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Seasonal dynamics in community structure, abundance, body size and sex ratio in two species of Neotropical annual fishes

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Seven ephemeral pools on the coastal plain of southern Brazil were found to be inhabited by three annual and 22 non-annual fish species. Two common annual species (*Austrolebias minuano* and *Cynopoecilus fulgens*) exhibited clear seasonal dynamics, with the appearance of young fishes in the austral autumn (May to June) and a decline in abundance over the seasonal cycle. The third annual species, *Austrolebias wolterstorffii*, was rare. No seasonal dynamics were observed in non-annual fishes. The relative abundance of non-annual fishes compared with annual fishes increased over the seasonal cycle, but they coexisted widely. The size structure of annual fishes suggested the presence of a single age cohort in most pools though a second age cohort was registered in one pool in August, coinciding with a large flooding. Strong sexual dimorphism in body size was found in *C. fulgens* throughout the seasonal cycle, while no sexual dimorphism in body size was found in *A. minuano*. Female-biased sex ratios were recorded in both common annual fish species in the last three sampling dates (in spring), but not during the first two sampling dates (in winter). The natural lifespan of annual fishes was <8 months. Annual fishes disappeared before habitat desiccation in half of the pools, while non-annual fishes were still present.

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Key words: coastal floodplain; coexistence; Lagoa do Peixe; life cycle; sex-biased mortality; temporary habitat.

INTRODUCTION

Remarkable diversity in the capacity to cope with marginal aquatic habitats has been reported for Cyprinodontiformes, which can inhabit or specialize on intertidal or ephemeral habitats with environmental conditions unsuitable for other fishes (Turko & Wright, 2015). These adaptations are especially pronounced in two large aplocheiloid families (suborder Aplocheiloidei), the Neotropical Rivulidae and African Nothobranchiidae (Loureiro & de Sá, 2015). In both families, there are several clades of annual killifishes capable of completing their entire life cycle in ephemeral (*i.e.*

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regularly desiccating) habitats (de Sá *et al.*, 2015). Their development involves diapause, an arrested stage that enables embryo survival after habitat desiccation (Wourms, 1972; Furness, 2015). Whether annualism and diapause are ancestral traits or have evolved independently in different killifish clades is currently unclear (Parenti, 1981; Costa, 1990; Murphy & Collier, 1997; Hrbek & Larson, 1999; Varela-Lasheras & Van Dooren, 2014; Furness *et al.*, 2015).

Annual killifishes typically inhabit pools in open grassland habitats in the tropical and temperate zones of Africa and South America, with forest-dwelling species relatively rare (de Sá et al., 2015; Reichard, 2015). While a basic understanding of the ecology of these fishes exists, further insights are necessary to identify similarities and dissimilarities among and within the annual killifish clades. Understanding these aspects of their biology are important because annual fishes have become established as model taxa in a range of biological disciplines, including biogerontology (Berois et al., 2014; Cellerino et al., 2016), alternative developmental pathways (Berois et al., 2014; Polačik et al., 2014; Podrabsky et al., 2015a), ability to cope with environmental stress (Berois et al., 2012; Podrabsky et al., 2015b), mechanisms of speciation (Reichard & Polačik, 2010; Polačik & Reichard, 2011; García, 2015) and sexual selection and mate choice (Haas, 1976; Polačik & Reichard, 2009, 2011; Passos et al., 2015). A detailed understanding of their ecology will be crucial in the identification of the evolutionary significance of traits of interest (i.e. diapause, environmental tolerance and sex-biased mortality) and will enable generalization of the ecological and evolutionary patterns across annual killifish clades.

Austrolebias Costa 1998 (Cynolebiasini tribe) is the largest genus of Neotropical annual killifishes, with 43 species currently recognized (Costa, 2014; Volcan *et al.*, 2014; Nielsen & Pillet, 2015). Most species are associated with grasslands in lowland floodplain habitats in Laguna dos Patos-Mirim and lowland floodplains of Uruguayan river drainages (Costa, 2010; García *et al.*, 2015), though at least three species occur on highland plateaus at 600–800 m a.s.l. (Costa, 2014) and a single species is recorded from the Amazon basin in Bolivia (Nielsen & Pillet, 2015). All *Austrolebias* are annual species (Costa, 2010).

