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# Distribution, habitat and conservation status of two threatened annual fishes (Rivulidae) from southern Brazil

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ABSTRACT: This study presents information on the distribution of *Austrolebias cyaneus* and *A. juanlangi*, 2 annual fish species of southern Brazil considered threatened. Furthermore, data are provided on the conservation status of these species and their habitat characteristics to assist in the development of management plans and in the selection of priority areas for conservation of annual fishes in southern Brazil. A total of 6 populations of *A. cyaneus* and 3 populations of *A. juanlangi* were sampled from small ponds less than 1000 $m^2$ in surface area. We sampled 104 specimens of *A. cyaneus*, distributed in the municipality of Rio Pardo and Minas do Leão in the basins of the Francisquinho, Capivari, and Dom Marcos streams, Jacuí River basin. *A. juanlangi* was recorded in the municipalities of Herval, Hulha Negra, and Pedras Altas, Jaguarão River basin, with 53 specimens being captured. At the sampling sites, the main threat to both species was rice cultivation. All pools were altered, isolated, and in an advanced state of degradation as a result of this monoculture. The restricted distribution area of the annual fishes and the advanced state of degradation and habitat loss of the wetlands sampled in this study justify the creation of conservation units in the areas of occurrence of Rivulidae species.

KEY WORDS: *Austrolebias cyaneus* · *Austrolebias juanlangi* · Endemic · Endangered species · Rivulidae · Wetlands · Habitat loss



## INTRODUCTION

The family Rivulidae (Cyprinodontiformes, Aplocheiloidei) currently comprises 27 genera and 324 possible valid species distributed in southern North America, Central America, and South America, and it is one of the largest families of freshwater fishes from the Neotropical region. Brazil has the greatest diversity, with about 200 species recorded and 60% of the total Rivulidae (Costa 2008). Despite the species richness of Rivulidae in Brazil, the family is considered the most endangered of all fish in the country (Rosa & Lima 2008). The causes vary according to the region of the country and are mostly related to population density and economic activity.

In the state of Rio Grande do Sul, 26 species of Rivulidae have been recorded, 20 of which belong to the genus *Austrolebias* (Cheffe et al. 2010, Volcan et al. 2010a), 5 to *Cynopoecilus* (Costa 2002a), and 1 to *Rivulus* (Costa & Lanés 2009). The main cause of habitat loss for annual fishes in the state is rice farming, which usually occurs in low areas and floodplains near rivers and streams precisely where there is a greater concentration of wetlands with rivulids (Reis et al. 2003, Rosa & Lima 2008). Of all freshwater fishes threatened with extinction in the state, about 40% are annual fishes (Reis et al. 2003). Despite the human threats and imminent risk of extinction of some species, few studies have focused on the distribution and conservation of this group of fishes (Costa 2002b, Reis et al. 2003, Volcan et al. 2009, 2010a,b,c,d).

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The advanced stage of loss and fragmentation of wetlands in southern Brazil (Maltchik et al. 2004, Guadagnin et al. 2005, Stenert et al. 2008) highlights the urgency of adopting conservation and protection measures in these environments. Locating and mapping the distribution and describing their habitats are critical for the development of strategies that promote conservation. Given the high level of endemism of the fish and their level of imperilment, the annual fishes are in urgent need of conservation assistance.

This paper presents current information on the distribution, abundance, and habitat of the annual fishes *Austrolebias cyaneus* (Amato, 1986) and *A. juanlangi* Costa, Cheffe, Salvia and Litz, 2006. These data were collected to assist in the development of management plans and in the selection of priority areas for conservation of endangered species of Rivulidae in southern Brazil.

## MATERIALS AND METHODS

**Study area.** According to the classification of IBGE (2004), the grasslands areas in the state of Rio Grande do Sul are located in 2 distinct biomes: Mata Atlântica, covering the grasslands that are located to the northeast, and the Pampa, which includes grasslands located in the central and southern half of the state. Around 65% of the territory of Rio Grande do Sul is located in the Pampa biome (IBGE 2004). Several areas of this biome have been prioritized for conservation based on species richness, endemism, and abiotic factors (MMA 2002). Nevertheless, this biome is poorly represented by protected areas (Overbeck et al. 2007).

