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Feeding ecology of *Rivulus luelingi* (Aplocheiloidei: Rivulidae) in a Coastal Atlantic Rainforest stream, southern Brazil

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Feeding habits of the killifish *Rivulus luelingi* collected in a black water stream of the Coastal Atlantic Rainforest in southern Brazil were investigated. Eight samplings were made between April 2003 and January 2004. The diet, assessed through a similarity matrix with the estimated contribution values of food items, included microcrustaceans, aquatic immature insects (larvae and pupae), aquatic adult insects, terrestrial insects, insect fragments, spiders, and plant fragments. Differences in the diet according to temporal variations (months) were registered, but changes related with size classes evaluated and high/low precipitation period were not observed. The species presented an insectivorous feeding habit, and its diet in the studied stream was composed of autochthonous (mainly aquatic immature insects) and allochthonous (mainly insect fragments) material.

Neste estudo foram investigados os hábitos alimentares do peixe anual *Rivulus luelingi* em um riacho de água escura da Floresta Atlântica Costeira do Sul do Brasil. Oito amostragens foram realizadas entre abril de 2003 e janeiro de 2004. A dieta, avaliada através de uma matriz de similaridade com os valores de contribuição estimados para os itens alimentares, inclui microcrustáceos, insetos imaturos aquáticos, insetos aquáticos e terrestres, fragmentos de insetos, aranhas e fragmentos de plantas. Diferenças relacionadas ao período amostral (meses) foram registradas, mas mudanças na dieta em função das classes de tamanho avaliadas e o período de alta/baixa precipitação não foram observadas. A espécie apresentou hábito alimentar insetívoro, e sua dieta no riacho estudado foi composta por itens autóctones (principalmente insetos imaturos aquáticos) e alóctones (principalmente fragmentos de insetos).

Key words: Diet, Feeding habits, Freshwater, Killifish, Neotropics.

Introduction

The Coastal Atlantic rainforest streams are located in the Atlantic Forest biome, one of the most biodiverse and endangered ecosystems in the world (Myers *et al.*, 2000). These drainages are inhabited basically by small-sized fish species, which dwell in streams or shallow water of rivers, showing sometimes a high rate of speciation and a high degree of geographic endemism (Castro, 1999). Despite the ever-growing increase in the distribution data (*e.g.*, Bizerril, 1994; Oyakawa *et al.*, 2006; Menezes *et al.*, 2007; Abilhoa & Bastos, 2009; Bertaco, 2009) and feeding ecology documentation (*e.g.*, Graciolli *et al.*, 2003; Mazzoni & Rezende, 2003; Abilhoa *et al.*, 2007, 2009; Mazzoni & Costa, 2007; Mazzoni *et al.*, 2010) of the small-sized fish species, there is still scarce information on several species.

Studies on the feeding habits of stream fishes have demonstrated that the forested streams are important for the foraging behavior of the neotropical small-sized fish species, since some important food items (aquatic immature insects, aquatic adult insects, and terrestrial insects) are directly and indirectly dependent on the riparian forest (*e.g.*, Casatti & Castro, 1998; Sabino & Zuanon, 1998; Graciolli *et al.*, 2003; Abilhoa *et al.*, 2008; Vitule *et al.*, 2008). In general, omnivory seems to be a predominant feeding strategy in small rivers (Pringle & Hamazaki, 1998), and food resources for fishes in the form of leaves, seeds, fruits, and insects are mainly supplied by the forest canopy (Esteves & Aranha, 1999).

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Rivulus luelingi Seegers, 1984 is a little-known killifish species recently redescribed, reaching about 23.9-37.6 mm SL (Costa, 2007). This species has a restrict distribution, inhabiting the shallowest parts of small streams, temporary swamps, flooded areas and man-made drainage ditches of the Coastal Atlantic Rainforest drainages in southern Brazil (Santos-Filho, 1997; Costa, 2007). Despite its remarkable life cycle (Costa, 2003, 2006; Mitcheson & Liu, 2008), there is little information on its natural feeding ecology. Available evidences for the family Rivulidae suggest that the group might have a generalist diet (Taylor, 1992; Shibatta & Rocha, 2001; Shibatta & Bennemann, 2003; Laufer *et al*., 2009), and differences in the diet richness and prey size are related to variations in body sizes (Santos-Filho, 1997; Laufer *et al*., 2009).

Besides alterations conditioned by the ontogenetic development, most studies on the feeding ecology of tropical freshwater fish have shown that several species also shift their diets according to temporal variations (seasons), and the increase of feeding activity can be related with the rainy period, when prey availability is higher (Prejs & Prejs, 1987; Hahn *et al*., 1997; Resende, 2000; Rezende & Mazzoni, 2005). In the present work we describe the feeding habits of individuals of *R. luelingi* collected in an Atlantic Rainforest black water stream, and also assess the influence of temporal variations (months), precipitation period (high/low) and size classes on the diet of the species.