Cynopoecilus Regan 1912 (Cynopoecilini tribe) comprises six species distributed mainly in the coastal plains of southern Brazil and Uruguay, with a single species recorded from central region of Rio Grande do Sul state, southern Brazil (Costa, 2002; Ferrer *et al.*, 2014). The genera related to *Cynopoecilus* (*Campellolebias* Vaz-Ferreira & Sierra 1974, *Leptolebias* Myers 1952 and *Notholebias* Costa 2008) are typically found in the Atlantic Forest biome of south-eastern and southern Brazil (Costa, 2009*a*). All *Cynopoecilus* have an annual life cycle. The occurrence of *Cynopoecilus* spp. in a grassland biome is evolutionarily recent (Ferrer *et al.*, 2014).

Two or more species of annual fishes often co-occur, with various degrees of dietary, spatial and functional niche overlap (Nico & Thomerson, 1989; Costa, 1998; Reichard *et al.*, 2009; Loureiro *et al.*, 2015; Reichard, 2015). The diet of sympatric annual fishes is often similar (Arim *et al.*, 2010; Polačik & Reichard, 2010; Polačik *et al.*, 2014) and opportunistic feeding was suggested as an important adaptation to ephemeral habitats (Polačik & Reichard, 2010; Gonçalves *et al.*, 2011), despite distinctive diet preferences when food availability permits (Nico & Thomerson, 1989; Laufer *et al.*, 2009; Polačik & Reichard, 2010). Given that the natural habitat of annual fishes is under increasing pressure from agriculture, urbanization and forestry development, annual killifish populations face high levels of habitat degradation (Volcan *et al.*, 2015) and have been

proposed as flagship species for small wetland conservation (Lanés *et al.*, 2014*a*, *b*), a move already implemented in Brazil (ICMBio, 2013).

The coastal plain of Rio Grande do Sul state in Brazil supports 13 species of Austrolebias (Volcan et al., 2015) and five species of Cynopoecilus (Costa, 2002; Ferrer et al., 2014). Many of these species were recently recognized as local endemics (Costa, 2010) and little is known of their biology. In Lagoa do Peixe National Park (LPNP) and adjacent regions, Austrolebias minuano Costa & Cheffe 2001 and Cynopoecilus fulgens Costa, 2002 were reported to coexist in a network of ephemeral pools on a flat grassland floodplain (Lanés et al., 2014a). The two species are known to have substantial dietary overlap (Keppeler et al., 2015), but little is known about their demography and interactions with other fish species. Lanés et al. (2014a) reported length-mass relationships, sex ratios and temporal trends in abundance based on a small sample of fishes, pooled across six sites and three seasonal cycles. In the present study, the seasonal dynamics, measured in absolute and relative terms, sex ratio and size structure of A. minuano and C. fulgens are analysed from independent samples over an entire seasonal cycle in 2014. It was hypothesized that seasonal patterns in demography would indicate a single age cohort and seasonal trends in abundance and body size distribution would demonstrate seasonal patterns consistent with the existence of a single age cohort. A further question was whether these species show seasonal dynamics in the sex ratio and if it differs between the species.

MATERIALS AND METHODS

STUDY AREA

The study was conducted in the LPNP and its environs. LPNP is located in the middle section of the external coastal plain of Rio Grande do Sul, southern Brazil $(31^{\circ} 02'-31^{\circ} 48' S; 50^{\circ} 77'-51^{\circ} 15' W)$. This region supports high biological diversity and a high concentration of wetlands (Lanés & Maltchik, 2010; Maltchik *et al.*, 2010). The LPNP covers *c*. 36 722 ha of estuarine, freshwater and terrestrial habitats in a region with a level topography and low altitude (<20 m a.s.l.). The main permanent water bodies are represented by Laguna dos Patos and Lagoa do Peixe; the sampled pools were located between these two water bodies. The region has a moist subtropical climate and monthly average temperatures ranging from 13° C in winter to 24° C in summer. The mean annual rainfall varies between 1200 and 1500 mm year⁻¹ (Tagliani, 1995). The terrestrial vegetation is classified as the Pampa Biome (IBGE, 2004) characterized by subtropical grasslands, but including components of southern Atlantic Forest composed largely of pioneer species and fragments of restinga forests.

SAMPLING

Samples were taken during a single seasonal cycle (20-21 May, 29 June to 6 July, 23-25 August, 12-14 October, 15-16 November and 3-4 January) and are referred to by their month (May, July, August, October, November and January). Seven pools were chosen for seasonal sampling (pools 1, 2, 4, 6–9). The pools were at least 0.7 km apart, except for pools 1 and 2 which were only 0.15 km apart, but clearly separated (Fig. 1). The first sampling (May) included only pools 1 and 2. Pool 6 was converted into a rice field between August and October and was not sampled in the last three dates. All pools were dry in January 2015, except for pools 8 and 9 that had shallow turbid water and a thick layer of aquatic floating *Azolla* sp. vegetation, which did not permit a standardized collection effort. No fishes were recorded in pools 8 and 9 in January 2015.