The study region was the Depressão Central, located in the Pampa biome, in the central and southern half of the state. This region has a slightly undulating topography with wide floodplains. The average altitude is 100 m, while the maximum altitude is between 250 and 300 m. The Depressão Central region is the warmest area in the state, and, according to the Köeppen classification, it has a subtropical climate, with annual precipitation around 1600 mm. However, the average rainfall varies markedly over the year and is more concentrated during the cooler months (July to November), when annual fishes are also reported in southern Brazil (Costa 2006).

**Fish sampling.** As part of a research project conducted by the Ichthyology Laboratory of the Instituto Pró-Pampa to investigate the ecology, distribution, and conservation of annual fishes in the Pampa biome in southern Brazil (Annual Fishes of Pampa project), 4 sampling campaigns were performed between September and November 2009.

To identify the location of wetlands, maps and aerial images were analyzed. Based on the results of the analysis, field studies were carried out to map the sites with Rivulidae. Fish were captured with a D-shaped hand net (60 × 50 cm, 2 mm mesh). To determine the abundance of each species in wetlands, 15 sweeps of 1 m were randomly performed at each sampling site.

The captured specimens were measured (mm), recorded, and returned to their natural habitat. Representative specimens were fixed in 10% formalin and stored in 70% alcohol. Since both species (*Austrolebias cyaneus* and *A. juanlangi*) have restricted distributions and belong to a morphologically homogeneous group (Costa 2002c, 2006), representative specimens were measured with the aid of digital calipers (0.01 mm) to verify whether they were consistent with the findings of relevant taxonomic studies (Costa 2006). Voucher specimens were deposited in the ichthyological collection of the Museu de Ciência e Tecnologia da PUCRS (MCP).

At the site of capture of annual fishes, GPS coordinates, altitude, and wetland area were estimated, and the characteristics of habitat and the physical and chemical parameters of water were assessed with the aid of portable devices; pH, dissolved oxygen (mg $l^{-1}$), conductivity (µS $cm^{-1}$), temperature (°C; Quimis Instruments), transparency (Secchi disk), and depth (cm) were recorded.

**Data analysis.** Correlations between environmental variables were tested using Pearson's coefficient. Stepwise multiple regression with backward selection was used to ascertain the extent to which the environmental variables (area, altitude, water temperature, depth, conductivity, pH, and dissolved oxygen) and richness and abundance of non-annual fishes explained patterns of abundance of *Austrolebias* species. The analyses were performed using Biostat 5.0 after confirming that data satisfied the statistical assumptions of the parametric tests used.

## RESULTS

In total, we sampled 9 wetlands with annual fish occurrence, 6 populations of *Austrolebias cyaneus* and 3 of *A. juanlangi* (Fig. 1). *A. cyaneus* is distributed in the municipalities of Rio Pardo and Minas do Leão, in the basins of the Francisquinho (MCP 45180), Capivari (MCP 45179), and Dom Marcos Streams, all sampling sites in the Jacuí River basin, Patos-Mirim Lagoon system (Fig. 2), totaling 104 specimens. In the floodplains of the Dom Marcos Stream, 1 population was sampled. In the floodplain of the Francisquinho Stream, 2 abundant populations were recorded, while in the flood-

