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## The diet of *Cynopoecilus fulgens* Costa, 2002 (Cyprinodontiformes: Rivulidae) in Southern Brazil wetlands

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### Abstract

Studies with annual fish diet have shown that the generalist habit is a dominant strategy within this group. *Cynopoecilus fulgens* (Cyprinodontiformes: Rivulidae) is a restricted range species, endemic to a small area in Southern Brazil, and aspects of its biology and ecology are poorly known. The main goal of the study was to determine the diet of *C. fulgens* throughout its life cycle and compare it with the prey availability in Southern Brazil wetlands. Specifically, we analyzed samples collected in different stages of ontogenetic development (immature and adult fishes) in various year seasons. A total of 52 food items were found in the diet of *C. fulgens*, and invertebrates comprised 96.4% of the counts observed. The richness of consumed and available prey changed along with the seasons. The seasonal variation of the consumed prey richness was mainly related to increase in fish size along the annual cycle. Microcrustaceans represented 75.6%, 80.9%, and 68.4% of the consumed prey in autumn, winter and spring, respectively. Prey composition varied with the seasons and it was not correlated to prey availability. Diet selectivity was different among male, female and immature fish. Our study reported the feeding habits of *Cynopoecilus fulgens* to improve knowledge of the biological and ecological traits of annual killifishes in Southern Brazil wetlands, where more than 90% of the original wetlands have been lost due to agricultural expansion.

**Keywords:** Body size, feeding selectivity, life cycle, killifishes, sexual dimorphism

### Introduction

The impact of wetland loss on biodiversity is evidenced by the decline and extinction of many wetland-dependent species, including fishes (Millennium Ecosystem Assessment 2005). Approximately 20% of the world's freshwater fish species have been listed as threatened, endangered, or extinct in the last few decades (see Millennium Ecosystem Assessment 2005). The Rivulidae family are a good example of wetland-dependent fish (Lanés 2011) and a large number of species of this family are threatened due to the combination of two main factors: (1) specialized life cycle (small body dimensions, low dispersion capacity, and limited spatial distribution), and (2) habitat loss (Fontana et al. 2003).

Most species of the Rivulidae family consist of annual-cycle fish, which exclusively inhabit intermittent wetlands. These species maintain viable

populations through dormant eggs that go through a diapause process over the dry season (Myers 1942; Costa 1990). The annual life cycle in fishes is exclusive of the Rivulidae family (endemic to the Americas), and Nothobranchiidae (endemic to sub-Saharan Africa). These species have strategies to minimize the dry season effects, such as fast growth, early sexual maturation (Arenzon et al. 1999), and high reproductive capacity (Gonçalves et al. 2011). Some species, such as *Cynopoecilus multipapillatus* Costa, 2002, are capable of reaching adulthood, to reproduce and to leave egg banks in sediments, in just 53 days (Arenzon et al. 1999).

Early sexual maturation and high reproductive capacity have been associated with abundant energy resources (Polačik et al. 2011). In fast-growing organisms, fatty acids provided through live prey are essential to satisfy the high energy demand and are required to promote growth (Tocher et al. 2003).

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The success of fish development is related to the availability of lipids, proteins, carbohydrates, vitamins, and minerals via the diet (Kanazawa 2003). In this sense, several studies showed the importance of the biochemical composition of the selected food items for fish development (Olivotto et al. 2006, 2011), since deficiencies in these nutritional requirements resulted in poor growth, low feed efficiency, anemia and high mortality of sea-water fishes (Sargent et al. 1999; Olivotto et al. 2003, 2006).

Studies with annual fish diet have shown that the generalist habit is a dominant strategy within this group (Laufer et al. 2009; Polačik & Reichard 2010). Such a trend can be explained by its trophic position (top predator) (Laufer et al. 2009; Arim et al. 2010; Polačik & Reichard 2010), and the significant stochastic component of the extreme habitats used by the fish (Polačik & Reichard 2010). A broad food spectrum is found in many species of freshwater fish when rapid changes in environmental conditions cause alterations in prey availability (Abelha et al. 2001). In this sense, the annual fish persistence may be related to the exploitation of different energy resources and a larger prey variety (Arim et al. 2007). Shibatta and Bennemann (2003) gave evidence that feeding flexibility is one of the factors that facilitates the survival of a rivulid species from a small lake in Central-West Region of Brazil.