Sampling was undertaken using a dip-net (D-shaped, $60 \text{ cm} \times 40 \text{ cm}$, 2 mm mesh size). Sampling effort at each site was standardized per time unit. The net was swept parallel to the bottom and all available habitats in the pool were sampled. Two people collected the fishes during



FIG. 1. Map of the study area within Rio Grande do Sul, Brazil. •, sampled pools 1–9; □, boundary to Lagoa do Peixe National Park; □, Laguna do Patos.

a series of 15 min sampling periods. Six sampling periods were typically undertaken in each pool, though fewer were completed if the entire pool area was already swept. For each sampling period, collected fishes were kept separate. Fishes were not released between sampling periods. After sampling, non annual-fishes were identified to species according to Malabarba *et al.* (2013) and annual fishes according Costa (2002, 2006). Systematic classification follows Betancur-R *et al.* (2013). Sex was determined in the annual fishes on the basis of external characters (fin size, shape and colouration). Fishes were measured for their total length (L_T) to the nearest 1 mm on a plastic slate with a ruler and released. This sampling design provided replicated samples (typically n = 6) for each pool and each date. In three pools (1, 2 and 4), *A. minuano* and *C. fulgens* were marked by removing a small piece of caudal fin tissue before release. On the following day, the same sampling effort was repeated in these pools. The number of marked and non-marked

	Pool 1	Pool 2	Pool 4	Pool 6	Pool 7	Pool 8	Pool 9				
Pool area (m ²)											
May	122	220	Not	Not	Not	Not	Not				
•			sampled	sampled	sampled	sampled	sampled				
July	1971	1672	4866	11 551	4866	1748	3958				
August	1258	859	3056	8981	3056	1449	3032				
October	1172	796	2989	NA^*	2989	1266	3402				
November	487	416	2260	NA^*	2260	902	1498				
January	16	0	0	NA^*	0	11	1503				
Maximum water depth (cm)											
May	15	15	Not	Not	Not	Not	Not				
•			sampled	sampled	sampled	sampled	sampled				
July	30	40	40	30	40	130	50				
August	27	28	37	53	76	110	40				
October	30	35	35	NA^*	62	70	39				
November	28	27	30	NA^*	35	65	29				
January	15	0	0	NA^*	0	22	23				
Water temp	perature	(°C)									
May	12.0	12.0	Not	Not	Not	Not	Not				
•			sampled	sampled	sampled	sampled	sampled				
July	18.0	22.0	19.0	15.0	22.5	20.5	16.1				
August	18.5	26.3	27.6	22.0	22.0	22.0	17.0				
October	25.7	25.0	29.2	NA^*	26.6	19.8	25.3				
November	31.8	28.2	31.0	NA^*	30.2	26.0	32.6				
January	34.0	NA [#]	NA [#]	NA^*	NA [#]	33.11	33				

TABLE I. Seasonal dynamics in water surface area, maximum water depth and water temperature measured in seven ephemeral pools in the Lagoa de Peixe region of southern Brazil

*Site was destroyed by conversion to rice field, pool parameters not measured.

#Site was dry (desiccated).

annual fishes captured was recorded and all fishes were released. For each pool the surface area (m^2), maximum water depth (cm) and water temperature (°C) were measured (Table I). To record habitat desiccation, data loggers (Hobo UA-002-08; Onset Ltd; www.onsetcomp.com) were placed on the substrata in the deepest water in pools 1, 2 and 3 and recorded water temperature every 3 h. Daily fluctuations in water temperature are minor in the pools and desiccation is marked by an abrupt increase in temperature fluctuation (Fig. 2). Pool size was measured during each sampling *in situ* using a portable GPS (precision 3 m) unit by walking around the pool boundaries to calculate pool area. The maximum pool water depth (cm) was obtained using a rule. Water temperature was measured using a HORIBA U-222 water-quality checker (www.horiba.com). Fish collection was conducted under IBAMA/ICMBio authorization (process number SISBIO number 43251–1).

DATA ANALYSES

The estimates of population size of *A. minuano* and *C. fulgens* were calculated using the Schnabel modification of the Lincoln-Petersen method for closed populations and repeated sampling (Schnabel, 1938). The method uses the number of marked and non-marked recaptured individuals and provides an estimate of overall population size and variance. The catch per unit effort (CPUE) for *A. minuano*, *C. fulgens* and all non-annual species combined



FIG. 2. Three hourly water temperature fluctuation June 2014 to January 2015 in (a) pool 1 and (b) pool 3 showing an abrupt increase in fluctuation amplitude during habitat desiccation (\longrightarrow) in December to January.

for each sampled pool and date was estimated as mean \pm S.E. of fishes captured per each sample (*i.e.* per 15 min of sampling) and was considered as a relative measure of population density.