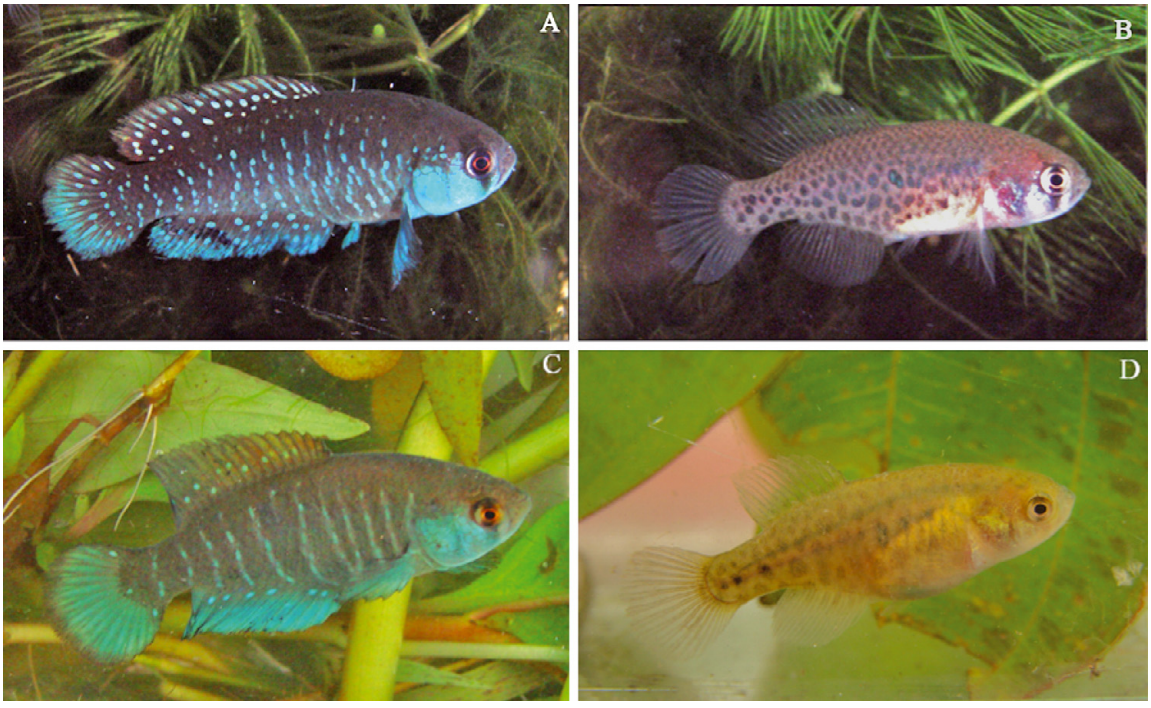


Fig. 1. *Austrolebias* spp. (A) Male and (B) female *A. cyaneus*, captured in the floodplain of Capivari Stream, Rio Pardo. (C) Male and (D) female *A. juanlangi* sampled in the Jaguarão River, Herval. Photos by M. V. Volcan

plains of the Capivari Stream, the occurrence of 3 populations was confirmed (Table 1).

The populations of *Austrolebias juanlangi* were recorded in the municipalities of Herval (MCP 45177), Hulha Negra (MCP 45178), and Pedras Altas (MCP 45181), located in the Jaguarão River basin, Patos-Mirim Lagoon system, and totaling 53 specimens (Table 1). Measurements of the 2 species are given in Table 2.

All sites were extremely shallow, with depths ranging from 8 to 26 cm (Table 3). The low value of transparency observed in the habitats of *Austrolebias juanlangi* (4.5 to 14 cm) was not recorded in pools inhabited by *A. cyaneus*, which were completely transparent. Dissolved oxygen was the parameter with the greatest variation in wetlands with *A. cyaneus* (3.7 to 9.4 mg $l^{-1}$), while conductivity showed the greatest variation in wetlands with *A. juanlangi* (59.4 to 164.9 µS $cm^{-1}$; Table 3).

Sites with *Austrolebias cyaneus* were located at altitudes ranging between 15 and 46 m, while *A. juanlangi* was recorded in highlands up to 152 m a.s.l. (Table 1). Both species were caught in small ponds less than 1000 $m^2$ in size. At the sampling sites, the main threat to both species is rice cultivation, since all pools were altered, isolated, and in an advanced state of degradation. Cattle occurred at all sampling sites, which showed typical signs of grazing and trampling.

