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ABUNDANCE, SEX-RATIO, LENGTH–WEIGHT RELATION, AND CONDITION FACTOR OF NON-ANNUAL KILLIFISH *ATLANTIRIVULUS RIOGRANDENSIS* (ACTINOPTERYGII: CYPRINODONTIFORMES: RIVULIDAE) IN LAGOA DO PEIXE NATIONAL PARK, A RAMSAR SITE OF SOUTHERN BRAZIL

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Background. The genus *Atlantirivulus* includes 11 species distributed along coastal river basins and lagoons of eastern and southern Brazil. Most killifishes are threatened with extinction mostly due to their restricted distribution ranges, small population sizes, and habitat loss. Poor knowledge on ecological- and life history information further increases the threats. *Atlantirivulus riograndensis* (Costa et Lanés, 2009) is a non-annual, small-sized fish endemic in the Laguna dos Patos basin and adjacent coastal areas in southern Brazil. There is no information on the life history and ecology of the species. In this paper, we analyzed population traits of *A. riograndensis* to support conservation efforts.

Materials and methods. The fish were captured with a D-shaped hand net in six sampling surveys carried out between 2008 (June, August, and October) and 2009 (March, August, and October) in the type locality of *A. riograndensis*. The variation of abundance, body size, and condition factor over time, sex ratio, and length–weight relations were analyzed.

Results. The estimated abundance was 1.97 specimens per 1 m^2. The TL means increased along the year, and males were larger than females. Strong differences in proportions of sexes were detected, with high bias for females. The species showed allometric positive growth (b = 3.3), and the general condition factor (CF) was 0.258 ± 0.003.

Conclusion. *Atlantirivulus riograndensis* inhabits small water bodies and apparently has a small population size. The population is female biased, and males are typically bigger than females. The species has an allometric positive growth. The body size variation is related to individual growth throughout each study year, and probably affected by high mortality of large individuals in dry season. This study presents new original data on basic traits of the natural history of *A. riograndensis*. The observed results may be used to assist the development of management strategies and conservation of the species and its habitat, mainly in a region where 90% of wetland systems were lost by agriculture, and the remaining areas are at high risk due to the expansion of exotic pine plantations.

Keywords: life history, freshwater fishes, Neotropical, wetlands, conservation

INTRODUCTION

The genus *Atlantirivulus* was recently established by Costa (2011) to comprise a well supported monophyletic group of killifish species formerly known as *Rivulus santensis* superspecies (Huber 1992), *R. janeiroensis* group (Costa 1998), or *R. santensis* species group (Costa 2004). Presently, *Atlantirivulus* includes 11 valid species (*A. depressus*, *A. haraldsiolii*, *A. janeiroensis*, *A. jurubatibensis*, *A. lazzarotoi*, *A. luelingi*, *A. nudiventris*, *A. riograndensis*, *A. santensis*, *A. simplicis*, and *A. unaensis*) distributed along coastal river basins and lagoons of eastern Brazil (Costa 2008a, b, Costa and De Luca 2009, Costa and Lanés 2009). This genus presents unique morphological features among aplocheiloid fishes, represented by a curved ventral process of angulo-articular and numerous infraorbital neuromasts arranged in a zig-zag pattern

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(Costa 2008a). Monophyly of *Atlantirivulus* was supported by morphology and corroborated by molecular analysis (Costa 2008a, 2011).

These species occur in small and shallow streams and pools within the forest or in open areas adjacent to forests, habitats extremely vulnerable to increased human settlement in the coastal area (Costa 2008b). Recently, studies have contributed to the knowledge of the diet of some *Atlantirivulus* species, suggesting an insectivorous feeding habit, composed of autochthonous (mainly immature aquatic insects) and allochthonic material (mainly insect fragments) (Abilhoa et al. 2010, Contente and Stefanoni 2010). Several species of *Atlantirivulus* presented restricted distribution ranges (Nogueira et al. 2010), and the biology and ecology of these species are little known (Abilhoa et al. 2010, Contente and Stefanoni 2010). For these reasons and mainly due to habitat loss, many *Atlantirivulus* species are threatened with extinction (Costa 2009).

Atlantirivulus riograndensis (Costa et Lanés, 2009) is a freshwater and non-annual small-sized fish (< 40 mm TL) endemic in the Laguna dos Patos and adjacent coastal areas of Rio Grande do Sul state, southern Brazil. This killifish was observed in few areas (Costa and Lanés 2009), and its low representation in ichthyologic collections suggests that it is a rare species with small populations. Like most of its congeners, information on the life history and ecology attributes of the species is lacking (Nogueira et al. 2010).