Material and Methods

Eight study trips were conducted from April 2003 to January 2004 in a 100 m forested stretch of a black water stream located in the City of Guaratuba, State of Paraná, southern Brazil (25º57'S 48º35'W) (Fig. 1). The study site comprises remaining areas of the Coastal Atlantic Rainforest, and cleared areas with floating vegetation and herbaceous riparian vegetation. Even though some stretches of the stream studied are impacted by anthropogenic disturbances (*e.g*., urban development), preserved areas of Atlantic Rainforest with different levels of regeneration are still common in the region. Rainfall and air temperature data were provided by Simepar Technological Institute's weather station in Guaratuba. The air temperature averages during the study period ranged from 15.8ºC (May 2003) to 24.8ºC (January 2004), and the monthly rainfall from 36 mm (August 2003) to 192.4 mm (January 2004). Based in the volume of rain, the samples were divided into low precipitation period (August and September 2003) and high precipitation period (April to June 2003 and October 2003 to January 2004).

Fishes were collected using dip nets, and captured specimens were fixed in the field in 10% formalin solution. In the laboratory all specimens were weighted (g), measured (cm) and dissected. Digestive tracts were removed and their contents analyzed. Voucher specimens were deposited in the fish collection of the Museu de História Natural Capão da Imbuia (MHNCI 11214). Fishes were collected with Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis (Ibama) authorization Proc. No. 02017.003718/2003-55.

In order to estimate the contribution of food items to stomach volume in the diet of each individual, an adaptation of the Hynes' (1950) method of points was performed, as proposed by Vitule *et al*. (2008). The frequency of occurrence method (Hyslop, 1980) was also performed for the diet analysis. The food items were grouped into broad categories according to their ecological characteristics and origin. Microcrustaceans (Cladocera, Copepoda and Amphipoda), aquatic immature insects (dipteran larvae/pupae, Odonata nymphs), and aquatic adult insects (Hemiptera Belostomatidae and Veliidae) were considered as autochthonous. Terrestrial insects (Formicidae, Lepidoptera larvae), insect fragments (mostly wings, elytra and head capsules of terrestrial groups), spiders, and plant fragments were considered as allochthonous.

A similarity matrix with the transformed estimated contribution values of food items was then generated using the Bray-Curtis similarity coefficient. Data was investigated through analysis of similarity (ANOSIM) and similarity percentage (SIMPER) methods performed by the Primer v6 software (Clarke & Gorley, 2006), using individuals as samples, and months, size classes and precipitation period as factors. The ANOSIM test compares similarities between two or more groups of sampling units (factors), and generates a statistic R, which varies between -1 and +1 (Clarke & Gorley, 2001). In the context of this study, the R value of zero represents the null hypothesis (no difference among a set of samples), which means that similarities within and between samples are the same; and R value of 1 indicates that the set of samples (items contribution) within months, size classes or precipitation period (factors) were more similar among themselves than between the levels of each factor.

One-way ANOSIM was used to test for differences in the diet among the size classes, sample period and precipitation period. The Two-way ANOSIM was used to test for differences in the diet between the months sampled across size classes and also between size classes across months sampled. The SIMPER analysis was used for identifying which food item account for observed differences between groups. Because of the lack of information on *R. luelingi* life history (size at first maturity, growth, and reproduction), size classes and corresponding length intervals were obtained from Sturges' formulation (Vieira, 1991).

Results

A total of 129 individuals were measured and dissected, ranging from 12.3-36.2 mm total length. The stomach contents were composed of 15 food items (Table 1). In relation to the origin of the alimentary items, most of them were autochthonous (10 items, 66.7%). Among the autochthonous ones, the most representative was aquatic immature insects (67.4% of occurrence and 56.5% of volume