Pearson's correlation analysis demonstrated that the wetland altitude was negatively correlated with temperature ($r = -0.79$, $p = 0.01$) and positively correlated with depth ($r = 0.88$, $p = 0.001$), pH was positively correlated with conductivity ($r = 0.93$, $p = 0.0002$) and depth ($r = 0.72$, $p = 0.02$) and negatively associated with temperature ($r = -0.87$, $p = 0.002$). Water temperature was negatively correlated with conductivity ($r = -0.69$, $p = 0.03$) and depth ($r = -0.77$, $p = 0.01$). The other environmental variables were not correlated ($p > 0.05$). Stepwise multiple regression analysis showed that the models incorporating wetland area, altitude, depth, pH, dissolved oxygen, water temperature, and conductivity failed to explain the variation in *Austrolebias* abundance and in the richness of fish species in the sampled wetlands ($p > 0.05$).

*Austrolebias cyaneus,* despite not occurring with sympatric Rivulidae species, occurred with 8 species of non-annual fishes: *Cyphocarax voga*, *Cheirodon ibicuhiensis*, *Cheirodon interruptus*, *Oligosarcus robustus*, *Rhamdia* aff. *quelen*, *Callichthys callichthys*, *Phalloceros caudimaculatus*, and *Synbranchus marmoratus*. *Austrolebias juanlangi* was recorded only with *Astyanax* aff. *eigenmanniorum*, *Cheirodon ibicuhensis*, *Cheirodon interruptus*, and *Hyphessobrycon luetkenii*. Multiple linear regression analysis revealed no influence of richness and abundance of non-annual fishes on the abundance of Rivulidae ($p > 0.05$), which were the most abundant species at all sampling sites.