In order to contribute to basic ecological information on *A. riograndensis* to support conservation and management strategies, we analyzed the variation of abundance, body size and condition factor over time, sex ratio, and length–weight relations in the type population of *A. riograndensis*.

MATERIALS AND METHODS

Study area. The study area is located in the Lagoa do Peixe National Park (LPNP), southern Brazil (Fig. 1). The LPNP (50º77′–51º15′W, 31º02′–31º48′S;) has 34 400 ha, 62 km in length, and an average width of 6 km. The LPNP is an important conservation unit considered as Ramsar Site and Biosphere Reserve (Lanés and Maltchik 2010). The Lagoa do Peixe is the main water body in the LPNP, characterized as a 35 km-long shallow lagoon (mean water depth = 1 m) with intermittent communication with the Atlantic Ocean in its southern part. The region presents flat topography and low altitude. Climate is moist subtropical, and the mean temperatures range from 14.6ºC (in winter) to 22.2ºC (in summer), with a mean annual temperature of 17.5ºC, and an annual precipitation ranging from 1150 to 1450 mm (Tagliani, unpublished[O]). Although belonging to Pampa Biome (Anonymous, unpublished[*]), the LPNP is located in a warm-temperate transition biogeographical region and, as a consequence, it contains botanical and faunal elements typical of temperate grasslands (Pampa Biome) and tropical rainforests (Atlantic Forest) (Klein 1998).

Fish samplings. The type locality of *A. riograndensis* was selected for study, which is characterized as a freshwater shallow pool situated in an open vegetation matrix at the border of a forest (areas of pioneer formations) and near of permanent lagoon called Pai João. Six sampling surveys were carried out between 2008 (June, August and October) and 2009 (March, August and October). On each sampling survey, fishes were collected using a D-shaped hand net (60 cm wide, 1 mm mesh). The chosen mesh-size was sufficient to capture all sizes of fish. Sampling was performed sweeping the net parallel to the pool bottom. Ten sweeps were carried out in each expedition. The sweeps were pooled into plastic buckets and the fish were euthanized with a lethal dose of phenoxyethanol and preserved in situ in 10% formaldehyde. In laboratory, the fish were preserved in 70% ethanol solution. Individuals were identified and sexed according to Costa and Lanés (2009). All individuals were measured (total length, TL, to the nearest 0.01 mm) and weighed (body weight, *W*, to the nearest 0.0001 g). Fish collections were made under IBAMA/ICMbio/SISbio authorization (18576-1) and part of the material was vouched (UFRJ 6639 and UFRJ 6640) in the Federal University of Rio de Janeiro. The water physical and chemical parameters (pH, dissolved oxygen, conductivity, and temperature) were taken on each sampling occasions using a Horiba H-10 Water Quality Checker.

Data analysis. Abundance was assessed by the total number of fish captured and expressed as catch per unit area (CPUA: number of individuals/total sampled area). One-way ANOVA was used to assess differences in TL means in all samples. For multiple comparisons of TL

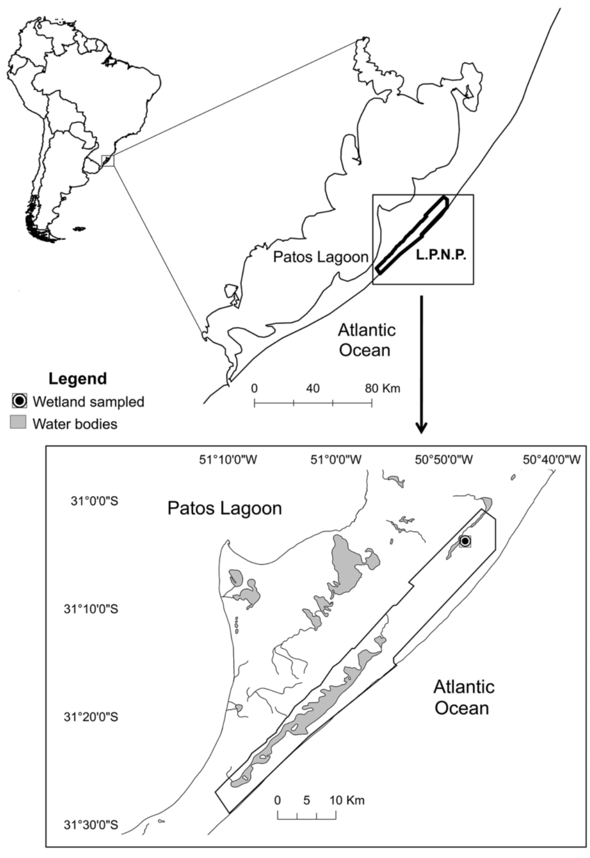

Fig. 1. Location of study site in Lagoa do Peixe National Park (LPNP), Rio Grande do Sul, southern Brazil

[*] Tagliani P.R.A. 1995. Estratégia de planificação ambiental para o sistema ecológico da Restinga da Lagoa dos Patos-Planície Costeira do Rio Grande do Sul. [Environmental planning strategy for the ecological system of the Lagoa dos Patos Restinga in the coastal plain of Rio Grande do Sul.] PhD Dissertation, Federal University of São Carlos, São Carlos, São Paulo, Brazil. [In Portuguese.]