Seasonal dynamics and sex differences in body size were examined using general linear models. Given the focus on sex differences in this analysis, seven unsexed juvenile individuals of A. minuano ($L_{\rm T} = 13 - 18$ mm; pool 6, July sampling) were removed from this analysis, but were included in the visualization of the seasonal dynamics in $L_{\rm T}$. Due to an unbalanced design (incomplete sampling across pools), a set of pool-specific analyses were used to investigate overall seasonal dynamics in pool-specific datasets. The models were constructed with the fixed effects date and sex and their interaction. The interaction was removed when non-significant (Engqvist, 2005). To test for differences among the pools, seasonal data were reduced to samples from July and August in A. minuano (i.e. the second and third sampling dates, covering the period of the most intensive growth) when data for four pools were available. For C. fulgens, samples from July, August and October (i.e. second, third and fourth sampling dates) from five pools were used. The model with the fixed effects date, sex, pool and their fully crossed second order interactions was constructed and subject to hierarchical backward elimination of non-significant terms. Test statistics of non-significant factors from the full model are reported. The statistics of significant terms from the final (*i.e.* minimum adequate) model are reported. Treating the effect of date as a continuous or categorical factor yielded quantitatively identical results; models with date treated as a categorical factor are reported in the results because a non-linear increase in $L_{\rm T}$ was assumed.

Relative species abundance was compared among pools using a generalized linear mixed model (GLMM) with binomial distribution and logit link function in the lme4 package in R (Bates *et al.*, 2015). Given correlated observation of relative species abundance from the same pools, sampling date was treated as a random factor in the analysis. Individuals were summed across the six sampling periods to provide a replicate; considering each sampling period count as a replicate yielded severely zero-inflated data. Statistical significance was obtained by comparing the full model (fixed factor: pool) with the null model (intercept only).

Seasonal dynamics of sex ratio was also analysed using a GLMM with binomial distribution and logit link function in the lme4 package in R (www.r-project.org). The fixed effects were date, species and their interaction. The random effect was population identity (n = 16 sex-ratio estimates over five populations of A. minuano, n = 21 and over six populations of C. fulgens). Models with date as a continuous or categorical variable provided concordant results; results on continuous fixed effects are reported to provide robust estimates of the interaction effect. The model assumptions were inspected by plotting residuals against fitted values. All analyses were performed in the R statistical environment 3.2.1 (www.r-project.org).



FIG. 3. Relative abundance by months of *Austrolebias minuano* (\blacksquare) and *Cynopoecilus fulgens* (\Box) in six ephemeral pools in the Lagoa de Peixe region in southern Brazil. Sample sizes for each estimate are given above each column.

RESULTS

FISH COMMUNITIES IN TEMPORARY POOLS

Annual fish species coexisted with a range of five to 12 non-annual fish species in the study pools. A total of 3083 individuals from 22 non-annual species were recorded (Table SI, Supporting information). Most species (50%) were characids. The most common non-annual species with 1672 individuals were *Phalloceros caudimaculatus* (Hensel 1868), 532 *Cheirodon interruptus* (Jenyns 1842), 318 *Astyanax cf. eigenmanniorum* (Cope 1894) and 242 *Hyphessobrycon* aff. *igneus* Miquelarena, Menni, López & Casciotta 1980 that occurred in all study sites. For all other species, except 161 *Hyphessobrycon boulengeri* (Eigenmann 1907) across six pools, <32 individuals were recorded. Potential fish predators included *Hoplias* aff. *malabaricus* (Bloch 1794), *Callichthys callichthys* (L. 1758), *Cichlasoma portalegrense* (Hensel 1870), *Gymnogeophagus rhabdotus* (Hensel 1870), *Synbranchus* aff. *marmoratus* Bloch 1795 and *Austrolebias wolterstorffi* (Ahl 1924). Pool-specific data on abundance and frequency of non-annual fishes are summarized in Table SI (Supporting information).

