Abundance variations and life history traits of two sympatric species of Neotropical annual fish (Cyprinodontiformes: Rivulidae) in temporary ponds of southern Brazil

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Abundance variations and life history traits of two sympatric species of Neotropical annual fish (Cyprinodontiformes: Rivulidae) in temporary ponds of southern Brazil

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We investigated abundance variations and life history traits (body size, sex ratio, length–weight relationships and condition factor) of two species of annual fish (*Austrolebias minuano* Costa and Cheffe and *Cynopoecilus fulgens* Costa) in temporary ponds of southern Brazil. Six samples were taken from small temporary ponds during three annual seasons over 2 years (2008–2009). A total of 104 individuals of *A. minuano* and 433 individuals of *C. fulgens* were collected. The abundance of both species tended to decrease from autumn to spring in the 2 years. The overall sex ratio of *A. minuano* and *C. fulgens* were 1.0 male to 1.8 females and 1 male to 1.04 females, respectively. Juveniles of both species occurred only in autumn over the two years and no adult was observed during this season. While the length–weight relationship of *A. minuano* indicated hyperallometric growth, the relationship of *C. fulgens* indicated values close to isometric growth.

Keywords: *Austrolebias*; *Cynopoecilus*; killifish; restricted range fish; endangered fish species

Introduction

The annual recurrence of a dry period is an important hydrological characteristic of temporary aquatic habitats (Williams 2006). Hydrological extremes (wet and dry) require specific adaptations of organisms that inhabit these environments, such as rapid growth, resistance structures, dispersal capacity and several adaptations related to environmental physical–chemical variations (Oertli et al. 2002, 2009; Sam Lake 2011). The animal species that live in these time-constrained habitats are dominated by macroinvertebrates and amphibian larvae (Laufer et al. 2009). Strictly aquatic species, like fish, are rare in temporary aquatic ecosystems (Drenner et al. 2009) and their occurrence is associated with the surface water exchange between these habitats and permanent lakes and streams (Medeiros and Maltchik 2001; Maltchik and Medeiros 2006) or when the species of fish have specific adaptations such as aestivation or aerial respiration (Magoulick and Kobza 2003).

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Several species of Rivulidae (endemic to the Americas) and Nothobranchiidae (endemic to Africa) are short-lived fish with specific adaptations for surviving long dry periods that also require a dry period to trigger eggs to hatch (Wourms 1972). Annual fish live exclusively in seasonal wetlands (Costa 2008) and have specific adaptations that allow them to inhabit temporary aquatic environments, such as embryonic diapause in the eggs, rapid growth, early sexual maturity (Arenzon et al. 1999) and high reproductive capacity (Gonçalves et al. 2011).

Rivulidae species survive during dry periods as dormant eggs with embryonic diapause (Myers 1942). Eggs remain in the sediments during dry periods in different embryonic stages (Wourms 1972), which reduces the risk of flash floods triggering all of the eggs in the pool to hatch. When the wet season begins, the eggs hatch and juveniles grow quickly, which allows them to reach reproductive maturity at 6–8 weeks old (Liu and Walford 1970; Errea and Danulat 2001; Costa 2006).

The two genera of annual fish in southern Brazil are *Austrolebias* Costa and *Cynopoecilus* Regan (Costa 2002, 2006). *Austrolebias* comprises approximately 40 species (Costa 2008; Loureiro et al. 2011), which are distributed throughout southern Brazil, southern Bolivia, Paraguay, Uruguay and northeastern Argentina (Costa 2010). *Cynopoecilus* has a more restricted distribution and includes five species in southern Brazil and Uruguay (Costa 2002). Studies of these two genera have focused on taxonomic aspects (Costa 1998; 2002; 2006; Loureiro and García 2008; Loureiro et al. 2011), diet (Costa 2009; Laufer et al. 2009; Arim et al. 2010; Keppeler et al. 2013), growth (Liu and Walford 1970; Errea and Danulat 2001; Arenzon et al. 2002), reproductive behaviour (Belote and Costa 2004; García et al. 2008) and biogeographic patterns (Costa 2010; García et al. 2012).

Despite the uniqueness of the life cycle of annual fish, information related to population dynamics (e.g. abundance variation), life history traits (growth, length–weight relationships, condition factor and sex ratio) and influence of environmental parameters based on temporal series of wild populations are extremely limited. Annual fish species generally have a restricted distribution, low vagility and are highly endangered (Costa 2008). Understanding the life history traits of annual fish is fundamental to effective species conservation (Volcan et al. 2009; Volcan et al. 2010; Lanés and Maltchik 2010). Furthermore, these fish have also become important models for laboratory studies of aging and senescence (Genade et al. 2005; Reichard et al. 2009).

Taking into account the limited knowledge about population dynamics and life history traits of annual fish, we investigated temporal variations in abundance, body size distribution, sex ratio, length–weight relationships and condition factor of two sympatric, endemic and endangered annual fishes (*Austrolebias minuano* Costa and Cheffe 2001 and *Cynopoecilus fulgens* Costa 2002) for 2 years in temporary ponds of southern Brazil. We also analysed the influence of environmental variables on the abundance and condition factor of these two species. Our approach is an exploratory analysis of annual fish ecology based on temporal series for further hypothesis testing.