[*] Anonymous 2004. [Vegetation map of Brazil and map of Brazil Biomes.] Instituto Brasileiro de Geografia e Estatística. [Brazilian Institute of Geography and Statistics.] www.ibge.gov.br (Downloaded on 22 May 2011).

means among the samples (sampling expeditions), the Gabriel's procedure was used. This post-test is indicated when sample sizes show little variation (Field 2009). A Student's *t*-test was applied to test for differences in means of TL between males and females. The sex ratio was analyzed by Chi-square test, in order to verify whether the proportion of males and females differed from the expected 1 : 1 rate. Because of the relatively small sample size, we performed a randomized test with Monte Carlo method to obtain the *P*-value for the Chi-square test (McDonald 2009).

The log-transformed length–weight relation (LWR):

$$logW = log(a) + b \times log(\mathrm{TL})$$

was used, and the relation between TL and W was determined by the method of least squares regression. LWR was calculated for all specimens (n = 71) and separately for males (n = 21) and females (n = 50). The confidence limits for b (CL 95%) were calculated to determine if the hypothetical value of isometry (3) fell between these limits (Froese 2006).

Condition factor (CF) coefficients across months and between males and females were calculated as the ratio between body weight and total length, as follows:

$$\mathrm{CF} = (W \cdot \mathrm{TL}^{-b}) \times 100$$

where b is the slope of LWR (Vazzoler 1996) by taking into account all captured specimens. One-way ANOVA was used to assess differences in CF mean throughout the samples. Gabriel's procedure was used for multiple comparisons between the samples. Differences between *CF* coefficients of males and females were analyzed by Student's *t*-test.

RESULTS

Individuals of *A. riograndensis* were found in shallow areas (maximum depth of 15 cm) with muddy substrate. Water samples showed high levels of dissolved oxygen (mean 8.62 ± 0.82 mg · L^{-1} SE) and low values of pH (mean 6.16 ± 0.11 SE), and conductivity (mean 15.5 ± 1.02 mS · m^{-1} SE). In all samples the salinity values were near 0%. The water temperature varied between 15.7ºC and 27.15ºC (mean 21.3ºC ± 2.36 SE) with higher values in summer and lowest values in winter. The variation of the physico-chemical parameters of water during the study period is summarized in Table 1. *Phalloceros caudimaculatus* (Hensel, 1868), representing the family Poeciliidae, was the only fish species that co-occurred with *A. riograndensis*.

A total of 71 individuals (mean 11.83 ± 1.62 SE per sampling expedition) of *A. riograndensis* were captured, including 50 females and 21 males (Table 2). The total CPUA was 1.97 specimens per m^2. There were significant differences in the TL mean between the samples ($F_{(5,70)}$ = 2.514, P = 0.03), with tendency of annual increase (Fig. 2). The greatest difference in TL mean occurred between March and October 2009 (P = 0.04). Males were generally significantly larger than females (t-test$_{(69)}$ = –2.258; P = 0.02), and this pattern was repeated in each month sampled. The population was characterized as highly female-biased (2.38 : 1, Female : Male), and the Chi-squared test demonstrated strong differences in sex proportions (χ^2 = 11.84; P < 0.001). Variations in regression parameters and minimum and maximum TL range for each sex and for all fish are presented in Table 2, and LWR of all specimens are plotted in Fig. 3. *Atlantirivulus riograndensis* showed

Table 1

Seasonal variation of physico-chemical parameters of water during the study period in the habitat of *Atlantirivulus riograndensis*, Lagoa do Peixe National Park, southern Brazil

Month	Dissolved Oxygen [mg · L^{-1}]	pH	Conductivity [mS · m^{-1}]	Temperature [ºC]	Salinity [%]
Jun 2008	6.09	6.41	16	15.94	0.01
Aug 2008	6.63	6.37	14	15.7	0
Oct 2008	9.86	5.73	20	26.63	0.01
Mar 2009	10.86	6.06	14	27.15	0
Aug 2009	10.31	6.48	13	16.5	0
Oct 2009	7.97	6.07	16	25.97	0.01

