



Species co-occurrence and population dynamics in annual fish assemblages in the lower Río Uruguay basin

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Abstract *Austrolebias* is a genus of annual killifishes from subtropical and temperate grasslands of South America. They coexist in assemblages of up to five species in pools with variable connection to permanent water bodies and complete their entire life cycle within 1 year, terminated by summer desiccation of their habitat. To quantify community and population characteristics of *Austrolebias* assemblages, we sampled a set of 18 pools in the lowlands of the confluence of Río Negro and Río Uruguay in western Uruguay, with 16 sampling trips conducted over the entire seasonal cycle in 2015. *Austrolebias bellottii* was a ubiquitous and generalist species, *A. nigripinnis* was more common in pools influenced by active floodplain, *A. elongatus* (a large predatory species) was rare overall, and four *A. alexandri* was found only in two pools. Unexpectedly, many pools desiccated in winter (mid season) and some of them supported a second cohort after their re-inundation. Fish abundance and fish density declined steadily prior to pool desiccations. There were

indications that adult *A. bellottii* can survive a short period of habitat desiccation in wet mud, leading to coexistence of an older *A. bellottii* cohort with a younger *A. nigripinnis* cohort. Males disappeared from populations at a higher rate, especially towards the end of the season. The overall lifespan of both common species was up to 7 months in pools that did not experience mid-season desiccation. This study provides fundamental demographic parameters for the annual fish in this region.

Keywords Annual fish · Community assembly · Cyprinodontiformes · Seasonal dynamics · Sex ratio

Introduction

Ephemeral pools are exploited by fishes of diverse life-history strategies. While most fishes exploit ephemeral habitats temporarily, others possess special adaptations to the fluctuating and challenging environmental conditions, such as air breathing and the ability to escape from desiccating pools via crawling or jumping (Polačik and Podrabsky 2015). Very few fish taxa, however, are adapted to complete their entire life cycle in ephemeral pools. Adaptations include dry-season aestivation of adult fish in wet mud (Delaney et al. 1974; Chew et al. 2004) and survival of embryos protected in diapausing stages in some species of cyprinodontiform fishes, named annual fishes.

Annual fish populations survive the dry period as eggs encased in the desiccated substrate. The eggs

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possess an ornamented external layer (Loureiro and de Sá 1996; Thompson et al. 2017) that protects the embryo from desiccation and enables gas exchange (Wourms and Sheldon 1976). The embryonic development of annual fishes comprises up to three diapausing stages (Wourms 1972a, b, c) that shield developing embryos from harsh environmental conditions and synchronize hatching with habitat inundation (García et al. 2018). After hatching, annual fish develop rapidly, reaching sexual maturity within a few weeks (Blažek et al. 2013; Vrtilek et al. 2018a), and reproducing daily to fully exploit temporary nature of their habitat. Annual life history appears to have evolved at least six times in Cyprinodontiformes (Furness et al. 2015). Most annual fishes occur in tropical regions, with the rainy season during the warmest phase of the year. However, some Neotropical killifishes have colonized ephemeral pools in subtropical and temperate regions, where the wet phase of pools occurs during the coldest period of the year and pools desiccate in summer (Berois et al. 2015).

In the present study, we investigated population and community characteristics of *Austrolebias* annual fishes from subtropical region of Uruguay. The genus *Austrolebias* (Rivulidae) is the most diverse genus of annual fishes in subtropical South America, with more than 40 species described (Loureiro et al. 2016; Alonso et al. 2018a). The centre of its range is in the lowlands of the La Plata and Los Patos basins. While many *Austrolebias* species are endemic to small areas and categorized as vulnerable, endangered or critically endangered (Rosa and Lima 2008; Volcán et al. 2009, 2010; Loureiro and Bessonart 2017; Alonso et al. 2018a), other species are common and geographically widespread (Costa 2009). The size of habitat where *Austrolebias* occur varies from small pools to extensive flooded meadows. *Austrolebias* are specialised to ephemeral pools where they complete their entire life cycle and their populations are not found in permanent habitats. Across most of their range (including our study area), the pools inundate in early autumn (March–April) once temperature decreases to the levels when precipitation exceeds evaporation and persist until late spring (November–December) when evaporation increases (García et al. 2018). The hatching of *Austrolebias* species is highly synchronised with pool inundation (March–April) (García et al. 2018), with juveniles attaining sexual maturity in approximately 8 weeks (Berois et al. 2012).