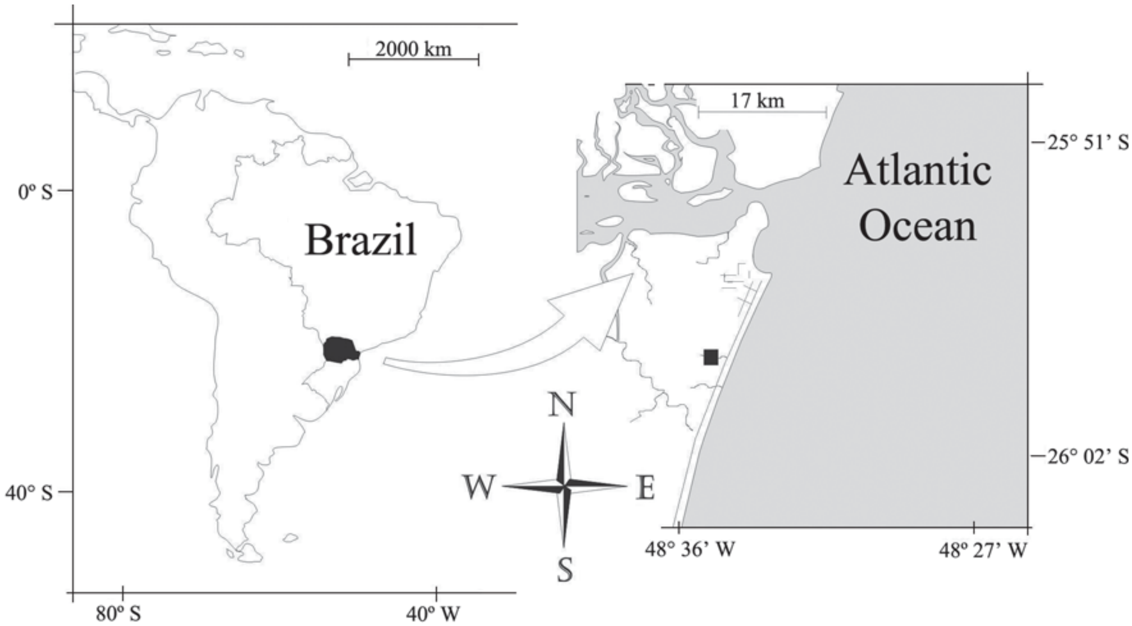

Fig. 1. Location of the study site in Guaratuba Munipality (black square), Paraná State coast (southern Brazil).

contribution). Chironomidae larvae and dipterans pupae were the main aquatic immature insects consumed. Insect fragments (50.4% of occurrence and 28.6% of volume contribution) were the most important allochthonous food item. Considering the consumption of both autochthonous (mostly aquatic immature insects and aquatic adult insects) and allochthonous (mostly terrestrial insects and insect fragments) material, the feeding habit of *R. luelingi* can be characterized as insectivorous.

Multivariate analysis of data did not reveal a clear pattern of separation of diet composition and estimated contribution according to size classes, precipitation period and monthly variations. The one-way ANOSIM test showed that the diet differed significantly among the sampling period, but the R value (0.097) was negligible small, indicating that the diets among the sampling period strongly overlapped. Two-way ANOSIM further indicated significant difference in similarity between the months sampled across size classes and also between size classes across months sampled, nevertheless with some degree of overlapping (Table 2). According to SIMPER results, most of dissimilarity among size classes and months' samples was produced by the items aquatic immature insects and insect fragments (Table 3 and 4).

Discussion

Our observations indicate that the most consumed food items for *R. luelingi* were aquatic immature insects and insect fragments. Davies *et al*. (1990) and Taylor (1992), studying *Kryptolebias marmoratus* (Poey,1880) in Florida mangrove marshes, and Shibatta & Bennemann (2003), studying *R. pictus* Costa, 1989 in small lakes of Brazilian savanna, also noted that aquatic and terrestrial invertebrates (mostly insects), are considered one of the main food items

Table 1. Food items found in 129 stomach contents of *Rivulus luelingi* from a Coastal Atlantic Rainforest stream in southern Brazil.

Origin	Food Items	Frequency of occurrence (%)	Points method (%)
Autochthonous	Aquatic immature insects	67.4 (total)	56.5 (total)
	Diptera - Ceratopogonidae (larvae)	6.2	5.7
	Diptera - Chironomidae (larvae)	58.9	26.2
	Diptera - Simulidae (larvae)	3.9	5.6
	Diptera (pupae)	53.5	12.4
	Odonata - Libelullidade (nymphs)	10.1	6.6
	Aquatic insects	10.8 (total)	8.5 (total)
	Hemiptera - Belostomatidae	6.9	6.3
	Hemiptera - Veliidae	3.9	2.2
	Microcrustaceans	1.6 (total)	2.8 (total)
	Cladocera	0.8	1.4
	Copepoda	0.8	0.8
	Amphipoda	1.6	0.6
Allochthonous	Insecta	2.3 (total)	1.8 (total)
	Hymenoptera - Formicidae	2.3	1.0
	Lepidoptera (larvae)	0.8	0.8
	Insect fragments	50.4	28.6
	Arachnida - Araneae	2.3	0.7
	Plant fragments	3.9	1.1

consumed by rivulids. In fact, most small-sized Atlantic Rainforest fishes are omnivorous and insectivorous (Costa, 1987; Sabino & Castro, 1990; Aranha *et al*., 1998; Vitule *et al*., 2008; Abilhoa *et al*., 2009), and in small to mid-order tropical streams, several fish species feed primarily on terrestrial insects (Costa, 1987; Allan, 1995; Sabino & Zuanon, 1998; Abilhoa *et al*., 2008).