The relative abundance of the two annual species, *A. minuano* and *C. fulgens*, varied among pools ($\chi^2 = 348.9$, d.f. = 5, P < 0.001). Species ratio within the pools was relatively stable across the seasonal cycle in pools 2, 6 and 8, but varied seasonally in pools 1 and 4 (Fig. 3). *Austrolebias minuano* was absent from pool 9 and rare in pool 8, but in all other pools the relative abundance of both annual species was similar. Another species of annual fish, *A. wolterstorffi*, was collected in the pools 6 (n=2), 7 (n=2) and 8 (n=3) in July and August. It was not included in population estimates due to its rare occurrence and low abundance.

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FIG. 4. Seasonal abundance estimates (*N*) of *Austrolebias minuano* (\blacksquare) and *Cynopoecilus fulgens* (\Box) calculated by Schnabel capture–mark–recapture method from (a) pool 1, (b) pool 2 and (c) pool 4 in the Lagoa de Peixe region in southern Brazil.

POPULATION SIZE

Estimates of population size of both annual fish species ranged between *c*. 100 and 400 individuals in pools 1, 2 and 4 early in the season and tended to decline progressively over the seasonal cycle (Fig. 4). In two cases, when the estimates were >1000 fish (pool 4), low recapture rates were recorded (*A. minuano*: 89 marked, six recaptured and 81 unmarked fish; *C. fulgens*: 63 marked, two recaptured and 58 unmarked fish). An unexpected decrease in population size in pool 2 during July may have been an artefact; pool size expanded by >700% (from 220 to 1657 m²) between May and July sampling after heavy rainfall and consequently, fish recapture may have been compromised.

POPULATION DENSITY

CPUE data demonstrated generally congruent seasonal dynamics in *A. minuano* and *C. fulgens*, with a progressive decline over the seasonal cycle (Fig. 5). The only exception was for pool 4 where the density of *C. fulgens* was low in July. In contrast, non-annual species did not demonstrate any consistent seasonal dynamics in CPUE (Fig. 5) and variation was probably due to their incursions into temporary pools during periods of connectivity with permanent waters. *Austrolebias minuano* and *C. fulgens* was four times more abundant in pool 8 and *A. minuano* was absent in pool 9. All three pools with data loggers desiccated between 16 and 26 November 2014 (Fig. 2).

SEX RATIO

There was clear seasonal decline in the number of males in both species (z = -4.25, P < 0.001), with no difference in the sex ratio between species (z = 0.05, P > 0.05) and no difference in male decline (date × species interaction: z = -0.62, P > 0.05) (Fig. 6).

BODY SIZE

There was seasonal increase in $L_{\rm T}$ in both annual fish species and in all pools (Table II, and Figs 7 and 8). In pool-specific analyses of *A. minuano*, size difference



FIG. 5. Relative abundance expressed as catch per unit effort (CPUE, the number of individuals 15 min⁻¹ of collection) of *Austrolebias minuano* (■), *Cynopoecilus fulgens* (□) and all non-annual species combined (■), from (a) pool 1, (b) pool 2, (c) pool 4, (d) pool 6, (e) pool 8 and (f) pool 9 in the Lagoa de Peixe region in southern Brazil.

between the sexes was limited to a single case. Female *A. minuano* were larger at the end of the season in pool 1. There was no sex difference in $L_{\rm T}$ of *A. minuano* in the overall analysis (main effect of sex: $F_{1,259} = 0.36$, P > 0.05), nor any interaction with pool and seasonal effects (both P > 0.05). There was a difference in $L_{\rm T}$ among pools ($F_{3,267} = 26.4$, P < 0.001) and in the change in $L_{\rm T}$ among pools (pool × date interaction: $F_{3,267} = 15.9$, P < 0.001), along with an overall seasonal increase in $L_{\rm T}$ ($F_{1,267} = 125.9$,



FIG. 6. Mean ± 95% C.I. proportion of male (a) Austrolebias minuano and (b) Cynopoecilus fulgens in the population by months, estimated as the least-squared means from the final general linear models, across ephemeral pools in the Lagoa de Peixe region in southern Brazil. The number of fish used for each estimate is given above the corresponding month.

P < 0.001). There was a limited increase in body size in pool 2 compared to substantial increases in pools 4, 6 and 8. Visual inspection of Figs 7 and 8 indicated that there was probably a second age cohort in pool 2 that hatched in July. Overall, the largest *A. minuano* male was 45 mm long and the largest *A. minuano* female measured 46 mm.