Material and methods

Study area

The coastal plain of southern Brazil has a high concentration of large and small wetlands (Maltchik et al. 2004). The study area (Figure 1) is located in the Lagoa do
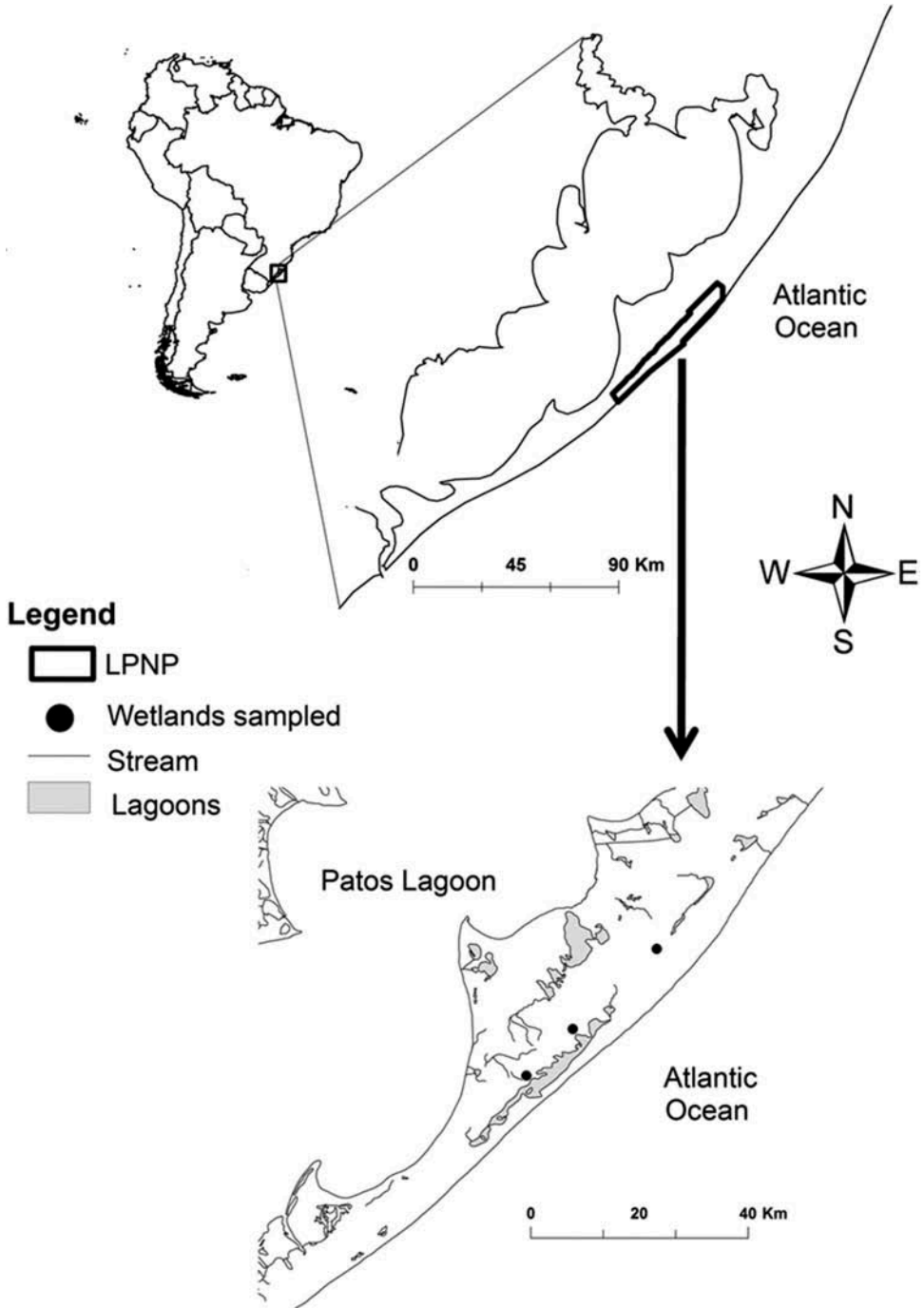
Figure 1. Map of South America and location of the Lagoa do Peixe National Park (LPNP) and study sites sampled in southern Brazil in 2008 and 2009.

Peixe National Park (LPNP), which is the only Ramsar site in southern Brazil. The LPNP was established in 1986 and designated as a Ramsar site and Biosphere Reserve because of its ecological importance for biodiversity conservation. The LPNP (31°02'—31°48' S, 50°77'—51°15' W) has an area of approximately 345 km^2 and protects estuarine, freshwater aquatic and terrestrial habitats (e.g. dune, grassland and shrubby–arboreal vegetation). The absence of hills and the low altitude (< 20 m above the sea level) throughout the study area make the climatic conditions (precipitation and temperature) similar across the study region (Rambo 2000).

Sampling

Six samples were taken from small temporary ponds distributed among three native grassland matrices (five ponds sampling for each grassland matrix). The terrestrial vegetation of each native matrix was characterized by grasslands, with sparse shrub and tree formations. Each grassland matrix (5 ha) represented a complex of approximately 20 small temporary ponds (small ground depressions) that each had an area between 0.1 and 0.2 ha and a depth of no more than 30 cm. The topography of all studied matrices was flat and the climate was subtropical humid, with average temperatures of 14.6°C in winter (June–August) and 22.2°C in summer (December–February), and an annual average of 17.5°C. Annual precipitation of three matrices varies between 1150 and 1450 mm, with an annual average of 1250 mm (Tagliani 1995). Three natural grassland matrices were sampled to better represent spatially the population dynamics and life history traits of both studied species. The matrices were located at least 20 km apart to avoid spatial autocorrelation. Samplings were performed during three annual seasons (autumn, winter and spring) over 2 years (2008 and 2009). The flooded area of ponds was greater in autumn and winter, decreasing during late spring until no surface water was observed during summer (December, January, February). No samples were taken in the summer because all temporary ponds were dry. Aquatic vegetation was abundant in the ponds and formed multi-specific stands without defined habitats. The main types of vegetation were floating rooted (*Leersia hexandra*, *Luziola peruviana*, *Ludwigia peploides*, *Ludwigia grandflora*, *Myriophyllum aquaticum*, *Nymphoides indica*, *Polygonum hydropiperoides*) and submerged (*Scirpus submersus*, *Urtricularia gibb)* (Rolon et al. 2011). Physical and chemical parameters of the water (pH, electrical conductivity, turbidity, dissolved oxygen, temperature, total dissolved solids and oxidation–reduction potential) were measured using a Horiba U-10 water-quality checker.