The Rivulidae family has approximately 324 species distributed in 43 genera, and the largest part of this diversity is found in Brazil. In Southern Brazil, the annual fishes are represented mainly by two genera: *Cynopoecilus* Regan and *Austrolebias* Costa. The genus *Cynopoecilus* (Tribe Cynopoecilini) has five species and is distributed between Rio Grande do Sul (Southern Brazil) and Uruguay (Costa 2002). *Cynopoecilus fulgens* Costa, 2002 is a restricted range species, endemic to a small area in the Coastal Plain of Rio Grande do Sul ( $\pm 250$  km) (Costa 2002), and aspects of its biology and ecology are poorly known. *Cynopoecilus fulgens* presents a small body size (less than 50 mm of total length) with pronounced sexual dimorphism. Previous studies revealed that males are larger than females (Costa 1995; Arenzon et al. 2001; Laufer et al. 2009). Furthermore, sexual differences in shape and position of anal, dorsal, pectoral and pelvic fins are typical in the species (for more details see Costa 2002).

The main goal of the study was to determine the diet of *C. fulgens* throughout its life cycle and compare it with prey availability in Southern Brazil wetlands. Specifically, we analyzed samples collected in different stages of ontogenetic development in various year seasons.

## Materials and methods

### Study area

The Southern Brazil region presents a high concentration of wetlands (Maltchik 2003) and high biological diversity (Rolon et al. 2008; Stenert et al. 2008). The study area is located in the Lagoa do Peixe National Park (LPNP – Ramsar site) (Figure 1) located in the Coastal Plain of Rio Grande do Sul. The park is one of the main wetland conservation units in Southern Brazil, with an area of approximately 34,400 ha. The vegetation is characterized by small fragments of forest and subtropical grassland areas. The local climate is humid subtropical with an average annual temperature of 17.5°C, varying from 13°C in winter to 24°C in summer. Annual precipitation ranges between 1200 and 1500 mm, with an annual mean of 1250 mm (Tagliani 1995). This region presents a flat topography and low altitude (less than 20 m above sea level).

### Study sites

Nine freshwater wetlands were selected using the following criteria: (1) palustrine and intermittent system; (2) wetland area ranging from 0.5 to 1.5 ha; and (3) water depth ranging from 30 to 40 cm. All study wetlands were inserted in a native grassland matrix. In each wetland, six collections were carried out in three seasons over two years (autumn – May 2008 and March 2009, winter – June 2008 and August 2009, and spring – October 2008 and November 2009). There was no summer sampling due to the lack of surface water in these intermittent wetlands at that time.

### Fishes

Fish were collected with a D-shaped hand net (60 × 30 cm; 2-mm mesh). Each sweeping covered an area of approximately 0.6 m<sup>2</sup>. The sampling effort varied from 25 (areas smaller than 1 ha) to 50 sweepings (areas larger than 1 ha) (Table I). The sampling effort was varied to guarantee that the entire wetland was sampled. Fish were anesthetized with a lethal dose of benzocaine and fixed in a 10% formalin solution at the collection site. Later in the laboratory, the specimens were washed in tap water and preserved in 70% ethanol.

A total of 713 individuals of *C. fulgens* were collected during the study period. From this total, 293 individuals were used for diet assessment (Table I). The sub-sampling of specimens was random to represent all wetlands and all three seasons of the two-year study. Individuals were