In *C. fulgens*, sex differences in $L_{\rm T}$ were major in all pools except pool 9 (Table II). In three pools, there was also a significant interaction between date and sex that indicated a steeper increase in $L_{\rm T}$ in males (Fig. 8). The overall analysis confirmed sexual dimorphism (main effect of sex: $F_{1,459} = 96 \cdot 5$, P < 0.001), with varying magnitude among pools (sex × pool interaction: $F_{4,459} = 15 \cdot 7$, P < 0.001). There was a difference in $L_{\rm T}$ among pools ($F_{4,459} = 22 \cdot 4$, P < 0.001) and in the seasonal dynamic in $L_{\rm T}$ among pools (pool × date interaction: $F_{7,459} = 5 \cdot 0$, P < 0.001), along with an overall seasonal increase in $L_{\rm T}$ ($F_{2,459} = 63 \cdot 4$, P < 0.001). The seasonal dynamics in $L_{\rm T}$ was concordant between males and females (sex × date interaction: $F_{2,459} = 1 \cdot 8$, P > 0.05). Pool-specific seasonal patterns in $L_{\rm T}$ and sex differences in $L_{\rm T}$ are illustrated in Fig. 8. The largest *C. fulgens* male measured 47 mm and the largest *C. fulgens* female was 46 mm.

SEASONAL DYNAMICS OF ANNUAL FISHES



Fig. 7. The total length (L_T)-frequency distribution by months of female (\blacksquare), male (\Box) and juvenile (\blacksquare) Austrolebias minuano in five ephemeral pools in the Lagoa de Peixe region in southern Brazil. Column: (a) pool 1, (b) pool 2, (c) pool 4, (d) pool 6 and (e) pool 8; row: (a) May, (f) July, (g) August, (h) October and (i) November.

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FIG. 8. The total length (L_T) -frequency distribution by months of female (\blacksquare), male (\square) and juvenile (\blacksquare) *Cympoecilus fulgens* in six ephemeral pools in the Lagoa de Peixe region in southern Brazil. Column: (a) pool 1, (b) pool 2, (c) pool 4, (d) pool 8, (e) pool 8 and (f) pool 9; row: (a) May, (g) July, (h) August, (i) October and (j) November.

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	Date			Sex			Interaction		
	d.f.	F	Р	d.f.	F	Р	d.f.	F	Р
Austrole	bias minu	ano							
Pool 1	4,49	65.2	***	1,49	14.1	**			
Pool 2	4,143	16.2	***	1,138	0.5				
Pool 4	1,76	103.2	***	1,76	0.5				
Pool 6	1,79	65.0	***	1,79	1.2				
Pool 8	1,44	92.4	***	1,42	1.3				
Cynopoe	cilus fulg	ens							
Pool 1	3,90	41.5	***	1,90	35.9	***			
Pool 2	4,144	5.4	***	1,144	55.8	***			
Pool 4	2,77	19.9	***	1,77	28.6	***			
Pool 6	1,75	45.0	***	1,75	85.1	***	1,75	22.2	***
Pool 8	2,162	105.8	***	1,162	101.8	***	2,162	10.4	***
Pool 9	3,100	2.6	0.054	1,100	0.5		3,100	6.5	**

TABLE II. ANOVA results of the sex-specific seasonal variation in total length (L_T) in two species of annual fishes from ephemeral pools in the Lagoa de Peixe region in southern Brazil. Interaction terms are only reported when significant

***P* < 0.01; *P* < 0.001

DISCUSSION

Temporary pools in coastal plains within the Pampa region of southern Brazil were shown to be inhabited by three annual killifish species and a range of non-annual fishes that varied seasonally in their abundance and species composition. This finding indicates that the pools are part of an interconnected system of ephemeral and permanent habitats. Annual species exhibited clear seasonal dynamics, with the appearance of young fishes in autumn and early winter (May to July) and a continuous decline in abundance and density over the seasonal cycle. An analysis of population size structure suggested a single age cohort in both *A. minuano* and *C. fulgens* in most pools, but indicated a potential for two age cohorts in one of the pools. There was strong sexual dimorphism in body size in *C. fulgens*. Female-biased sex ratios were recorded in the second part of their life cycle.

The coexistence of annual fishes with non-annual species demonstrated that *A. min-uano* and *C. fulgens* are capable of completing their life cycle and maintaining viable populations under competition with the poeciliid *Phalloceros caudomaculatus* Hensel 1968 and a range of characid species. This finding is in contrast to six annual killifish species from ephemeral pools in Venezuelan Llanos region (the Orinoco basin) where immigration of non-annual fishes to temporary pools with an elevated water level resulted in the complete disappearance of annual species (Nico & Thomerson, 1989). In the Mozambican savanna in southern Africa, annual *Nothobranchius* Peters 1868 killifishes rarely coexist with non-annual species because their pools are isolated (Reichard *et al.*, 2009), although exceptionally large floods may enable dispersal of non-annual species into temporary pools. In years when non-annual fishes invade their habitat, *Nothobranchius* populations have low abundance or disappear completely (Reichard, 2015). Similarly, *Nothobranchius caprivensis* Watters, Wildekamp & Shidlovskiy 2015 from the Zambezi

