaves; small body	Tolerant
Americabaetis labiosus	Stone face	Clingers; stout legs and claws with numerous denticles	Very sensitive
Baetodes sp.	Stone face	Swimmers/clingers; tubercles on top of abdomen help swimming; hydrodynamic body	Somewhat sensitive
Camelobaetidius anubis	Stone face	Swimmers/clingers; setae in the cerci help swimming, spatulated claws used for clinging	Somewhat sensitive
Camelobaetidius sp.	Stone face	Clingers; spatulated claws used for clinging	Somewhat sensitive
Cloeodes irvingi	Stone bottom/pool areas	Swimmers/clingers; creep behind stones	Sensitive
Cryptonympha dasilvai	Stones with moderate water current	Clingers; stout legs and claws with numerous denticles	Somewhat sensitive
Paracloeodes eurybranchus	Pool areas	Swimmers/clingers; legs not stout, tarsal claws with denticles poorly developed	Sensitive
Zelusia principalis	Riffle/pool	Swimmers/clingers; legs not stout, tarsal claws with denticles poorly developed	Very sensitive

2002; Nolte, Tietbohl, & McCafferty, 1996; Salles & Lugo-Ortiz, 2003b; Salles, et al., 2004). In-field observations suggest that *C. irvingi*, when associated to stony substrates, live in areas less exposed to the water flow.

The species *Americabaetis alphus*, differently from *A. labiosus*, was found predominantly in litter in riffle areas. These findings are in accordance with Francischetti, Salles, Da-Silva, & Nessimian, (2004), when studying biological aspects of these species. *A. alphus* was associated to areas with slow water flow, frequently living beneath litter, while the more robust species *A. labiosus* is more often found associated to stony substrates influenced by faster water current.

Our results showed that *Zelusia principalis* and *Paracloeodes eurybranchus* were often associated with pool substrates, but they were also found in riffle areas. Little is known about the biology of both species, but in-field observations indicate that species of *Paracloeodes* seems to be more often associated to pool areas.

4.2 Site preference of Baetidae species

Since this is the first approach to using baetid species to measure anthropogenic impact in streams in Brazil, there is little information about their sensitivity to impacts. The higher abundance of baetids in intermediately impaired areas corroborate the classification of the family Baetidae as 'somewhat sensitive' in biotic indices worldwide (Armitage, Moss, Wright, & Furse, 1983; Hilsenhoff, 1988). However, considering taxa richness, all nine species occurred in sites of classes 1 and 2, while richness in the most impaired site was low (and often with low abundance). Therefore, taxa richness of Baetidae, in spite of being a simple parameter, was a valid indicator of water quality. In this study it was possible to verify that not all species have the same response to impacts, and it was possible to identify which species were associated to unimpaired and impaired areas.

Based on the distributional pattern of baetid species and as verified in the CCA ordination, species could be assigned to one of the five tolerance classes: 'Very sensitive' – for those restricted to unimpaired sites; 'sensitive' – for those predominantly associated to areas of classes 1 and 2 (high RCE values and dissolved oxygen concentration); 'somewhat sensitive' – for those well represented in classes 2 and 4 sites; 'tolerant' – for those found with high abundance in sites of Class 4 and the most impaired site (G); and 'very tolerant' – for those restricted to impaired sites.

No baetid species found in this study was assigned to the 'very tolerant' class. Since these species are frequently associated with stony substrates, in this study one of the main factors restricting baetid distribution was the high sedimentation observed in sites of classes 4 and 5. Tolerance classification, together with information on the preferred substrate of each species, is a first approach towards a biological monitoring program considering species-level in Neotropical region (Table III).

In a time where rapid bioassessment tools are growing in importance, analyses at family or order-level are often preferred, because of difficulties on taxonomy and better cost-effectiveness ratio (Resh, 1995; Barbour et al., 1999). However, information on lower taxonomic levels allows complementary refined results for specific responses, especially in the chronically misleading 'intermediately impaired sites.' The advantages of using a species-level approach were clear with the two species of *Americabaetis*. We verified that these species occupy different substrates (*A. labiosus* occurred in stony substrates, while *A. alphus* occurred in litter in riffle areas), and have different tolerance to the measured stress (90% of individuals of *A. labiosus* occurred in the two least impaired sites, while *A. alphus* occurred with higher abundance at intermediately impaired sites and seem to be the only baetid species able to live in the most impaired site in this study).

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