Table 2

Minimum and maximum total length range, regression parameters, and condition factor for *Atlantirivulus riograndensis* in Lagoa do Peixe National Park, southern Brazil

		TL [mm]		Regression Parameters						
Sex	n	Min	Max	a	95% CI of a	b	95% CI of b	r^2	P	CF
M + F	71	14.75	38.03	–2.59	–0.40 to 0.60	3.32	3.12 to 3.46	0.971	0.0001	0.258 ± 0.003
M	21	20.33	37.5	–2.58	–3.74 to 0.63	3.30	2.89 to 3.71	0.938	0.0001	0.251 ± 0.013
F	50	14.75	38.03	–2.65	0.32 to 1.16	3.36	3.24 to 3.49	0.984	0.0001	0.261 ± 0.006

n = sample size, a and b = parameters of the log-transformed length–weight relations, r^2 = coefficient of determination, P = probability value, TL = total length, CF = condition factor, M = males, F = females, M + F = mixed sexes, Min = minimum length, Max = maximum length.

allometric positive growth ($b > 3$). All regressions were strong ($r^2 > 0.9$) and highly significant ($P < 0.0001$). However, b ($b = 3.3$) for males was highly variable ($CI_{95\%}$ from 2.89 to 3.71).

The condition factor calculated for all fishes was 0.258 ± 0.003. Although females have higher coefficients of CF (Table 2), there was no significant difference in means between the sexes (t-test$_{(69)}$ =1.412; $P = 0.162$). In relation to the variation of *CF* means between the months, we found a significant difference ($F_{(5,59)} = 2.960$ $P = 0.019$). However, the magnitude of the variation appears not to be so strong (mean values ranging from 0.243 ± 0.011 SE in October 2009 to 0.275 ± 0.006 SE in August 2008) (Fig. 4).

DISCUSSION

The body-size differences observed in *A. riograndensis* during the studied period are a reflection of the individual growth throughout each study year. Lanés (unpublished*) found the same pattern in annual species of killifishes in temporary wetlands of the LPNP. In summer and late spring, water temperature and evaporation rate are higher in the study area (Klein 1998). The lack of large individuals in the beginning of 2009 was probably due to a high mortality over this period, as consequence of intrinsic species aspects (e.g., senescence, active and aggressive reproductive behavior) and ecological factors (e.g., decrease of water level and greater vulnerability to predation). Errea and Danulat (2001) observed the growth rate of annual rivulid species over the year, and suggested that the low temperature prolonged the life and delayed the senescence of the species. Simpson (1979) observed that the increase of temperature was a decisive factor for the killifish mortality. Besides, the reduction of water depth may have also increased the predation by water birds (Costa 1998). However, further experimental studies are necessary to validate these questions.

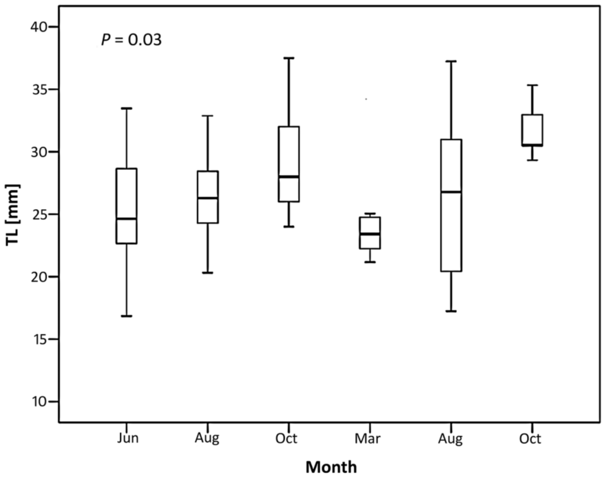

Fig. 2. Seasonal variation of total length (TL) of *Atlantiruvulus riograndensis* in Lagoa do Peixe National Park, southern Brazil

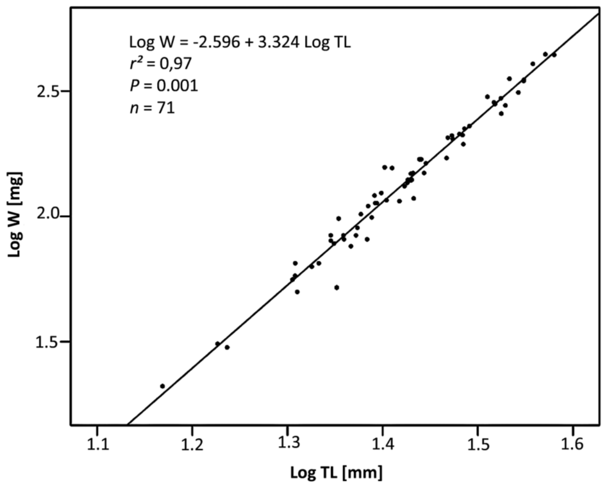

Fig. 3. Log-transformed length–weight relations for *Atlantirivulus riograndensis* from Lagoa do Peixe National Park, southern Brazil; n = number of individuals, r^2 = coefficient of determination, P = significance value, TL = total length