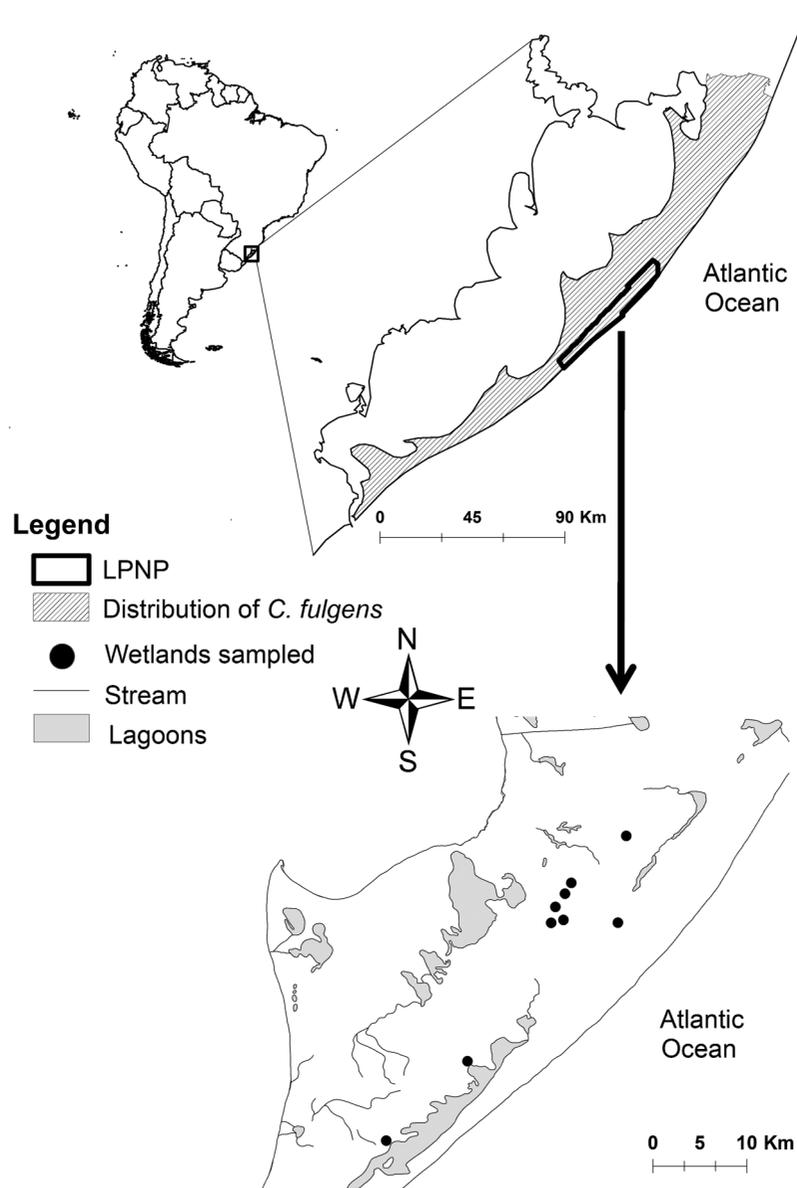


Figure 1. Location of the Lagoa do Peixe National Park and the intermittent wetlands studied.

measured (in mm) and classified in demographic groups according to the presence of visible sexual characters (coloration pattern and fin configuration; Costa 2002), as follows: male, female and immature. Individuals were considered immature when there was no possibility of defining sex, and the size was lower than 17 mm total length (TL).

#### *Invertebrates*

Aquatic invertebrates were collected using a 30 cm-wide dip-net (250  $\mu$ m). The net was swept at random places, in areas 1 m long and covering approximately 0.3 m<sup>2</sup>. Sampling efforts varied from 9 (in areas smaller than 1 ha) to 18 sweepings (in areas larger

than 1 ha) (Table I). Sampling efforts were varied to ensure that the entire wetland was sampled. Sweeps were pooled into 3.5-L plastic jars and fixed *in situ* with 10% formalin. In the laboratory, the samples were washed through a 250- $\mu$ m sieve and leaves, stems, and other debris were removed. The resulting material was preserved in 80% ethanol.

#### *Analysis of stomach contents*

The gastrointestinal tracts of fishes were removed and observed with a stereomicroscope. The contribution of each food item to fish diet was estimated using the numerical method proposed by Hyslop (1980). The ingested and available invertebrates were

Table I. Sampled wetlands, fish and invertebrate (prey) sampling effort, and the abundance of collected individuals (fish and invertebrate) according to the annual season, age and sex in the Lagoa do Peixe National Park, Southern Brazil.

Sampled sites	Sampling effort (sweeping)		Abundance of collected individuals																	
	<i>C. fulgens</i>	Prey	Autumn						Winter						Spring					
			<i>C. fulgens</i>		Prey		<i>C. fulgens</i>		Prey		<i>C. fulgens</i>		Prey		<i>C. fulgens</i>		Prey			
I	M	F	I	M	F	I	M	F	I	M	F	I	M	F	I	M	F			
1	50	18	9	3	0	939	3	9	8	4941	0	5	7	2105						
2	50	18	6	0	0	575	0	11	1	1474	0	0	0	1722						
3	50	18	3	3	0	326	0	10	8	870	0	0	4	1892						
4	25	9	6	0	0	2219	3	9	9	1046	0	6	6	288						
5	25	9	9	0	0	1075	3	6	6	450	0	6	6	261						
6	25	9	13	0	0	582	3	7	8	229	0	6	6	202						
7	25	9	6	0	0	114	3	9	9	145	0	6	6	74						
8	25	9	0	0	0	452	0	2	2	115	0	2	8	90						
9	25	9	3	0	3	342	0	7	7	132	0	6	6	288						

*C. fulgens* = *Cynopocillus fulgens*.

identified to the lowest possible taxonomic level. In most cases, aquatic macroinvertebrates (arthropods, worms, flatworms, leeches and molluscs) were identified to family level (Merritt & Cummins 1996; Fernández & Domínguez 2001). Microcrustaceans were identified to class or order levels. Fish, invertebrates and the gastrointestinal contents were stored in the reference collection of the Laboratory of Ecology and Conservation of Aquatic Ecosystems at University of Vale do Rio dos Sinos (UNISINOS).

#### Data analysis

To evaluate temporal patterns of diet composition the seasonal samples (autumn, winter and spring) were grouped across the two study years (2008 and 2009). The Kruskal-Wallis test was used to evaluate: (1) the differences in richness of ingested and available prey among the three seasons, and (2) the differences in body size between males and females, besides annual seasons. The Mann-Whitney test was used for multiple comparisons between groups. To avoid Type I error inflation, the Bonferroni correction was applied, where the significance value was divided by the number of Mann-Whitney comparisons carried out (three tests  $P = 0.017$ ) (Field 2005). Jonckheere-Terpstra trend test replaced Mann-Whitney in cases where the data appeared to show trends across the treatments. The Jonckheere-Terpstra test is more powerful than the Mann-Whitney test for comparing two samples against ordered alternatives (Hollander & Wolfe 1999).