We collected fish with a D-shaped hand net (60-cm width, 1-mm mesh), anaesthetized them with clove oil and fixed them immediately in a 10% formalin solution. We performed a total of 50 sweeps in each wetland system distributed as 10 sweeps per pond. Each sweep covered an area of 0.6 m^2, resulting in a sampling effort of 300 sweeps, covering an area of 180 m^2 per wetland system throughout the study. We identified the fish to the species level and classified them by demographic groups (juveniles, females, males) according to the presence of visible sexual characteristics (patterns of coloration and fin shape) (Costa 2002, 2006). We measured the fish to the nearest 0.01 mm (standard length, L_S) and weighed (W) them to the nearest mg with a digital scale to an accuracy of 0.0001 g. The specimens were captured under the

authorization of the Brazilian Ministry of Environment (MMA/IBAMA/ICMBio, 18576–1).

Data analysis

Abundance values were square-root transformed to meet the assumptions of normality and equality of variances. We used a 3×2 contingency table to determine whether the sex ratio changed over the three seasons, and a chi-squared test to analyse the overall sex ratio for both species. We used a two-way analysis of variance (ANOVA) to test the effects of season and year on the abundance, standard length (L_S), and condition factor (K) of *A. minuano* and *C. fulgens*, with post-hoc Tukey tests for multiple comparisons between the annual seasons.

We performed a simple linear regression to evaluate the length–weight relationship [$W = \log (a) + b * \log (L_S)$] throughout the study period. We calculated the 95% confidence interval (95% CI) for b to determine whether the hypothetical isometric value ($b = 3$) remained between those limits (Froese 2006). The slope of the regression line (b) calculated for each species (mean value of all individuals collected) was used to estimate the condition factor [$K = (W/L_S^b) * 100$].

The seven environmental variables (Table 1) were subjected to a principal component analysis (PCA), which used the mean values collected for each wetland in each season over the 2 years of the study. For PCA ordination all variables were standardized by z-scores. We analysed the influence of the environmental variables on the abundance and condition factor for each of the two species with a multiple linear regression using scores of the first three axes of the PCA ordination. In this analysis, the condition factor was the mean value for all individuals collected in each wetland per season.

Results

Abiotic variation during the study period

The range of values for each environmental variable per season is shown in Table 1. The environmental variation was lower among seasons than among ponds, except for water temperature in the winter. Environmental variables were reduced to three principal components that explained 80.1% of the variation in the abiotic characteristics. The first component, which represented 37.5% of the variation was related to

Table 1. Range of variation (min–max) of water quality parameters for the study seasons in temporary ponds of Lagoa do Peixe National Park, southern Brazil.

Abiotic parameter	Autumn	Winter	Spring
pH	6.03–7.42	6.31–8.88	6.46–8.24
Electrical conductivity (mS/cm)	15–21.17	11.3–26	10.4–24
Turbidity (NTU)	47–124.35	14.25–245	44–179.7
Dissolved oxygen (mg/L)	5.91–9.36	9.65–12.64	6.09–12.18
Temperature (°C)	18.41–27.83	10.25–19.1	19.98–29.68
Total dissolved solids (g/L)	0.1–0.96	0.07–1.92	0.12–2.6
Oxidation reduction potential (mV)	99.5–274.14	243.2–339.7	144.9–403

dissolved oxygen ($r = 0.964$), oxidation–reduction potential ($r = 0.903$) and temperature ($r = -0.792$). The second component (23.8%) was positively correlated with pH ($r = 0.802$) and total dissolved solids ($r = 0.727$), and the third component (18.8%) was associated with conductivity ($r = 0.875$).

Austrolebias minuano

A total of 104 individuals (57 in 2008 and 47 in 2009) were collected during the study period. The majority of individuals were observed in autumn (63.4%) and winter (26.9%); only nine adult individuals were collected in spring. The abundance tended to decrease from autumn to spring in the both years of the study (Figure 2A), but this was not statistically significant (ANOVA, d.f. = 2,12, $p = 0.090$). The lack of significance may be related to the high variability observed in autumn (Figure 2A). There was no difference in *A. minuano* abundance between years (ANOVA, d. f. = 1,12, $p = 0.866$).

The L_S of *A. minuano* ranged from 8.87 mm (autumn 2009) to 44.38 mm (spring 2009). Mean L_S was similar between the two years (ANOVA, d.f. = 1,98, $p = 0.06$), but varied among seasons (ANOVA, d.f. = 1,98, $p < 0.001$). L_S was lower in autumn than in spring ($p = 0.001$) in both study years (ANOVA, df. = 1,98, $p = 0.627$, Figure 3A). The highest mean L_S values were observed in the end of the annual cycle (spring, Figure 3A).

The overall sex ratio of *A. minuano* was 1.0 male to 1.8 females, and it did not deviate from the expected 1 : 1 rate ($\chi^2 = 2.941$; $p = 0.086$). Although females have predominated in autumn and spring (Figure 4A), there were no significant seasonal differences in sex ratio ($\chi^2 = 1.860$; $p = 0.173$). Juveniles occurred only in autumn over the 2 years and no adult was observed during this season.

The length–weight relationship (Figure 5A) was estimated as log $W = -2.732 + 3.701$ log L_S (linear regression: $r^2 = 0.99$; $p = 0.001$), which indicates hyperallometric growth ($b = 3.701$; 95% CI = 3.64 to 3.76). The mean condition factor was $K = 0.19 \pm 0.01$ SE. Although the condition factor was similar among females, males and juveniles over the study period (ANOVA, d.f. = 2,100, $p = 0.602$), it changed among seasons (ANOVA, d.f. = 2,100, $p = 0.001$, Figure 6A) and was higher in spring than in winter ($p = 0.001$) and autumn ($p = 0.002$). The condition factor was similar between the two study years ($p = 0.092$). Physical and chemical water variables were not associated with abundance or condition factor ($p > 0.05$).