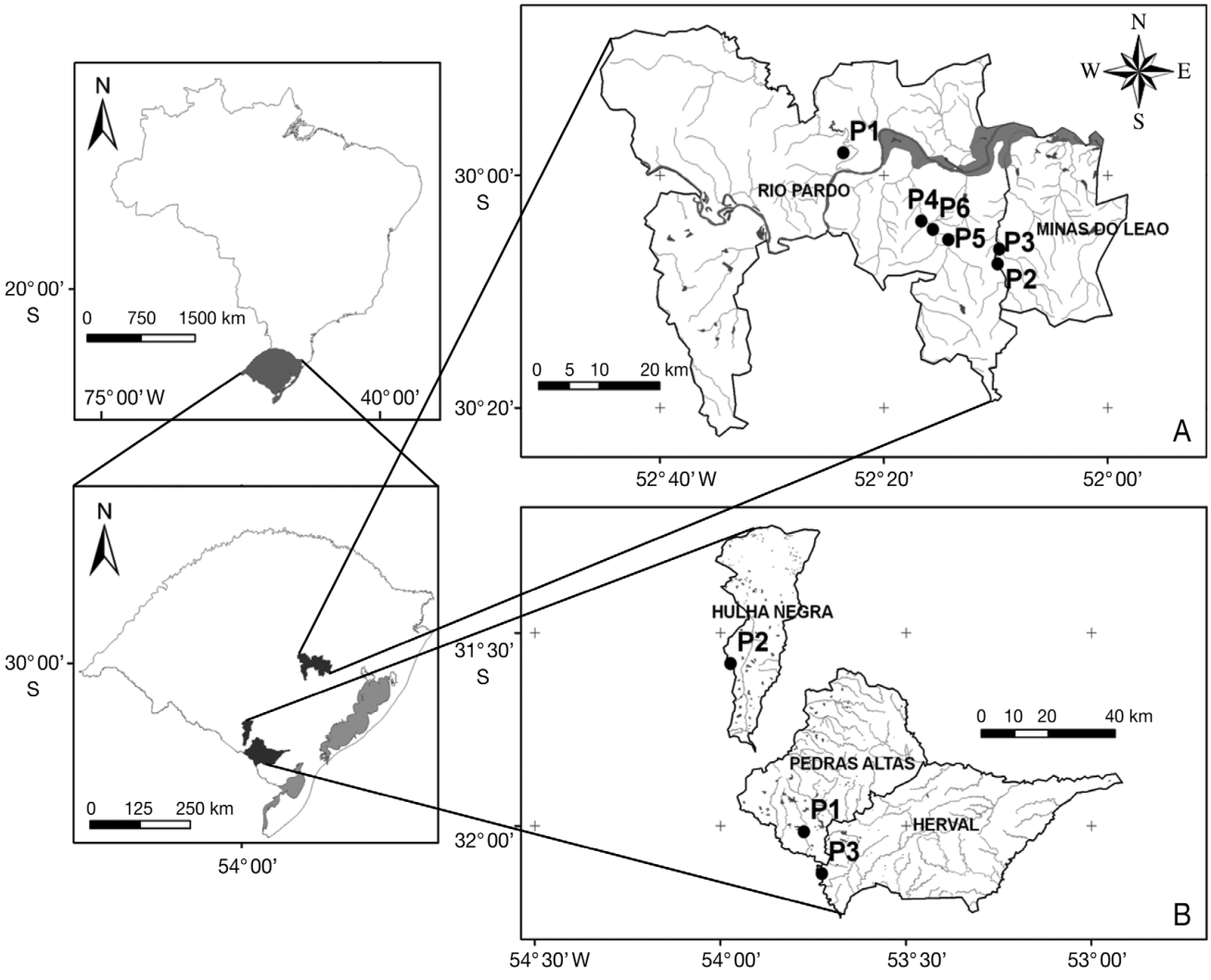


Fig. 2. *Austrolebias* spp. Distribution area of 2 annual Rivulidae fishes in Rio Grande do Sul, southern Brazil. (A) Location of the 6 sampling sites with *A. cyaneus*; (B) sampling sites with *A. juanlangi*. Map by M. P. Corrêa. Source: FEPAM (2005)

Table 1. *Austrolebias* spp. Municipality, basin, sampling sites (see Fig. 2 for locations of sites), coordinates, altitude (Alt. = meters above sea level), estimated area ($m^2$), abundance (N) and standard length (SL, mm) of males and females of *A. cyaneus* and *A. juanlangi* in southern Rio Grande do Sul, Brazil

| Municipality | Basin | Site | Coordinates | | Alt. | Area | Male | | Female | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | South | West | | | n | SL | n | SL |
| ***A. cyaneus*** | | | | | | | | | | |
| Minas do Leão | Francisquinho Stream | P1 | 30° 06′ 24″ | 52° 09′ 41″ | 39 | 800 | 9 | 21.6–22.4 | 12 | 22.0–23.8 |
| Minas do Leão | Francisquinho Stream | P2 | 30° 07′ 40″ | 52° 09′ 51″ | 37 | 40 | 17 | 22.0–37.5 | 13 | 18.0–23.3 |
| Rio Pardo | Capivari Stream | P3 | 30° 03′ 59″ | 52° 16′ 41″ | 15 | 200 | 3 | 21.2–30.2 | 6 | 19.6–27.2 |
| Rio Pardo | Capivari Stream | P4 | 30° 05′ 35″ | 52° 14′ 14″ | 46 | 1500 | 9 | 21.0–31.6 | 8 | 20.0–24.7 |
| Rio Pardo | Capivari Stream | P5 | 30° 04′ 41″ | 52° 15′ 36″ | 26 | 2000 | 2 | 29.4–31.6 | 4 | 22.4–23.3 |
| Rio Pardo | Dom Marcos Stream | P6 | 29° 58′ 05″ | 52° 23′ 37″ | 18 | 300 | 7 | 23.2–31.5 | 14 | 23.0–31.8 |
| ***A. juanlangi*** | | | | | | | | | | |
| Pedras Altas | Jaguarão River | P1 | 32° 00′ 56″ | 53° 46′ 28″ | 84 | 700 | 6 | 31.5–34.7 | 10 | 24.0–26.3 |
| Hulha Negra | Jaguarão River | P2 | 31° 34′ 51″ | 53° 58′ 18″ | 152 | 800 | 2 | 32.4–36.2 | 8 | 24.8–34.0 |
| Herval | Jaguarão River | P3 | 32° 07′ 28″ | 53° 43′ 34″ | 75 | 1500 | 11 | 21.8–26.9 | 16 | 19.3–22.7 |