The frequency of occurrence and relative abundance of ingested preys were calculated according to Amundsen et al. (1996). The graphical analysis of feeding strategy of Costello (1990) modified by Amundsen et al. (1996) was carried out to compare the relative importance of ingested items over three seasons. The graphical analysis of Costello plots the importance of each ingested item, where the x axis represents the frequency of occurrence and y represents the abundance of a prey taxon.

A permutation multivariate analysis of variance (PERMANOVA) was used to compare differences in prey composition among the three seasons and between males and females. The difference in prey composition was tested using Bray-Curtis dissimilarity matrix, and 9999 permutations to test the model for significance. The analyses were performed using the Vegan Package (Oksanen et al. 2009) and the Labdsv Package (Roberts 2007) in the R Statistical Program version 2.9.0 (R Development Core Team 2009).

The correlation matrix between ingested prey and available prey was analyzed by Mantel test, using Bray-Curtis dissimilarity. We verified whether differences of ingested prey over the three seasons were influenced by prey availability. Selectivity for a particular prey species is determined by comparison of its relative abundance in diet and habitat. The selectivity index of Paloheimo (1979) was used to evaluate the degree of feeding selectivity of *C. fulgens*. The index was calculated by the equation:

$$\text{NRF} = (r_i/P_i) / \left( \sum r_i/P_i \right) \quad (1)$$

where NRF is the normalized forage ratio,  $r_i$  is the relative frequency of prey  $i$  in diet, and  $P_i$  is the frequency of the same prey  $i$  in the environment. The interpretation of the selectivity value is made through the reference value that is represented by the equation  $1/n$ , where  $n$  is the number of food types in the environment. If  $\text{NRF} > 1/n$  it represents positive selection, if  $\text{NRF} < 1/n$  it represents negative selection, and if  $\text{NRF} = 1/n$  it represents no selection. Occasionally a prey category occurred in the diet but not in the environment sample or vice-versa. In these cases, when the prey abundance in the environment was  $> 5\%$  but this prey was not consumed, the selection was considered negative. And when the prey abundance in diet was  $> 5\%$  but it was not present in the environment, the selection was considered positive. When the percentage of prey abundance comprised  $< 5\%$ , the selection was considered null. In this index, the selectivity value is based on normalized feed ratio and it is independent of the relative abundance of prey. This index was used to evaluate the prey selectivity among male, female and immature fishes, besides seasonal samplings.

## Results

### Fish size

From the 293 individuals analyzed, only four showed empty stomachs. Fish size varied among immature (Median = 12.22 mm, Max. = 16.52, Min. = 8.63), females (Median = 25.06 mm, Max. = 48.3, Min. = 13.97), and males (Median = 32.75 mm, Max. = 48.73, Min. = 17.4). Males were larger than females ( $U = 2686.5$ ,  $P < 0.001$ ). Length of *C. fulgens* changed across seasons [ $H(2) = 115.181$ ,  $P < 0.001$ ], and the largest length was observed near the end of the annual cycle (Spring) ( $J = 21085$ ,  $Z = 10.832$ ,  $P < 0.001$ ) (Figure 2). No immature fish were sampled in spring (Table I).

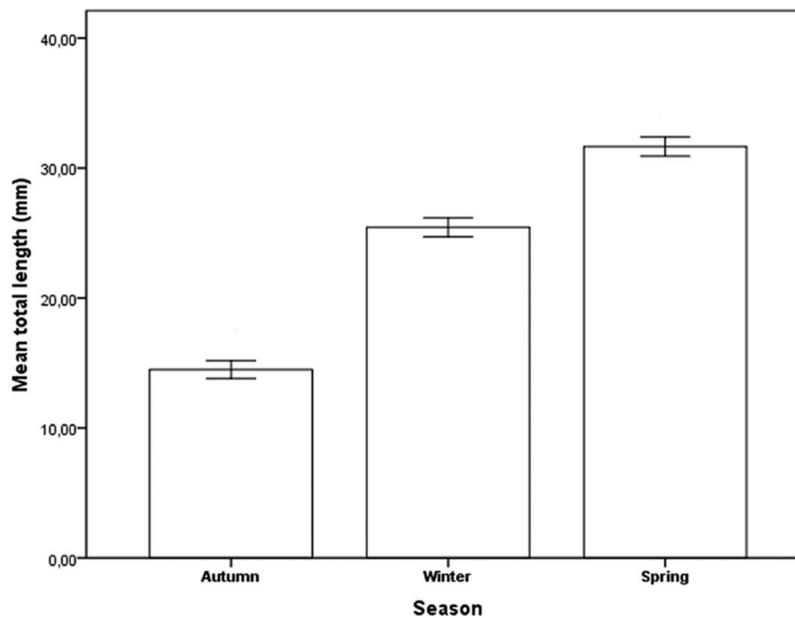


Figure 2. Mean total length of *Cynopoecilus fulgens* throughout the study seasons.