floodplain co-occurred with non-annual fishes only in a single pool that was heavily modified by human activities and in roadside culverts at the peak of seasonal flooding (Tweddle et al., 2014). Other Nothobranchius populations from the coastal wetland plain, however, coexist with non-annual species in a grassland matrix of ephemeral and permanent swamps, marshes and streams. Here they exploit different habitats within the interconnected system, resulting in the relatively rare co-occurrence of annual and non-annual species at the mesohabitat scale (Reichard, 2015). In the present study, simultaneous collection of annual and non-annual species in the same net sweep suggested coexistence in the same microhabitats, although microhabitat use was not directly recorded. It is hypothesized that the major difference between tropical (Nothobranchius, Austrofundulus Myers 1932) and subtropical to temperate (Austrolebias, Cynopoecilus) habitats is in the higher habitat complexity (more vegetation) of the latter, perhaps related to a higher abundance of prey. Additionally, environmental conditions (primarily related to water temperature and oxygen availability) may be more extreme in tropical ephemeral pools (Podrabsky et al., 1998; Reichard et al., 2009).

It is notable that non-annual species were abundant in all pools in November (late spring) while annual fishes either disappeared (pools 4, 7 and 8) or were rare (pools 1, 2 and 9). Given that there was no marked change in the non-annual fish community (Table SI, Supporting information), the disappearance of annual fishes is attributed to natural mortality. Their abundance declined progressively during the winter, but in November, the water level and surface area of the pools were low and environmental conditions deteriorated. In two pools where fishes were present during sampling on 14-15 November and that contained data loggers (pools 1 and 2), water disappeared before 26 November; *i.e.* <2 weeks after sampling. Water temperatures rarely reached 25°C before mid November, but increased steeply thereafter (Fig. 2). Austrolebias spp. are known to be sensitive to higher water temperatures (Errea & Danulat, 2001; Fonseca et al., 2013; Polačik & Podrabsky, 2015). Data from the current study indicate that annual fishes may disappear from the pools before they dry. The lifespan of Austrolebias, primarily inhabiting the subtropical and temperate zone of the Neotropics, is confined to cold periods of the year, with their habitats desiccating due to higher summer evaporation rather than seasonal changes in precipitation. This observation is in contrast to annual killifishes from tropical and subtropical regions of the Neotropics and Africa that complete their life cycle during the rainy season in summer, when water temperature may be as high as 37° C (Podrabsky et al., 1998; Reichard et al., 2009; Reichard, 2015).

Present estimates of population abundance based on capture–mark–recapture (CMR) were relatively consistent in two smaller pools, but less consistent in a third, larger pool. No estimates were available for the two smaller pools during extensive flooding in July and August when capture rates were too low to permit a reliable estimate of population density based on recaptures. Therefore, it appears that the CMR-based estimate of annual fish population abundance may be relatively reliable for smaller pools (<1000 m²), but unreliable for larger pools where recapture rates are low. The CMR-based population estimates were generally congruent with the CPUE estimates and agreed with variation in the species ratio. They demonstrated stronger seasonal decline in pool 1 than in pool 2 and changes in the species ratio in pools 1 and 4. It should be stressed, however, that the estimates were not independent as they

ultimately arise from the same set of individuals. Nevertheless, congruence between temporal dynamics in the CMR and CPUE estimates adds support to their credibility.

The two studied annual species widely coexisted in all but a single pool. The limited number of replicated pools did not permit a quantitative comparison of their habitat requirements. The pool with the exclusive occurrence of C. fulgens in the study was situated closest to the Atlantic Ocean coast (5.5 km), but showed no extreme values in any of the environmental variables that were measured. Austrolebias minuano has been recorded in this pool in previous years (Lanés et al., 2014a). Austrolebias minuano and C. fulgens have a similar diet and it was proposed that their concurrent occurrence is facilitated by a high abundance of their chief prey (planktonic crustaceans and chironomids) (Keppeler et al., 2015). Up to four and five species of Austrolebias and a Cynopoecilus species may coexist in temporary pools in southern Brazil (Volcan et al., 2015) and Uruguay (Arim et al., 2010), respectively and Canavero et al. (2014) argued that differences in their diet are related to body size rather than taxonomic designation. Despite some coexisting species showing limited morphological differences (Laufer et al., 2009), others appear to represent different functional guilds (e.g. large predatory and small non-predatory species and slender and deep-bodied phenotypes), specialized for different ecological niches (Costa, 2009b; Loureiro et al., 2015). This is comparable with the situation in Venezuelan and African annual killifish communities (Nico & Thomerson, 1989; Polačik & Reichard, 2010; Polačik et al., 2014).