The larger body size observed in males corroborates the pattern of sexual dimorphism commonly observed in other species of Rivulidae (Costa 2003). The difference observed in the sex ratio, with female biased populations, is supported by previous observations for other aplocheiloid species (Laufer et al. 2009, Olaosebikan et al. 2009, Reichard et al. 2009, Okyere 2012). The reasons for this disproportion are unknown. For several fishes, genetic, physiological, and environmental conditions influence the sex ratio (Devlin and Nagahama 2002).

The lack of studies of length–weight relation and condition factor of *Atlantirivulus* make difficult to compare our results. Only one single species of Rivulidae has available information of length–weight relation (Froese and Pauly 2012). *Atlantirivulus riograndensis* population showed an allometric positive growth ($b > 3$), indicating that this species increases more in weight than predicted by its increase in length (Froese 2006). Females had higher values for b. The greatest slope found in females suggested a higher growth rate, and it was probably related to

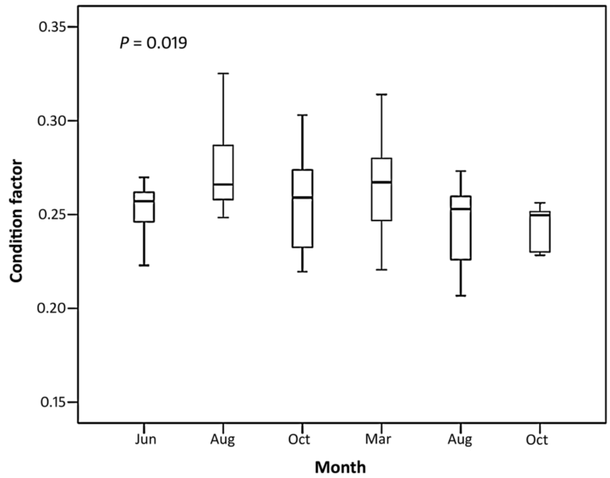

Fig. 4. Seasonal variation in condition factor (CF) for *Atlantirivulus riograndensis* from Lagoa do Peixe National Park, southern Brazil

the greater weight of the gonads of females compared to males. A greater variation of this parameter for males may be related to smaller sample size and reduced body size ranges (Froese 2006).

Low values of condition factor are usually interpreted as results of spawning seasons (Froese 2006). During our study, the lowest values of *CF* were found in late spring (October 2008 and 2009), when only large individuals were found and the water temperature was higher. This result may be related to the fact that killifishes have high energy demand to support physiological adaptations to stress conditions (Lin and Dunson 1999), especially when they are adults and are exposed to high temperatures (Liu and Walford 1966, Errea and Danulat 2001). However, we found a significant but slow magnitude along the seasonal samples. The fast growth of rivulid species, associated with longer and undefined reproductive period and continuous production of eggs (Arenzon 1999, 2001, Arezo et al. 2007, Gonçalves et al. 2011) may be a plausible explanation for this low variation. Nevertheless, data on the feeding and reproductive dynamics of the species are needed to better understand this variation of body condition.

Our results contribute to the knowledge of biology and ecology of *A. riograndensis*, providing information on life history, abundance, sex-ratio, length–weight relations, and condition factor, thus helping conservation efforts for these poorly known and highly endangered species. Wetlands fragmentation and habitat loss are the main threats for the Rivulidae conservation in Brazil (Rosa and Lima 2008). In southern Brazil, 90% of the wetland systems have already been lost mainly due to agriculture, and the remaining ones are still at high risk due to the expansion of rice fields and exotic *Eucalyptus* and pine plantation (Maltchik et al. 2010). Studies conducted in wetlands of LPNP have shown that pine plantation changed the hydroperiod of wetlands causing a negative effect on macrophytes (Rolon et al. 2011), macroinvertebrates (Stenert et al. 2012), amphibians (Machado et al. 2012), and probably on rivulid populations.

Additional studies are needed to search for abiotic and biotic factors regulating the populations of *A. riograndensis*, and to assess whether the apparent rarity of the species is not due to lack of studies on their potential habitats and failures in their detection.

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