#### Food offer

A total of 23,104 invertebrates distributed among 39 macroinvertebrate taxa (33 aquatic and six terrestrial), and four microcrustacean taxa, were collected during the study period (Table II). While aquatic macroinvertebrates represented the majority of the individuals collected (84.4%), microcrustaceans were represented by only 3348 individuals. Dogielinotidae was the most abundant taxon, representing 20.1%, 49.3%, and 53.4% of the macroinvertebrates collected in autumn, winter, and spring, respectively. Chironomidae and Planorbidae were also abundant over the study period. Terrestrial arthropods also occurred in the wetlands, representing 0.5% of the invertebrates collected (Table II).

#### Fish diet

A total of 52 food items were found in the diet of *C. fulgens*, distributed among invertebrates, macroalgae, seeds, plant debris, fish scales, and sediment (Table II). Invertebrates comprised 96.4% of diet of *C. fulgens* (Table II). Among the invertebrates, the microcrustaceans were the most abundant food items observed and they were distributed among Cladocera [%Fi (relative frequency) = 84.9, %Ai (relative abundance) = 54.7], Copepoda (%Fi = 65.1, %Ai = 15.6), and Ostracoda (%Fi = 35.8, %Ai = 4.4). The second most abundant group observed in the diet of *C. fulgens* was the immature aquatic insects, distributed among Chironomidae, Ephemeroptera, Trichoptera, Coleoptera, and other

Diptera (Table II). Dogielinotidae, Hydracarina, and Planorbidae were also abundant in the diet of *C. fulgens*. The major component of the fish diet was composed of autochthonous taxa (Table II).

The richness of consumed prey changed along the seasons [ $H(2) = 52.054$ ,  $P < 0.001$ ], and it was higher in winter and spring than in autumn ( $U = 1602.5$ ,  $P < 0.001$ ; and  $U = 1253$ ,  $P < 0.001$ , respectively) (Figure 3). The richness of available invertebrates also showed seasonal variation [ $H(2) = 7.151$ ,  $P < 0.05$ ], and it was greater in autumn (mean = 18.65) than winter (mean = 14.83), which in turn was greater than spring (mean = 11.94) ( $J = 293.5$ ,  $Z = -2.642$ ,  $P < 0.01$ ) (Figure 3).

Prey composition varied along the seasons ( $F = 0.944$ ,  $P = 0.001$ ), and it was not correlated with prey availability ( $r = 0.249$ ,  $P > 0.05$ ). Microcrustaceans represented 75.6%, 80.9%, and 68.4% of the consumed prey in autumn, winter and spring, respectively (Table II). Cladocera was the most frequent and abundant consumed invertebrate taxon in the three study seasons (Figure 4). Chironomidae, Hydracarina and Dogielinotidae were the most abundant macroinvertebrate taxa consumed throughout the life cycle of *C. fulgens* (Table II). The composition of the consumed prey was not different among males and females ( $F = 1.9797$ ,  $P = 0.055$ ).

#### Diet selectivity

Diet selectivity varied among seasons. While Cladocera was the most selected prey in autumn,

Table II. Stomach contents of *Cynopoeilus fulgens* and prey availability in intermittent wetlands of Lagoa do Peixe National Park, Southern Brazil. I = Immature, M = Male, F = Female, and PA = Prey Availability. "0" = not present in the diet, and "-" = lack of immature fishes and item not sampled in the environment.