While some pools in subtropical and temperate grassland are inhabited by only a single species of

Austrolebias, coexistence of two or three species is common and some assemblages comprise five species coexisting in a single pool (Loureiro et al. 2016). Coexisting species typically belong to different phylogenetic clades and morphotypes, including a large predatory morphotype. The predatory *Austrolebias* morphotype (elongated and robust body, with a total of five species) may feed on other annual fishes (Costa 2009; Loureiro et al. 2016) and are a rare component of the assemblage (Costa 2009). Other annual fish morphotypes correspond to medium or small-sized species with compressed and ellipsoidal to elongate bodies and are considered generalist predators with broadly overlapping diets (Laufer et al. 2009; Keppeler et al. 2015). Given that *Austrolebias* species live in small replicated water bodies, they represent an ideal system to understand aspects of community assembly (Arim et al. 2010) and community ecology (Laufer et al. 2009; Canavero et al. 2014; Keppeler et al. 2015; Reichard et al. 2018). However, there is limited information on seasonal dynamics in temperate and subtropical annual fish assemblages, most of which derives from the Atlantic coastal lagoon region (Lanés et al. 2014, 2016). Specifically, there is a lack of information on the seasonal dynamics (that represent their entire life cycle) of the widely distributed species of Río Uruguay basin.

The pools where *Austrolebias* occur can be entirely isolated from permanent water bodies, or may be temporarily connected to lakes, streams or rivers during occasional floods, enabling the invasion of non-annual fishes such as characids and poeciliids (Vaz Ferreira et al. 1966; Lanés et al. 2016). *Austrolebias* populations appear adapted to these incursions (Lanés et al. 2016), though non-annual fishes are reported to eliminate annual fish populations in the tropics (Nico and Thomerson 1989). In the Atlantic coastal lagoons and the Río Paraná basin of subtropical South America, other genera of annual fishes occur with *Austrolebias* but in the Río Uruguay basin, where the current study was conducted, *Austrolebias* is the only annual fish genus that occurs in this region.

In this study we aimed to describe the seasonal patterns of species co-occurrence and sex ratio of annual fishes that coexist in the wetlands of the Río Negro basin in Uruguay (Río Uruguay/La Plata basin). We predicted existence of one core species with a common occurrence in most pools co-occurring with other, more specialised annual fish species. We further predicted a steady seasonal decrease in annual fish abundance (as a response

to deterioration habitat conditions and the negative effects of ageing) rather than a sudden steep decline in the terminal phase of the pools (Vrtílek et al. 2018b). Lastly, we predicted a seasonal decrease in the proportion of males in the populations as a response of male-male competition for females (Passos et al. 2014).

Three *Austrolebias* species were expected to be found in our study area. *Austrolebias bellottii* (Steindachner, 1881) (maximum size 70 mm) and *Austrolebias nigripinnis* (Regan, 1912) (maximum size 40 mm) are sympatric and sometimes syntopic species with wide distributions, from the middle and lower Paraná and Uruguay river basins to the south of Buenos Aires province in Argentina (García et al. 2012; Volcán et al. 2017). The third species, *Austrolebias elongatus* (Steindachner, 1881) (maximum size 150 mm) is a large predatory species with a narrower distribution that coexists with *A. bellottii* and *A. nigripinnis* across most of their southern range, approximately from the mouth of the Río Negro to the south of Buenos Aires province (Alonso et al. 2016). In Uruguay *A. elongatus* is only found in wetlands between the mouths of the Río Negro and the Río San Salvador in the vicinity of Villa Soriano.

Material and methods

Study area

We selected 26 study pools in the lowlands of the lower Río Negro, in its confluence with the Río Uruguay (33°23'56"S, 58°19'13"W, 1–7 m above sea level) in the surroundings of Villa Soriano town (Uruguay) (Fig. 1). This region has a moist, subtropical climate. The monthly average temperature ranges from 10.4 °C in winter to 23.5 °C in summer, with an annual average of 17 °C (<https://es.climate-data.org/location/50536/>). The mean annual rainfall in the 6 years preceding the study (2009–2014) was 1266 mm (maximum 1748 mm in 2014, minimum 814 mm in 2013), with the highest monthly precipitation in austral summer (February: 224 mm) and lowest in winter (June: 42 mm) (data provided by the National Meteorology Institute, Uruguay). In 2015 (study year), the region received a typical annual rainfall of 1029 mm, but experienced particularly dry episodes (41.5 mm in February, 52 mm in May, and 35.5 mm in July; data obtained from local pluviometers). Total monthly precipitation and their interannual variation are given in García et al. (2018).