Cynopoecilus fulgens

A total of 433 individuals (223 in 2008 and 210 in 2009) were collected during the study period. The majority of *C. fulgens* individuals were collected in autumn (65.12%), and 19.16% and 15.7% were collected in winter and spring, respectively. Abundance tended to decrease from autumn to spring (Figure 2B) in both years, although this was not statistically significant (ANOVA, d.f. = 2,12, $p = 0.119$). The abundance did not change between the two study years (ANOVA, d.f. = 1,12, $p = 0.799$).

The L_S of *C. fulgens* ranged from 6.44 mm (autumn 2009) to 43.54 mm (spring 2008). Mean L_S varied during the year (ANOVA, d.f. = 2,427, $p < 0.001$), and it was lower in autumn than winter and spring (Figure 3B) in both years. L_S did not differ
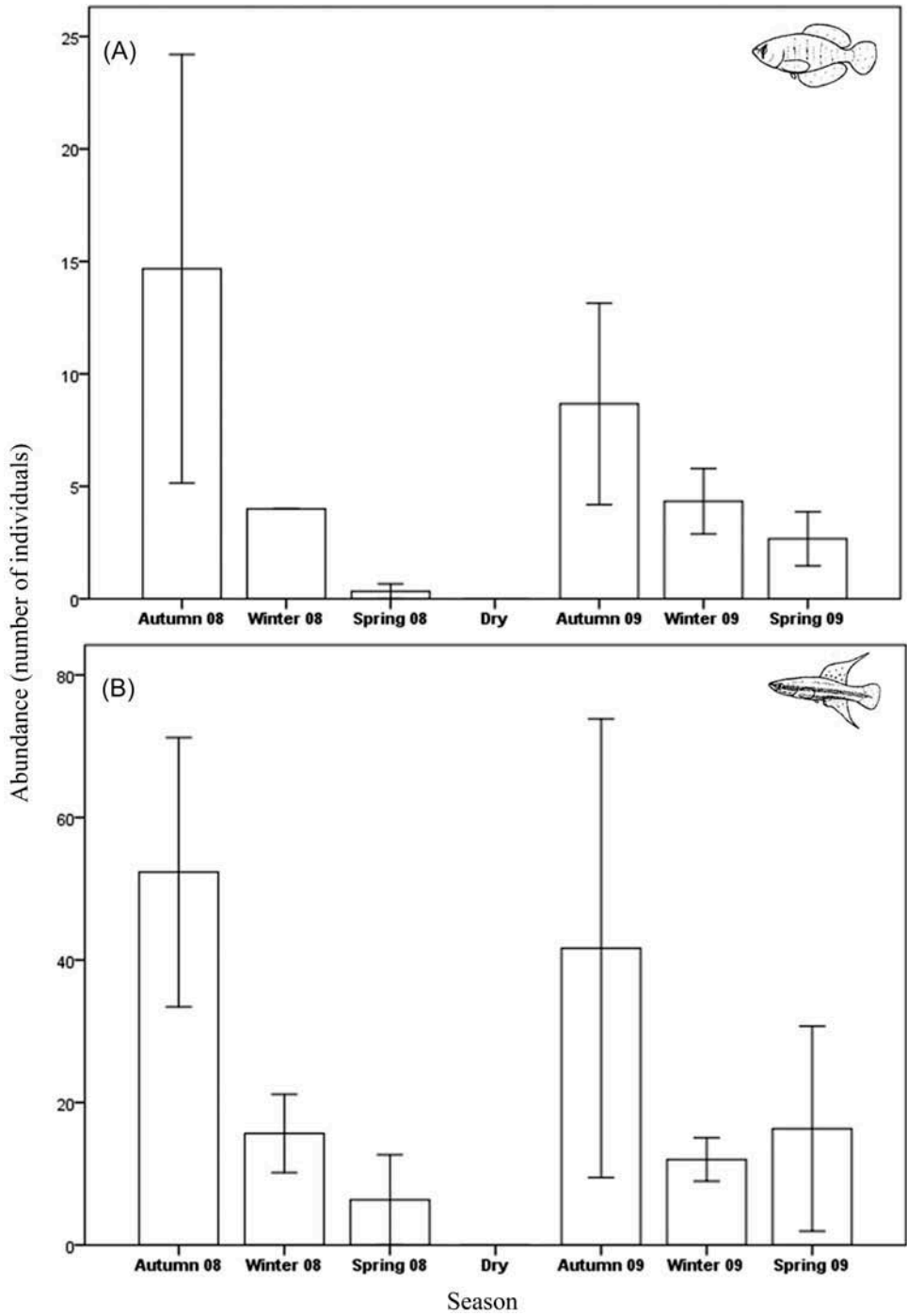
Figure 2. Mean abundances ± SE of *Austrolebias minuano* (A) and *Cynopoecilus fulgens* (B) sampled in temporary ponds of Lagoa do Peixe National Park, southern Brazil, in 2008 and 2009.
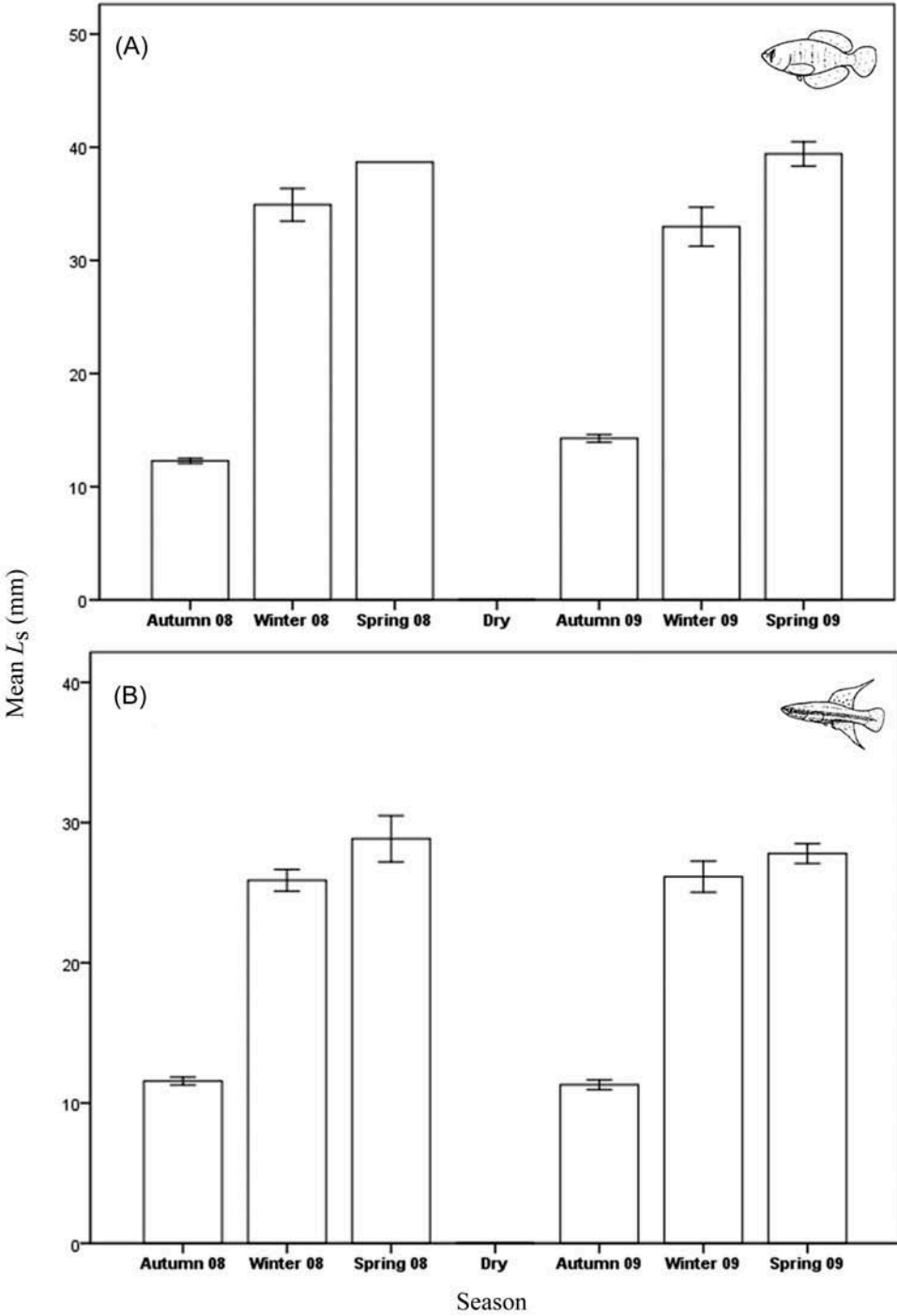
Figure 3. Mean standard length (L_S) ± SE of *Austrolebias minuano* (A) and *Cynopoecilus fulgens* (B) sampled in temporary ponds of Lagoa do Peixe National Park, southern Brazil, in 2008 and 2009.