Despite the fact that the analysis of similarities (one-way and two-way) revealed that the intake of insect preys (aquatic immature stages and terrestrial species) by *R. luelingi* changed during the studied period, no relation between the estimated contribution of these items and high/low precipitation period was observed. Thus our results fail in demonstrated the expected seasonal variation in the feeding habits of the small-sized fish species (Lowe-McConnell, 1987; Prejs & Prejs, 1987) as already registered for the Atlantic Rainforest streams (Deus & Petrere-Júnior, 2003; Mazzoni & Rezende, 2003; Abilhoa *et al*., 2007; Vitule *et al*., 2008; Abilhoa *et al*., 2009) and suggest similarities in the allochthonous matter input between rain and dry seasons.

Table 2. Results of the One-way and Two-way Crossed ANOSIM.

Factors		R value	p value
One-way	Size classes	0.032	0.096
	Months – sample period	0.097	0.002
	Precipitation period	0.038	0.147
Two-way crossed	Months (across all size classes)	0.168	0.001
	Size Classes (across all months)	0.114	0.016

Table 3. SIMPER results, showing the contribution percentages of the most representative item abundance for each size class considered.

Factor Size classes (mm)	n	Average similarity	Item abundance - percentual contribution: Aquatic immature insects Autochthonous	Insect fragments Allochthonous
12.3-15.2	3	22.22%	-	100
15.3-18.2	6	30.37%	36.16	58.96
18.3-21.2	10	24.95%	25.26	73.47
21.3-24.2	22	32.74%	66.58	33.42
24.3-27.2	32	36.94%	70.86	26.70
27.3-30.2	33	27.03%	57.60	42.69
30.3-33.2	19	48.14%	89.84	10.16
33.3-36.2	4	14.29%	100	-

Table 4. SIMPER results, showing the contribution percentages of the most representative item abundance for each month sampled.

Factor Month	n	Average similarity	Item abundance - percentual contribution: Aquatic immature insects Autochthonous	Insect fragments Allochthonous
April	12	32.47%	68.27	31.73
May	16	37.32%	15.16	84.84
June	33	36.35%	49.83	50.17
August	12	35.30%	95.89	-
September	18	39.94%	82.38	10.97
October	22	36.28%	39.77	59.56
November	9	29.19%	81.77	11.71
January	7	51.73%	91.55	-

Furthermore, we did not observe diet changes related with size classes, even though shift in food habits during ontogenetic development is well known in fishes (Nikolsky, 1963; Amundsen *et al*., 2003; Rezsu & Specziár, 2006; Alcaraz & García-Berthou, 2007), and was already registered for Rivulidae (Laufer *et al*., 2009) and also in a laboratory experiment performed with *R. luelingi* (Santos-Filho, 1997). In the present study, we believe that the pattern observed was due to the great abundance and availability of insect preys (the most consumed food items) in the environment studied, however, studies on the availability of feeding resources are necessary to corroborate this hypothesis.

The great amount of insect items (aquatic immature stages and terrestrial species) and the anatomical characteristics of *R. luelingi*, such as the fusiform body shape, large eyes dorsolaterally placed on the head, and upward turned mouth, point toward to the surface picking behavior as the foraging tactic (Sazima, 1986). Surface pickers feed on items that fall on the water from the streamside vegetation (*e.g*., insects, leaves, and seeds), and according to Santos-Filho (1997), *R. luelingi* is in fact a visually oriented predator, strongly attracted by prey movement. We believe that this feeding behavior occurs mainly in the riparian forested areas of the stream studied, probably due to a reduced ability for continuous swimming, in a similar way to the one observed for several characin species in Atlantic Rainforest streams (Costa, 1987; Aranha *et al*., 1998; Casatti & Castro; 1998; Abilhoa *et al*., 2007).

The relevance of aquatic immature stages of insects, terrestrial insects and insect fragments to its natural diet highlights the importance of the marginal forest to the feeding habits of stream fishes, results also recorded for several stream-fishes (Esteves & Lobón-Cerviá, 2001; Graciolli *et al*., 2003; Abilhoa *et al*., 2007; Vitule *et al*., 2008). In fact, most fish species use food items of allochthonous origin (Lowe-McConnell, 1999) and changes in the riparian vegetation can cause alteration in the feeding habits of freshwater fishes, affecting many links of the trophic chain (Barrela *et al*., 2000). The present work is an important register on the feeding ecology of *R. luelingi*, a significant component of temporary ponds communities and streams ecosystems.

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