The study area is composed of five sections that form clusters of pools with similar characteristics and spatial proximity (Fig. 1, Table 1). The “Rincón” cluster (section A, six pools) is located in the Río Negro floodplain and was occasionally flooded from the river. Depending on the flood magnitude, the river flooded different sets of pools. Small floods affected only 3A, 4A and 6A. medium floods also affected 1A and 5A. Pool 2A was not affected by flooding at all during the sampling season (García pers. obs.). The “Canaleta” cluster of pools (section B, two pools) are located in the depressions of an unused road (pools 7B and 8B), surrounded by grassland with cattle ranching. The “Ruta” includes a single pool (9C), adjacent to agricultural fields (soybean crops). The “Escuela” cluster (section D, three pools) is close to agricultural fields, but located in an area with extensive livestock production and no agricultural development. The “Curupí” cluster (section E) with six studied pools (13E–18E) is located in a cattle-ranging area with agricultural production in the surroundings.

Sampling

Samples were taken over the entire seasonal cycle between 18 May 2015 and 1 January 2016. Sampling was conducted every 2 weeks, except for a 20-day interval between trips 7 (9–10 Aug) and 8 (29 Aug–1 Sep). Hatching dates were estimated from otolith incremental growth rings. The first cohort hatched in late April, with a second cohort in early August in the pools subject to mid-season desiccation (García et al. 2018). A total of sixteen sampling trips were undertaken, with collection of fish across the entire study area. Initial sampling covered 26 pools but eight pools dried out early in the season (before three samplings were conducted) and were excluded from the analyses. The pools were at least 0.3 km apart, except for pools 11D and 12D that were only 0.1 km apart but clearly separated. Of the 18 pools sampled between May and July, 13 desiccated and despite a later flooding (30 Jul – 1 Aug), only six of these supported a second cohort of fish. In three pools (10D, 15E and 18E), the first-cohort fish persisted despite indications of earlier desiccation, and in one of these (10D) a second cohort co-occurred with the first cohort. Hence, we only sampled 14 pools in the second part of the season (seven where the first cohort persisted, six with a second cohort and one pool where the first and second cohorts coexisted). Between 25 and 27 October (trip 12), a flood partially covered the “Rincón” cluster,

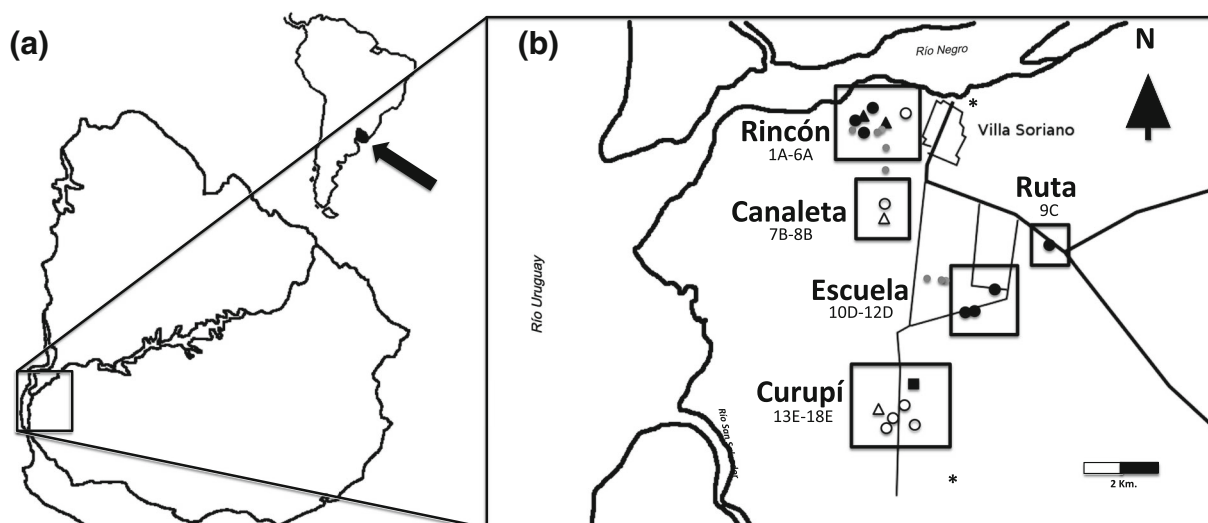


Fig. 1 Location of the study area in western Uruguay (a), with individual pools and their assignment to geographical clusters (b). Symbols of individual pools designate *Austrolebias* assemblages with only *A. bellottii* (open circle), *A. bellottii* and *A. nigripinnis* (black circle), *A. bellottii* and *A. elongatus* (open triangles),