Food items	Abundance											
	Autumn				Winter				Spring			
	I	M	F	PA	I	M	F	PA	I	M	F	PA
Autochthonous												
Macroinvertebrates												
Aquatic insects (holometabolous)												
Larvae												
Chironomidae	72	126	1	826	23	214	150	654	—	110	110	422
Culicidae	0	0	0	12	0	0	3	2	—	1	0	2
Other Diptera	1	0	0	19	0	0	0	16	—	1	0	23
Dytiscidae	1	0	0	70	0	18	9	8	—	1	7	12
Hydrophilidae	0	2	1	79	1	59	11	14	—	20	18	31
Noteridae	0	0	0	16	0	0	0	0	—	0	0	0
Other Coleoptera	0	0	0	0	0	0	0	0	—	0	2	0
Hydroptilidae	0	0	0	70	0	6	1	35	—	6	2	142
Leptoceridae	0	0	0	5	0	2	3	2	—	0	0	73
Polycentropodidae	0	0	0	0	0	3	0	1	—	0	0	0
Other Trichoptera	4	0	0	0	0	10	11	30	—	9	5	0
Lepidoptera	0	0	0	37	0	0	0	8	—	0	0	22
Pupae												
Culicidae	0	0	0	0	0	0	0	0	—	1	2	0
Diptera	0	0	0	0	0	7	4	0	—	2	1	0
Other Pupae	0	0	0	0	0	0	3	0	—	0	0	0
Adult												
Coleptera	0	0	0	339	0	0	0	190	—	0	0	91
Aquatic insects (hemimetabolous)												
Baetidae	0	2	0	216	0	15	2	361	—	6	6	264
Caenidae	0	0	0	119	0	2	0	91	—	9	12	168
Other Ephemeroptera	3	0	1	20	0	4	2	0	—	7	5	0
Corixidae	0	0	0	159	0	1	1	112	—	2	1	57
Notonectidae	0	0	0	5	0	1	0	17	—	0	0	5
Belostomatidae	0	1	0	39	0	7	2	15	—	4	1	49
Odonata	0	0	0	80	0	0	0	130	—	0	0	74
Other Hemiptera	0	0	0	135	0	0	0	43	—	0	0	36
Other aquatic arthropods												
Acarina	0	0	0	557	0	0	0	81	—	1	0	85
Hydracarina	15	1	0	178	169	95	103	46	—	4	13	7
Dogielinotidae	2	7	0	948	0	60	35	3945	—	43	134	3614
Amphipoda	0	0	0	0	1	21	0	4	—	0	2	0
Anostraca	0	0	0	0	0	1	0	15	—	0	0	0
Other Crustacea	0	0	0	4	0	0	0	4	—	0	0	0
Other aquatic invertebrates												
Turbellaria	0	0	0	8	0	0	0	178	—	0	0	27
Nematoda	5	0	0	281	1	4	6	80	—	5	7	1
Oligochaeta	4	0	0	187	0	0	3	204	—	4	0	66
Hirudinea	1	0	0	51	0	0	0	107	—	0	1	194
Other Annelida	0	0	0	0	0	0	0	0	—	0	0	2
Planorbidae	5	0	0	231	8	35	14	1584	—	4	7	1233
Other Mollusca	0	0	0	37	0	5	7	18	—	3	23	66
Microcrustaceans												
Cladocera	568	9	19	202	299	1878	1383	799	—	428	513	41
Copepoda	82	48	38	693	66	607	304	381	—	125	186	66
Ostracoda	25	2	0	892	24	233	74	131	—	30	22	49
Conchostraca	0	0	0	81	0	0	0	13	—	0	0	0

(Continued)

Table II. (Continued).

Food items	Abundance											
	Autumn				Winter				Spring			
	I	M	F	PA	I	M	F	PA	I	M	F	PA
<b>Allochthonous</b>												
<b>Terrestrial Arthropoda</b>												
Araneae	0	0	0	5	0	0	1	78	—	1	1	0
Hymenoptera	0	0	0	7	0	1	0	0	—	0	0	0
Delphacidae	0	0	0	3	0	2	0	0	—	0	0	0
Cicadellidae	0	0	0	8	0	1	0	3	—	0	0	0
Orthoptera	0	0	0	4	0	0	0	0	—	0	0	0
Collembola	0	0	0	1	0	0	0	2	—	0	0	0
<b>Other items</b>												
Macroalgae	1	0	0	—	0	18	6	—	—	7	8	—
Seed	6	0	2	—	2	19	15	—	—	3	1	—
Plant debris	0	0	0	—	2	14	23	—	—	4	11	—
Fish scales	0	1	0	—	0	7	2	—	—	8	7	—
Sediment	10	14	0	—	1	65	27	—	—	39	8	—

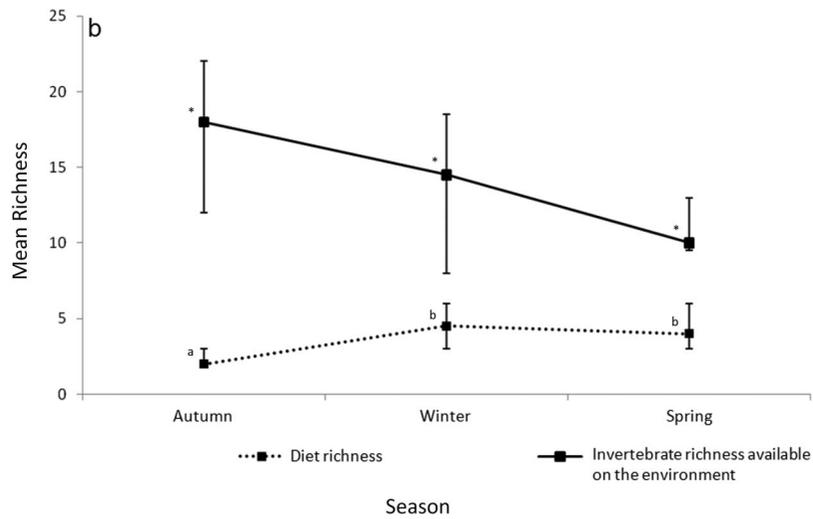


Figure 3. Richness of invertebrates observed in the diet of *Cynopoecilus fulgens* and in the environment throughout the study seasons. Different letters means significant statistical difference. \*Decreasing trend ( $J = 293.5$ ,  $Z = -2.642$ ,  $P < 0.01$ ).