Figure 4. Sex ratio for *Austrolebias minuano* (A) and *Cynopoecilus fulgens* (B) sampling in temporary ponds of Lagoa do Peixe National Park, southern Brazil, in 2008 and 2009.

Figure 5. Length–weight relationship for *Austrolebias minuano* (A) and *Cynopoecilus fulgens* (B) sampled in temporary ponds of Lagoa do Peixe National Park, southern Brazil, in 2008 and 2009.

Figure 6. Condition factor (K) ± SE of *Austrolebias minuano* (A) and *Cynopoecilus fulgens* (B) sampled in temporary ponds of Lagoa do Peixe National Park, southern Brazil, in 2008 and 2009.
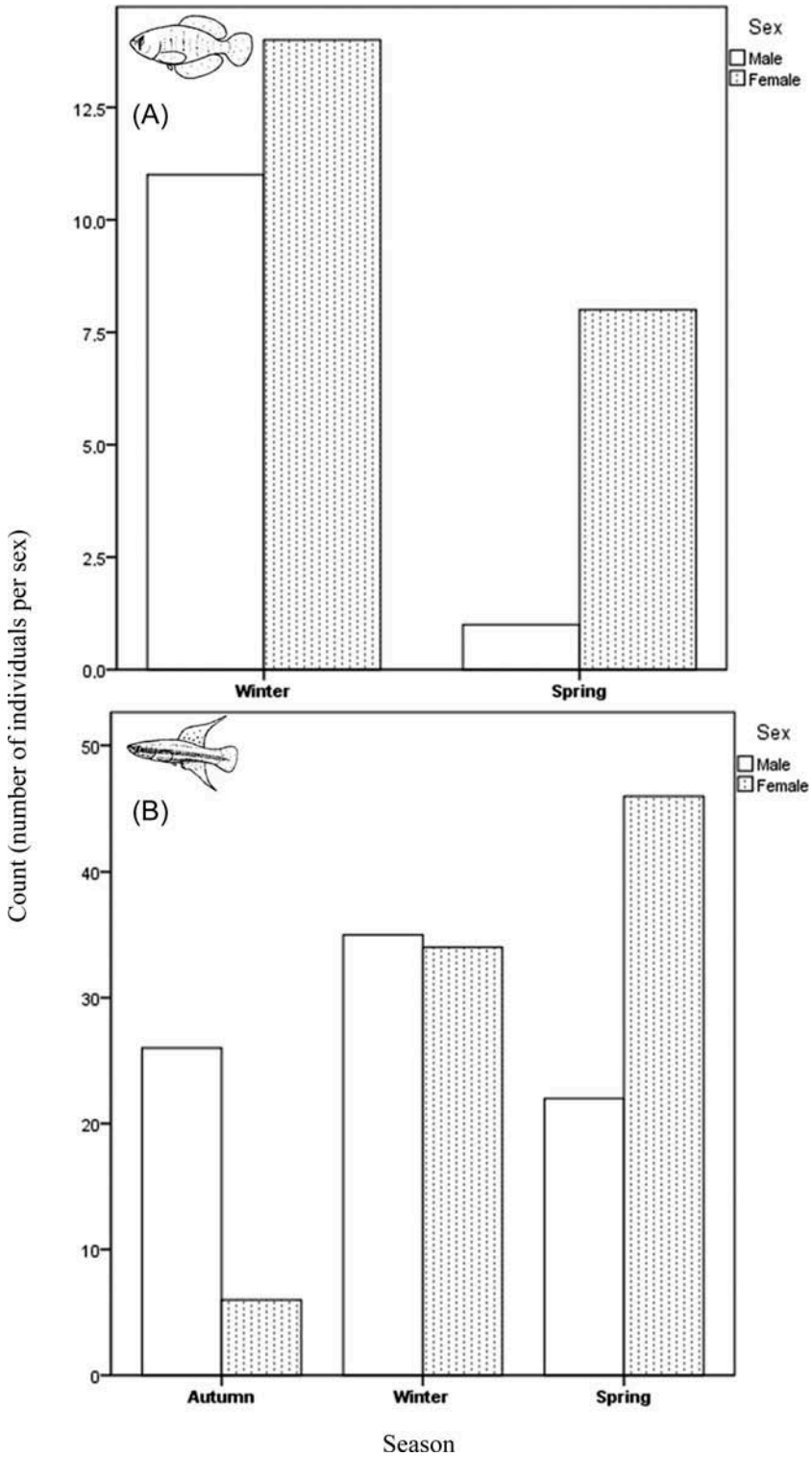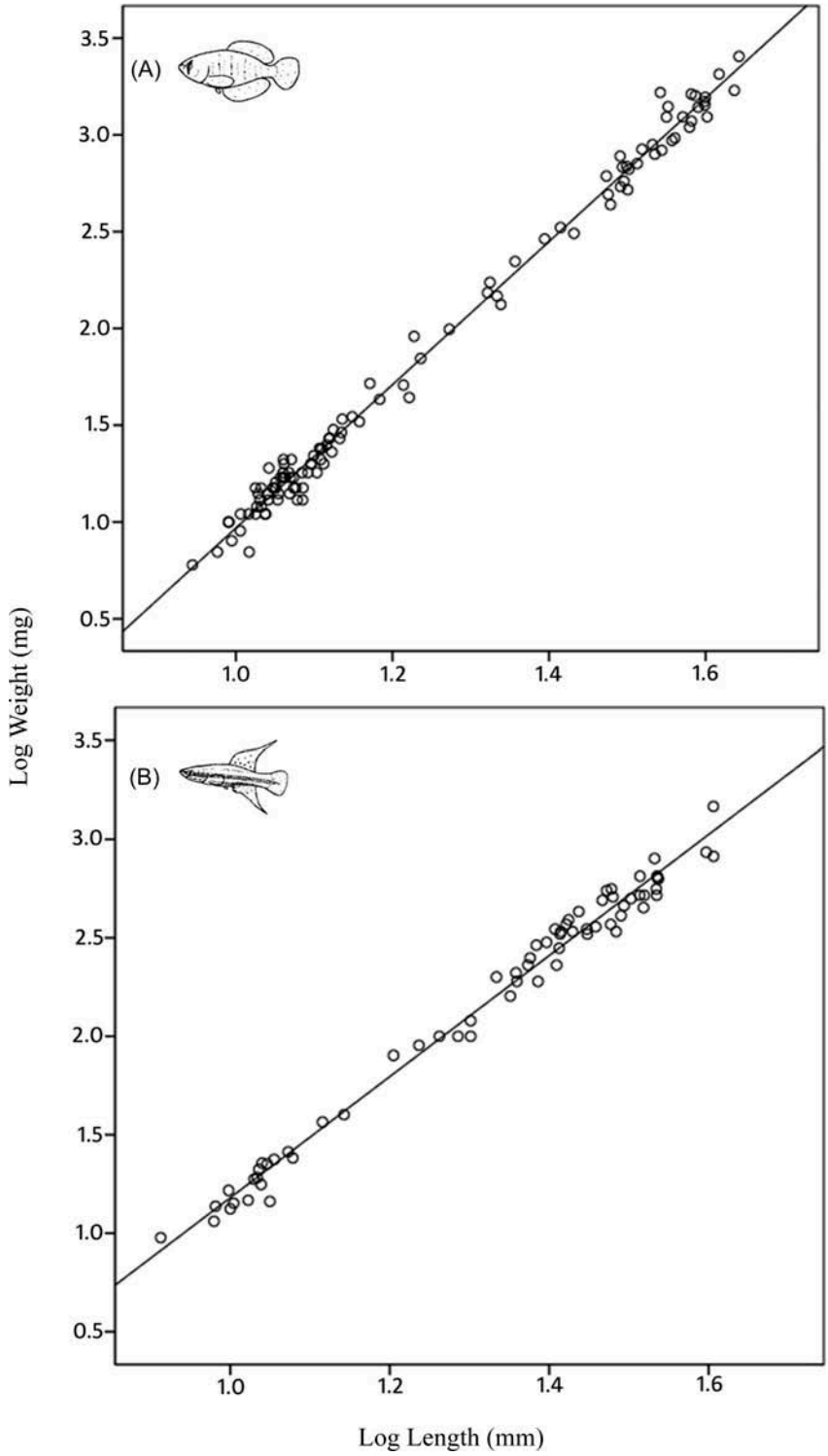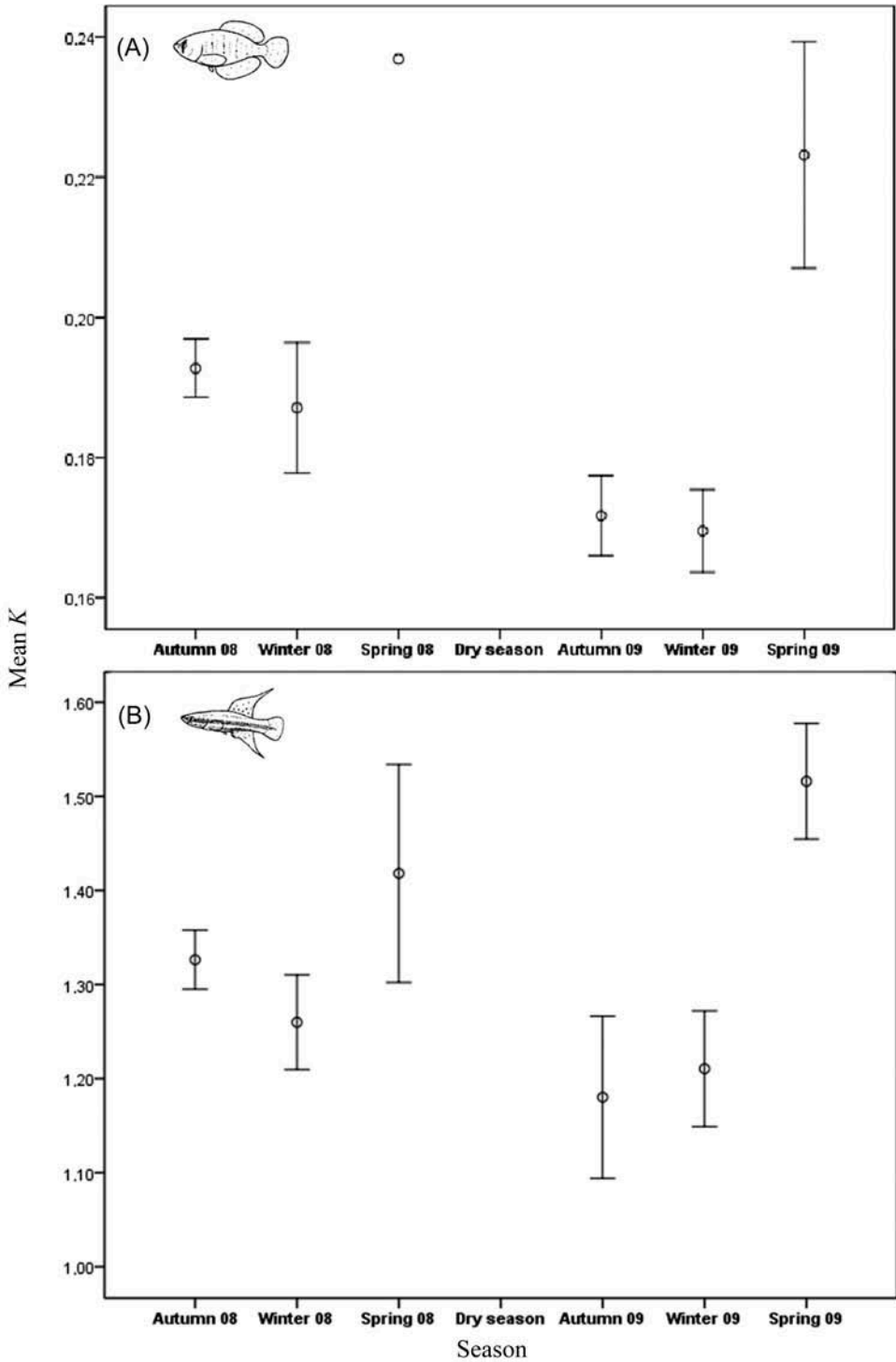
between the first and second year (ANOVA, d.f. = 1,427, *p* = 0.541), and there was no interaction between season and year (ANOVA, d.f. = 2,427, *p* = 0.706).