Table 2. *Austrolebias* spp. Morphometric data (n in parentheses)

| Measurement | *A. cyaneus* | | *A. juanlangi* | |
|---|---|---|---|---|
| | Males (3) | Females (2) | Males (4) | Females (5) |
| Standard length (SL, mm) | 29.3–31.3 | 22.2–23.6 | 23.9–34.1 | 22.6–29.7 |
| Body depth (%SL) | 36.1–38.7 | 36.4–38.7 | 32.9–35.7 | 33.2–35.9 |
| Caudal peduncle depth (%SL) | 15.4–17.3 | 14.9–16.1 | 14.7–15.8 | 12.8–14.4 |
| Pre-dorsal length (%SL) | 47.9–52.0 | 59.7–62.2 | 48.9–54.4 | 56.7–63.7 |
| Pre-pelvic length (%SL) | 40.6–42.0 | 48.3–55.4 | 43.1–48.1 | 49.6–54.2 |
| Length of dorsal fin base (%SL) | 41.2–43.5 | 31.1–33.9 | 38.9–43.4 | 23.4–29.6 |
| Length of anal fin base (%SL) | 41.7–42.8 | 23.9–29.7 | 37.2–40.2 | 21.5–26.3 |
| Caudal fin length (%SL) | 21.1–24.3 | 22.5–24.6 | 21.4–26.4 | 23.3–26.5 |
| Pectoral fin length (%SL) | 21.7–25.3 | 22.5–24.3 | 20.2–23.0 | 18.3–23.5 |
| Head length (HL, mm) | 32.3–36.7 | 33.8–34.3 | 26.2–32.3 | 28.9–31.9 |
| Head depth (%HL) | 86.4–101.0 | 95.1–101.3 | 86.1–109.5 | 91.1–98.7 |
| Head width (%HL) | 53.6–60.6 | 64.0–65.4 | 65.3–77.6 | 63.9–73.4 |

Table 3. Environmental variables related to physical-chemical characteristics of the wetlands with annual fishes in southern Brazil

| Water parameter | *Austrolebias cyaneus* | | | *Austrolebias juanlangi* | | |
|---|---|---|---|---|---|---|
| | Min. | Max. | Mean ± SD | Min. | Max. | Mean ± SD |
| Dissolved oxygen (mg $l^{-1}$) | 3.7 | 9.4 | 7.2 ± 2.4 | 5.9 | 8.2 | 6.9 ± 0.8 |
| pH | 5.1 | 5.5 | 5.2 ± 0.2 | 6.11 | 7.16 | 6.61 ± 0.4 |
| Temperature (°C) | 25.3 | 33.4 | 27.7 ± 3.8 | 14.3 | 19.1 | 16.2 ± 1.9 |
| Conductivity (µS $cm^{-1}$) | 33.2 | 45.8 | 38.2 ± 4.7 | 59.4 | 164.9 | 117.6 ± 46.1 |
| Depth (cm) | 8.0 | 14.0 | 10.8 ± 2.6 | 8.0 | 26.0 | 18.5 ± 6.1 |
| Transparency (cm) | 8.0 | 14.0 | 10.8 ± 2.6 | 4.5 | 10.0 | 6.7 ± 1.8 |

## DISCUSSION

*Austrolebias cyaneus* is endemic to the lower Jacuí River, Patos Lagoon system, and prior to this study, only a single population had been recorded, in the floodplain of the Dom Marcos Stream, municipality of Rio Pardo, Rio Grande do Sul (Costa 2006). Based on the fish captured in this study, we extend the distribution of *A. cyaneus* to a new municipality (Minas do Leão) and 5 new populations, divided into 2 new subbasins (Capivari and Francisquinho Streams).

The annual fish *Austrolebias juanlangi* has been recorded in Brazil in only 3 municipalities: Aceguá, Bagé, and Jaguarão in areas in the floodplains of the Jaguarão River, the Patos-Mirim Lagoon system, and the Negro River in the Uruguay River basin (Costa 2006). In Uruguay, the species has been recorded in the Taquarí River, and some populations were found in the Negro River (Costa 2006). Capture of specimens from 3 populations has expanded the distribution of this species to the municipalities of Herval, Hulha Negra, and Pedras Altas, which are all part of the Jaguarão River basin.