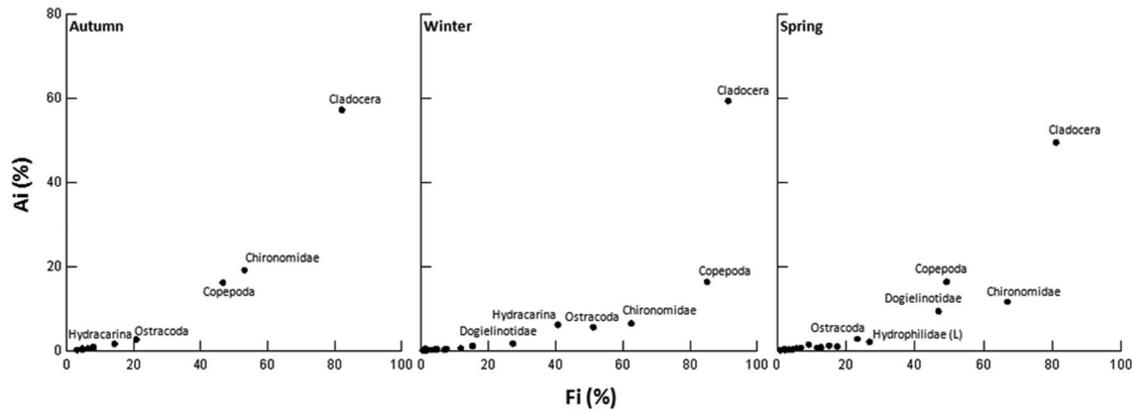


Figure 4. Feeding selectivity of *Cynopoecilus fulgens* throughout the study seasons. Fi (%) = relative frequency of prey; Ai (%) = relative abundance of prey.

Table III. Paloheimo selectivity index for study season and across males (M), females (F) and immature (I).

Prey taxa	Seasons			Sex/Ontogenic		
	Autumn	Winter	Spring	I	M	F
Chironomidae	1.528	0.88	1.881	0.744	1.258	0.962
Hydrophilidae (larvae)	0.22	17.387	17.304	0.178	5.98	2.921
Other holometabolous insects – larvae	0.152	0.878	0.402	0.255	0.456	0.454
Other holometabolous insects – pupae	Absent	0.052**	0.052**	Absent	0.052**	0.052**
Other holometabolous insects – adult	0*	0.052**	0.052**	0.052**	0.052**	0.052**
Other hemimetabolous insects	0.049	0.182	0.283	0.022	0.154	0.105
Cladocera	18.812	6.649	85.607	10.043	11.809	12.885
Copepoda	1.548	3.828	17.11	1.598	3.637	3.247
Ostracoda	0.186	3.783	3.266	0.555	1.314	0.628
Other microcrustaceans	0.052**	0.106	Absent	0.052**	0.049	0.052**
Dogielinotidae	0.054	0.036	0.178	0.005	0.069	0.139
Hydracarina	0.573	11.858	7.286	9.525	2.301	3.521
Other aquatic arthropods	0*	0.052**	0.083	0.052**	0.007	0.052**
Turbellaria	0.052**	0.052**	0.052**	0.052**	0.052**	0.052**
Nematoda	0.091	0.186	21.857	0.193	0.132	0.252
Annelida	0.107	0.116	0.071	0.127	0.164	0.052
Mollusca	0.096	0.063	0.109	0.081	0.079	0.113
Terrestrial Arthropoda	0.052**	0.038	0.052**	0.052**	0.113	0.149
Other	0.052**	0.052**	Absent	0.052**	0.052**	0.052**

Reference index: 0.052.

\*Negative Selection – Absent in the diet and present in the environment (> 5%).

\*\*Neutral Selection – Present in the diet (< 5%) and absent in the environment, or absent in the diet and present in the environment (< 5%).

Absent: Absent in the environment and diet.

adult holometabolous insects and other aquatic arthropods showed negative selection (Table III). In winter, Hydrophilidae larvae were the most selected prey, followed by Hydracarina, Cladocera, Copepoda, and Ostracoda. In spring, Cladocera was the most selected food item, followed by Nematoda, Hydrophilidae larvae, Copepoda, Hydracarina, and Ostracoda. The other prey showed absence of selection in the three seasons (Table III).