The same proportion of males to females was found over the entire study period; the overall sex ratio was 1 male to 1.04 females (χ^2 = 0.053; *p* = 0.817). However, there was a significant deviation from the expected 1 : 1 sex ratio in some seasons (χ^2 = 20.938; *p* < 0.001). Although males were more abundant than females in autumn, females outnumbered males in spring (Figure 4B).

The length–weight relationship (Figure 5B) was estimated as *log* $W = -1.893 + 3.074\ log\ L_S$ (linear regression: r^2 = 0.98; *p* = 0.001), which indicated values of slightly positive allometric growth very close to isometry (*b* = 3.07; 95% CI = 2.98 to 3.16). The mean condition factor was $K = 1.32 \pm 0.05$ SE. The condition factor varied among females, males and juveniles over the study period (ANOVA, d.f. = 2,430, *p* < 0.001), and it was higher in females than males and juveniles (*p* < 0.001). The condition factor varied among seasons (ANOVA, d.f. = 2,427, *p* = 0.002), and it was higher in spring than autumn and winter (*p* < 0.001) (Figure 6B). A similar result was observed in both study years and there was no significant interaction between season and year (ANOVA, d.f. = 2,427, *p* = 0.226) and between years (ANOVA, d.f. = 1,427, *p* = 0.538). Physical and chemical water variables were not related to abundance or condition factor (*p* > 0.05).

Discussion

The abundance of *A. minuano* and *C. fulgens* tended to decrease from autumn to spring in both years. The decrease in abundance over the year may be a result of the natural aging and senescence associated with early development (Errea and Danulat 2001; Arezo et al. 2005), rapid growth and the intense reproductive activity of these species (Arenzon et al. 2001; Volcan et al. 2012). Competitive interactions (intra- and inter-specific) and predation by other non-annual fish, macroinvertebrates and birds also may have played an important role in the decreasing abundance (Winemiller and Jepsen 1998; Walsh and Reznick 2008; Costa 2009). Competition or predation by non-annual fish is a major factor in eliminating adult annual fish in seasonal ponds (Nico and Thomerson 1989). Vaz-Ferreira et al. (1966) observed a higher abundance of annual fish in the beginning of the wet period and that a decrease over the year was associated with the increase of non-annual fish abundance and richness. *Hoplias* aff. *malabaricus* (Bloch) is the most abundant piscivorous fish in the study area (Loebmann and Vieira 2005), and rivulid predation by *Hoplias* has been documented (Corrêa et al. 2012). Aquatic birds may also play an important role as predators of annual fish in temporary habitats (Costa 1998, 2009), mainly when the water level decreases (Winemiller and Jepsen 1998; Casciotta et al. 2005; Almirón et al. 2008). The abundance reduction observed over the year of two annual fish species was not associated with the disappearance of their habitats, because all sampling was performed with surface water present. The increase of body size observed over the year, the occurrence of juveniles only at the start of the annual hydrological cycle, and the decrease of abundance observed over the year (without recruitment events) of the two annual fish species suggest a single age cohort per year. However, this should be investigated in further hypothesis testing.