A. bellottii, *A. nigripinnis* and *A. alexandri* (black triangles) and *A. bellottii*, *A. nigripinnis* and *A. elongatus* (black square). Grey circles represent pools that desiccated early in the season and were not followed later. Asterisks indicate position of local pluviometers

making it inaccessible for sampling. Samples from that trip are missing for pools 3A, 4A and 6A. Data loggers (Hobo UA-002-08) were deployed in 12 pools during the first day of sampling and were used to estimate the timing of pool desiccation (Lanés et al. 2016; Reichard et al. 2017; García et al. 2018).

Sampling was undertaken using a square dip-net (20×15 cm, 2 mm mesh size). The net was swept parallel to the bottom and sampling effort representatively covered all regions of the pool and its various habitat types. Sampling effort varied across sites and sampling period, due to wide variation in fish density and pool area. We aimed to collect at least 20 fish from each pool. Sampling effort was recorded by measuring time spent collecting fish (median = 26 min, mean = 32, standard deviation = 23.8) and pool surface area. Pool area was measured in situ during each sampling by walking around the pool boundaries using a portable GPS unit to map the outline of the pool surface. After sampling, all annual fish collected were photographed on a tray (8×8 cm) with a wet surface and standard and total body length were measured against a scale. Fish were then returned to the pool. Species and sex were determined from photographs on the basis of external characters (fin size, shape and colouration). Species can be clearly separated from the photographs, including juveniles.

Data analysis

Species co-occurrence was visualised using Venn's diagram in Venny 2.1 (<http://bioinfo.gp.cnb.csic.es>). Seasonal dynamics in the proportion of *A. bellottii* to *A. nigripinnis* was modelled using Generalised Linear Mixed Models (GLMM) with a binomial distribution and logit link function. The response variable was the ratio between the two species. The sampling trips were modelled as continuous variable. The random factor was pool identity. Only samples that contained at least four fish were used to calculate species ratio.

Fish density (a proxy for population density) was calculated by dividing the number of fish by sampling time and expressed in all samples as the number of fish collected over 30 min (the mean sampling time). Fish abundance (a proxy for population abundance) was estimated by multiplying fish density by pool surface area. The two measures represent distinct aspects of the population but were positively correlated (Pearson correlation on log-transformed data: $r_{170} = 0.523$, $p < 0.001$). Fish density and fish abundance estimates were completed for all *Austrolebias* fish pooled and for *A. bellottii* and *A. nigripinnis* separately. Density and abundance estimates for *A. bellottii* and *A. nigripinnis* (wherever they coexisted) as well as estimates for a single species and overall estimates were highly

Table 1 Overview of sample pools, including geographic location, surface area (mean, minimum and maximum m²) over the entire study period (18 May 2015–1 Jan 2016), the presence of annual fish species, incidence of mid-season desiccation, the presence of a second cohort, and persistence of the first cohort throughout the entire year

Pool	Region	Latitude	Longitude	Mean area	Min area	Max area	Species*	Desiccated	2nd cohort	1st cohort persistence
1A	Rincón	33°23'34.58"S	58°20'39.20"W	1202	671	1512	AB, AN	Yes	Yes	No
2A	Rincón	33°23'40.55"S	58°19'40.84"W	739	404	1144	AB	Yes	No	No
3A	Rincón	33°23'41.57"S	58°20'47.09"W	1020	11	2643	AB, AN, AA	Yes	Yes	No
4A	Rincón	33°23'48.89"S	58°20'58.52"W	935	200	2327	AB, AN	Yes	Yes	No
5A	Rincón	33°23'51.23"S	58°20'19.64"W	1658	7	3367	AB, AN, AA	Yes	Yes	No
6A	Rincón	33°24'0.17"S	58°20'48.77"W	1353	6	2992	AB, AN	Yes	Yes	No
7B	Canaleta	33°24'59.32"S	58°20'18.60"W	161	4	702	AB	No	No	Yes
8B	Canaleta	33°25'15.75"S	58°20'20.20"W	407	4	1203	AB, AE	Yes	No	No
9C	Ruta	33°25'40.21"S	58°17'12.73"W	52	9	160	AB, AN	Yes	Yes	No
10D	Escuela	33°26'17.63"S	58°18'14.72"W	81	6	259	AB, AN	Yes	Yes	Yes
11D	Escuela	33°26'33.78"S	58°18'37.51"W	138	44	218	AB, AN	No	No	Yes
12D	Escuela	33°26'34.49"S	58°18'41.41"W	37	17	63	AB, AN	No	No	Yes
13E	Curupí	33°28'1.48"S	58°19'46.85"W	212	11	533	AB, AN, AE	No	No	Yes
14E	Curupí	33°28'29.20"S	58°20'3.15"W	107	29	234	AB, AN	Yes	No	No
15E	Curupí	33°28'29.18"S	58°20'23.98"W	595	45	2364	AB, AE	Yes	No	Yes
16E	Curupí	33°28'52.50"S	58°20'4.61"W	233	78	341	AB	Yes	No	No
17E	Curupí	33°28'55.37"S	58°19'53.94"W	466	105	1102	AB	No	No	Yes
18E	Curupí	33°29'1.44"S	58°20'10.78"W	911	27	2444	AB	Yes	No	Yes