While describing *Austrolebias juanlangi*, Costa (2006) distinguished it from *A. affinis*, whose distribution was restricted to Uruguay. Nevertheless, Rosa & Lima (2008) included *A. affinis* in the list of endangered fish species in Brazil, but failed to include *A. juanlangi* among the species of Brazilian fauna in danger of extinction. The suppression and changes observed in its biotopes and the restricted distribution area of the species justifies its inclusion in the next list of species at risk of extinction in Brazil. In this context, *A. cyaneus* is included in the Red Book of Endangered Species of Fauna of Rio Grande do Sul and Brazil (Reis et al. 2003, Rosa & Lima 2008).

The presence of rice culture at all sampling sites with annual fishes recorded in the present study is considered by many authors to be the main cause of decline of populations of *Austrolebias* in Brazil (Costa 2002b, Reis et al. 2003, Rosa & Lima 2008, Volcan et al. 2009, 2010a,b,c,d). Besides these threats, there is much speculation in the region regarding exotic forestry, an aspect that deserves consideration in the implementation of future projects to prevent the loss of the remaining biotopes.

As observed in the present study, the small size of wetlands was also recorded by Maltchik et al. (2003), who inventoried about 3500 wetlands in southern Brazil and found that around 70% of them were less than 1 $km^2$ in size. This factor is probably a reflection of years of suppression, mainly caused by rice culture. In their study of wetlands of the coastal region of Rio Grande do Sul, Maltchik et al. (2010), observed that most areas are fragmented and small, and that although presenting lower biological richness than relatively larger wetlands, they have a different species composition, suggesting that small wetlands deserve attention, especially in regions where agriculture and forestry are expanding.

According to Errea & Danulat (2001) and Arenzon et al. (2002), annual fishes are generally exposed to marked fluctuations in abiotic conditions in their natural habitats, requiring several adjustments related to their life cycle. Survival under highly variable environmental conditions such as drought stress, low oxygen levels, and wide temperature fluctuations are typical characteristics of this group (Errea & Danulat 2001). In this study, multiple linear regression analysis revealed no relationship between

the environmental variables and abundance of annual fishes. *Austrolebias* species are considered rare (Nogueira et al. 2010), and some species are only known for their type locality (Costa 2008). It is possible that due to the low number of samples and wide variation in environmental conditions, the parameters used to characterize the wetlands could not explain the variation in the abundance of annual fishes.

The abundance and richness of non-annual fishes recorded in the present study did not influence the abundance of *Austrolebias.* The co-occurrence of these species (annual and non-annual fishes) was reported in other studies in southern Brazil and Uruguay (Vaz-Ferreira et al. 1966, Quintela et al. 2007). The coexistence of different species in temporary pools may likely be explained by the connection of water bodies during the rainy season (Vaz-Ferreira et al. 1966, Errea & Danulat 2001). All species of sympatric occurrence with Rivulidae recorded here are common to the Patos Lagoon system (Malabarba 1989, Reis et al. 2003).

According to Rosa & Lima (2008), because the species of *Austrolebias* were recorded in small ponds like those mentioned in this study, the protection of relatively small areas could ensure the preservation of the species. However, according to Volcan et al. (2010b,c), fragmentation and habitat loss prevent the connection of wetlands inhabited by Rivulidae and dispersal of species in floodplains. Thus, according to Volcan et al. (2010b,c), besides protecting their restricted habitats, it is necessary to establish and create large protected areas, mainly along river corridors and associated wetlands, such as the Jacuí and Jaguarão River basins, in order to enable connectivity and dispersal populations of annual fishes, assisting in their conservation.

Finally, none of the captured species occurs in protected areas. These species are endemic to Pampa (Costa 2006), and this biome is in general scarcely represented by protected areas, being considered neglected compared to other biomes of Brazil (Overbeck et al. 2007). Accordingly, areas with records of Rivulidae species could provide support for defining priority areas for the conservation of wetlands and biota that depend on these threatened ecosystems in southern Brazil.

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