The existence of a single sized cohort in most pools indirectly indicates that both species hatch relatively synchronously early after flooding of the pools and form a single age cohort. In pool 2, however, there was an indication of a second age cohort that hatched between sampling in July and August, apparent in both species (Figs 7 and 8). This coincided with a large flooding of the area in July, where a new set of eggs may have been flooded. This narrative is supported by data for Cynopoecilus multipapillatus Costa, 2002 [designated as Cynopoecilus melanotaenia (Regan 1912) in the original study], a closely related species from an adjacent, northern region of the coastal plain. On the basis of a bimodal body size distribution of adult males, Arenzon et al. (2001) suggested the existence of a second age cohort that hatched in the austral winter (August) in this species. Similarly, Arenzon et al. (1999) hypothesized continuous seasonal hatching of C. multipapillatus while the current study does not indicate continuous hatching in C. fulgens. During the first sampling in May, fishes that had recently achieved sexual maturity were encountered in the two sampled pools. Body size of annual fishes in the other five pools in early July was comparable with the size of annual fishes from pools 1 and 2 in late May. The other five pools were only located during the second sampling date, but it is possible that annual fishes hatched in June. It has been suggested for African annual fishes from southern Mozambique and for Austrolebias viarius (Vaz-Ferreira, Sierra de Soriano & Scaglia de Paulete 1965) from eastern Uruguay that their hatching may not be coincident with the initial habitat inundation, but rather with strong precipitation associated with major cyclones in the second part of the rainy season (Errea & Danulat, 2001; Watters, 2009; Polačik et al., 2011; Reichard et al., 2015). In contrast to Mozambican Nothobranchius, there appears to be low hatching synchrony across a relatively small spatial scale of the range of annual killifishes. At least in the present study year (2014), hatching apparently occurred from early May to June. At the same time, hatching within the pool was probably synchronous rather than continuous, as suggested by the body size distribution. Both species studied attain sexual maturity at a body size of 18-21 mm at the

age of c. 8 weeks (Arenzon et al., 1999; Volcan et al., 2012), though the sexes can be distinguished in larger juveniles, especially in C. fulgens.

Female-bias in the sex ratio was reported from Neotropical and African annual fishes (Reichard et al., 2009; Passos et al., 2014), despite equal sex ratios in captivity (Reichard et al., 2014). This finding implies higher male mortality in wild populations, though existing data did not permit determination of whether males disappear from the populations suddenly, during a period of harsh environmental conditions, such as in Trinidadian guppies Poecilia reticulata Peters 1859 (Arendt et al., 2014), or more gradually. The present study demonstrated that the increase in female bias was gradual, with males of both species disappearing from populations proportionally quicker than females. Significant female-biased sex ratios, however, were not recorded until August in C. fulgens and October in A. minuano. The cause of higher male mortality remains to be investigated. In African annual killifish, Reichard et al. (2014) primarily considered the roles of higher predation on sexually ornamented and more active males and on male-male aggression. Sexual dimorphism in colouration is apparent in both C. fulgens and A. minuano (Costa, 2002, 2006), while sexual dimorphism in body size was only apparent in C. fulgens. Given that male Cynopoecilus also appear to be more aggressive to rival males (Nielsen, 2010), the present study does not provide more support for any one of the male-biased sources of mortality. Given that the steepest decline in male numbers coincided with environmental deterioration (e.g. increasing water temperature), inherently higher male frailty (e.g. due to hormonal differences and more rapid functional senescence) is another plausible explanation for male-biased mortality.

In summary, the present study demonstrated the existence of a single age cohort in both annual fish species in most pools, but appearance of a second age cohort in one pool. Seasonal decline in abundance was concordant between species, with a natural lifespan of <8 months. In contrast to other annual killifish taxa (*e.g. Austrofundulus* spp. and *Nothobranchius* spp.), *A. minuano* and *C. fulgens* co-existed within a rich community of non-annual fishes. Further research should address the roles of environmental conditions, predation and intra-specific encounters on sex-specific mortality.

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Supporting Information

Supporting Information may be found in the online version of this paper: TABLE SI. List of all fish species with their systematic assignment (Betancur-R *et al.*, 2013) collected in seven ephemeral pools in the Lagoa de Peixe region in southern Brazil

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