*AB *A. belottii*, AN *A. nigripinnis*, AE *A. elongatus*, AA *A. alexandri*

positively correlated (Pearson correlation on log-transformed data: all $p < 0.001$). Therefore, estimates of fish density and fish abundance are only presented for all species pooled.

Given the mid-season desiccation of some pools, three datasets were assembled. First, the early-season data were collated from all pools until the period of mid-season desiccation (trips 1–6, 18 May to 27 July). This dataset contained data from 13 pools (pools A1, A4, A5, 16E and 18E had less than three datapoints for fish density and abundance and were excluded from this dataset). Second, complete seasonal data (no mid-season desiccation) were available for six pools. Third, data on the second cohort were available for another six pools.

Seasonal dynamics in pool surface area, fish density and fish abundance were analysed using Linear Mixed Models (LMM) with Gaussian distribution. All three response variables were log-transformed to standardize residuals. The models had a single fixed factor (sampling trip 1–16, describing seasonal progress). Pool identity was modelled as a random factor (intercept). The models were run with sampling trips coded as continuous variable (i.e. trip 1–16). To provide date-specific model estimates and to visualize potential sudden changes in the seasonal dynamics, sampling trip was additionally coded as a categorical factor. The models for all three subsets (pool area, fish density, fish abundance) had identical structure. Using mixed models where variation among pools can be incorporated by assigning separate intercepts for each pool, we were able to provide a single overall estimate of population dynamics.

Sex ratio was also modelled using GLMM with binomial distribution and logit link function. The response variable was ratio between males and females, fixed factor was sampling trip (continuous variable: 1–16) and random factor was pool identity. Separate models were run for *A. bellottii* and for *A. nigripinnis* and for each subset (no desiccation, early season, second cohort). For all models, assumptions were inspected visually from residual plots. The analyses were performed in the *lme4* package in the R statistical environment version 3.3.3 (R Development Core Team 2015).

Results

Overall, we collected 7679 fish from 26 pools during 16 sampling trips (18 May 2015–1 Jan 2016). Given that

eight pools were excluded due to their desiccation early in the season, the analyses are based on 18 pools and 7355 fish.

Co-occurrence

Austrolebias bellottii was found in all 18 pools that contained annual fish ($n = 4434$ fish), *A. nigripinnis* was found in eleven pools ($n = 2524$ fish), *A. elongatus* in three pools ($n = 18$ fish), and *Austrolebias alexandri* (Castello and López, 1974) was found in two pools ($n = 4$) (Fig. 2). Suboptimal photographs of some fish sampled during the first collection trip precluded species identification of 375 individuals but these were used in analyses of overall fish density and abundance.

Austrolebias bellottii was always common, and *A. nigripinnis* was common in five pools (1A, 3A–6A) located in the active floodplain of the Río Negro (“Rincón” section), in all pools of the “Escuela” section (10D–12D) and in one pool of the “Curupí” section (13E). However, after the mid-season dry period, fish abundance strongly decreased in the “Escuela” pools. No *A. nigripinnis* was found later in the season in the