Selection among the invertebrate taxa changed across males, females and immature fish. Immature fish ingested Cladocera and Hydracarina preferentially, and showed a negative selection for Dogielinotidae (Table III). Males showed preference for Cladocera, Hydrophilidae larvae, and Copepoda, and showed a negative selection for other aquatic arthropods. Females were more selective for Cladocera, Hydracarina, and Copepoda (Table III). The other prey items showed values close to absence of selection.

## Discussion

### Fish diet

The diet of *Cynopoecilus fulgens* was characterized by the dominance of autochthonous food items. Our result was also observed in other studies of annual fish (Laufer et al. 2009; Gonçalves et al. 2011). The

absence of allochthonous organisms is due to the study area matrix (grasslands are areas lacking forest vegetation and exposed to solar radiation). Other genera of the Rivulidae, such as *Atlantirivulus* (a non-annual killifish), which inhabits permanent wetlands surrounded by forest, tend to ingest a high number of allochthonous organisms (Abilhoa et al. 2010).

The presence of macroalgae, seeds, plant debris and sediment was very rarely observed in the diet. This result may be due to their accidental ingestion during foraging. Our results showed that males and females had fish scales in their gastrointestinal contents. The presence of fish scales may represent aggressive behavior associated with reproduction and territorialism, typical to members of the Rivulidae family (Shibatta & Rocha 2001). Gonçalves et al. (2011) observed lepidophagy in *C. melanotaenia* males, and they associated it with reproductive behavior.

### Seasonal changes in fish size, food offer and fish diet

The longest fish length was observed near the end of the annual cycle, when the fish doubled their size. Moreover, the richness of the ingested prey was also greater at the end of the annual cycle. The positive relationship between fish size and richness of ingested prey can be associated with the need of larger fish to diversify their diet to sustain larger energy demands

(Arim et al. 2010). The seasonal variation of prey richness was mainly related to increase in fish size through the annual cycle, and not with prey availability. Studies have shown that as a predator body size increases, larger prey items are progressively added to the diet, and the frequency of smaller species tends to decrease, but not necessarily be eliminated (Woodward & Hildrew 2002; Laufer et al. 2009).

Our study showed that prey selection varied among annual seasons. Cladocera was the most frequent food category consumed across all seasons. Gonçalves et al. (2011) found similar results for *C. melanotaenia*. Chironomidae was also frequently consumed across the seasons, with positive selection indexes in all collections. Studies have shown that Chironomidae is extremely abundant in subtropical wetlands (Stenert et al. 2008; Maltchik et al. 2010b), becoming a significant food item to *C. fulgens*. Although Dogielinotidae was the most abundant taxon in the study wetlands through the seasons each year, *C. fulgens* showed a positive selective value only when the fishes were adult (spring).

#### *Changes in fish diet in relation to intersexual and ontogenetic factors*

Prey selection was different among males, females and immature fish. Our results showed that females tend to ingest a larger number of Dogielinotidae, whereas males tend to ingest more Chironomidae, Ostracoda, and Hydrophilidae larvae. Selander (1966) reported that the morphological differences between sexes can lead to a low diet overlapping and lower intraspecific competition rates. Although our study has shown that males of *C. fulgens* were larger than females, smaller females tended to select for bigger prey (Dogielinotidae) than larger males. The length gradient of females analyzed may have not been enough to limit the ingestion of larger prey, mainly in the end of the annual cycle (spring) when the largest length was observed. On the other hand, immature fish eat only the prey which can fit in their mouths, e.g. Cladocera, Hydracarina and Copepoda. However, the high energy demand of young fishes may have influenced the selected food items since live prey enrichment is a limiting factor in larval development and metamorphosis (Olivotto et al. 2011).

#### **Conclusions and conservation implications**

The Rivulidae family has several endangered species, mostly due to their high endemism, small size of populations, and occurrence in habitats which are extremely sensitive to human pressures (Contente &

Stefanoni 2010). However, information on annual fish to allow their conservation is still scarce (Polačik & Reichard 2010; Volcan et al. 2010, 2011). Most available information is based on short-scale studies (Laufer et al. 2009; Arim et al. 2010) developed in laboratories (Arezo et al. 2007), or on taxonomic investigations (Costa 1998, 2002). Moreover, ecological studies carried out in their natural habitats and based on temporal series are scarce (Arenzon et al. 2001; Laufer et al. 2009; Lanés 2011). In this sense, such ecological studies are essential to support conservation efforts (Lanés & Maltchik 2010). Our study reported the feeding habits of *Cynopoecilus fulgens* to improve knowledge of the biological and ecological traits of annual killifishes in Southern Brazil wetlands, where more than 90% of the original wetlands have been lost due to agricultural expansion (Maltchik et al. 2010a).

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