Although males of *A. minuano* were less abundant than females, the proportion of sexes did not vary over the study period. This result may be related to the low representativeness of adults in our samples. Females of *Austrolebias* are generally more abundant than males (Laufer et al. 2009; Lanés et al. 2013), and strong female-biased populations are also observed in annual killifish of Africa (Nothobranchiidae) (Reichard et al. 2009; Polačik et al. 2011). The sex ratio of *C. fulgens* varied over the annual cycle. Males outnumbered females at the beginning of the wet period (autumn) but decreased in proportion in spring. This pattern suggests that males tend to have higher mortality rates in adulthood, perhaps because their sexually dimorphic colouration is more evident in this period. Promislow et al. (1992) showed that although sexual dimorphism provides a mating advantage for males, it also increases their predation risk. The greater number of males of *C. fulgens* in autumn may be an adaptive strategy to compensate for a large difference in mortality between the sexes. Other studies have shown contrasting results for related species. While Gonçalves et al. (2011) observed differences in the sex ratio of *Cynopoecilus melano-taenia* (Regan), with a male-biased population (2 : 1), Laufer et al. (2009) observed a 1 : 1 sex ratio for the same species. Neither of study analysed seasonal variations in sex ratio.

Based on the slope (*b*) of the relation between length and weight, *A. minuano* showed hyperallometric growth (the fish increases more in weight than predicted by its increase in length) (Froese 2006; Froese et al. 2011). *Austrolebias viarius* (Vaz-Ferreira, Sierra-de-Soriano and Scaglia-de-Paulete), *Austrolebias arachan* Loureiro, Azpelicueta and García, and *Austrolebias nigrofasciatus* Costa and Cheffe showed similar patterns of growth (Errea and Danulat 2001; Volcan et al. 2012; Lanés et al. 2013). *Cynopoecilus fulgens* had hyperallometric growth, but the values found were very close to isometry, which suggests that all dimensions of this species increase at the same rate (Froese 2006). The differences observed in the values of *b* in the two species are associated with different body shapes and types of growth. While *A. minuano* has a more laterally compressed body shape (Costa and Cheffe 2001), *C. fulgens* has a more fusiform body type (Costa 2002).

The condition factor of *A. minuano* and *C. fulgens* was highest at the end of the life cycle (spring). The lower values observed in autumn and winter suggest that these species invest more in growth in the beginning of the life cycle. Arenzon et al. (2002) reported similar observations for *Cynopoecilus* and Liu and Walford (1975) and Errea and Danulat (2001) for *Austrolebias* species. Although spring differed significantly from the other seasons, the values of *K* were similar across seasons (with lower values in winter). This result may be related to the rapid growth of annual fish species and a continuous reproductive period after reaching sexual maturity (Wourms 1972; Arenzon et al. 1999).

Environmental factors did not influence abundance and condition factor of *A. minuano* and *C. fulgens*. Annual fish are generally exposed to extremely variable environments with marked fluctuations in abiotic conditions which require life history adaptations (Arenzon et al. 2001; Errea and Danulat 2001; Volcan et al. 2012; Gonçalves et al. 2011). Annual fish are considered some of the most remarkable extremophile organisms among vertebrates (Berois et al. 2012). Schwartz and Jenkins (2000) suggested that assemblages in temporary waters were not clearly related to abiotic conditions, which indicates that the community structure is a result of annual habitat desiccation. Laufer et al. (2009) suggested that the temporal limitations of the habitat seem to determine the variations in ecological traits of annual killifish species.

The overall abundance of *A. minuano* was much lower than that of *C. fulgens* (1 : 4.16). This pattern corroborates some studies performed in other areas where the genera *Austrolebias* and *Cynopoecilus* co-occur (Quintela et al. 2007; Gonçalves et al. 2011). On the other hand, dominance of *Austrolebias* over *Cynopoecilus* was also observed (Laufer et al. 2009; Arim et al. 2010; Volcan et al. 2010), demonstrating that this pattern is subject to regional and local variation and should be further investigated. Natural selection is generally strong in small intermittent wetlands and competitive exclusion should be expected between annual fish species. However, several annual fish species were observed in a single temporary pond (Laufer et al. 2009). Although both species have shown a similar pattern of variation in the abundance and body size, they belong to distinct phylogenetic lineages, with significant differences in body shape, colour pattern and reproductive characteristics (egg morphology and courtships). These variations suggest two different annual life cycle strategies between co-occurring species (Laufer et al. 2009; Arim et al. 2010).

The Rivulidae family has a high number of endangered species in Brazil (Rosa and Lima 2008) due to the many endemic species, small population sizes and occurrence in habitats that are extremely vulnerable to human activities (Volcan et al. 2009; 2011; Lanés 2011; Lanés et al. 2012; Costa 2012). This study reports on the temporal dynamics and life history traits of two sympatric species of Neotropical annual fish that depend exclusively on temporary ponds for their survival. The conservation of small and seasonal ponds and wetlands is essential to maintain annual fish species and these systems should be included in conservation programmes in southern Brazil, where more than 90% of wetland systems have already been lost and those that remain are still at high risk.

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