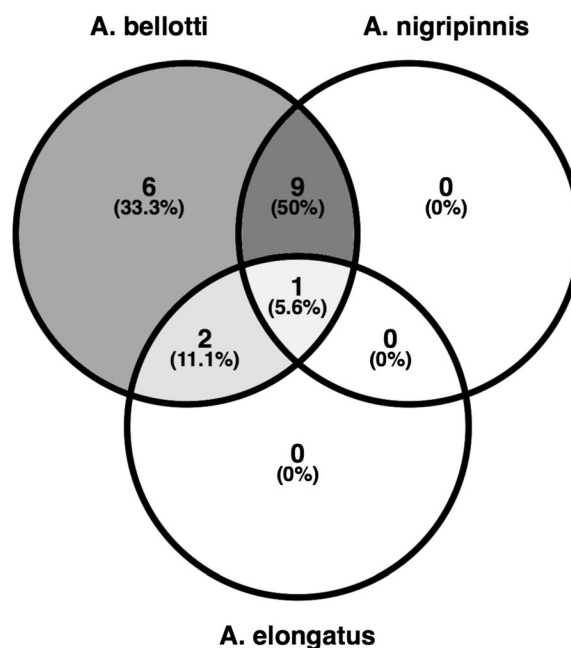


Fig. 2 Quantification of species co-existence in *Austrolebias* communities in Villa Soriano (Uruguay) region. *Austrolebias alexandri* was omitted from this analysis due to its sporadic occurrence

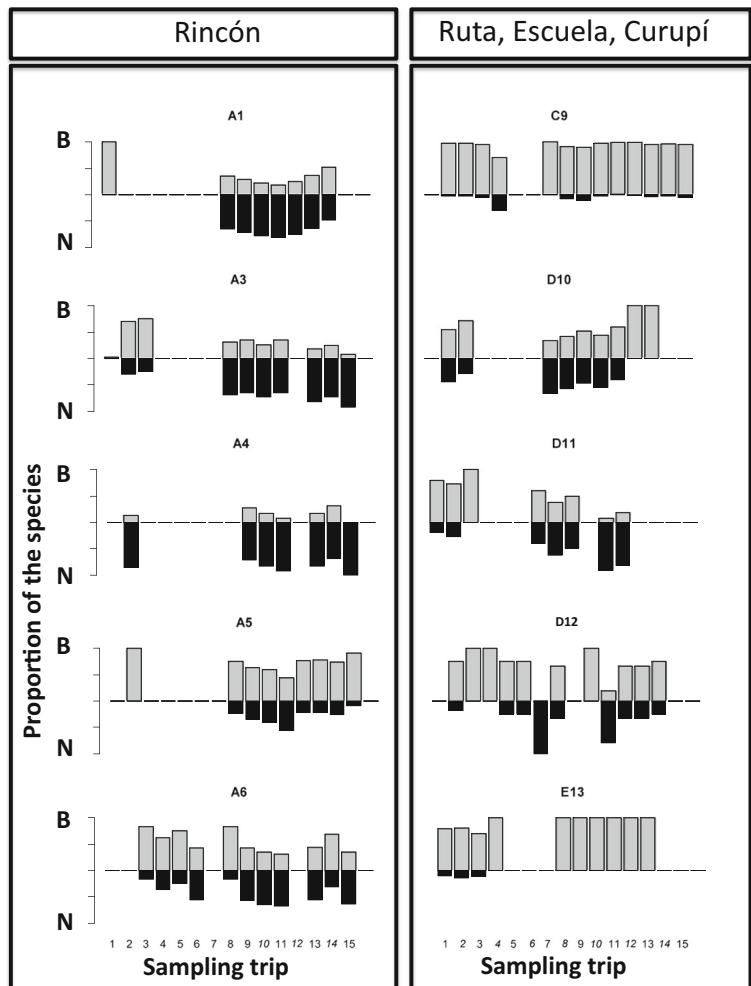
“Curupí” pool (13E). In addition, *A. nigripinnis* was rare (relative abundance 3.5%) in one pool (9C, “Ruta” section) and only a single individual was found in pool 14E (“Curupí” section). Overall, there was a seasonal decline in the proportion of *A. bellottii* to *A. nigripinnis* in the pools where they coexisted (GLMM with binomial error; estimate ± 1 standard error: -0.074 ± 0.012 , $z = -6.43$, $p < 0.001$, $n_{\text{samples}} = 93$, $n_{\text{pools}} = 11$; Fig. 3).

Austrolebias elongatus was rare in all pools where present. Its abundance was relatively highest in the “Curupí” section (pools 13E and 15E), and it was also found in the “Canaleta” region (pool 8B). It was absent from the Río Negro floodplain (“Rincón” section). *Austrolebias elongatus* was only found until 30 June (trip 4). *Austrolebias alexandri* was occasionally captured in the Rincón section (pools 3A, 5A) where it coexisted with *A. bellottii* and *A. nigripinnis*.

Seasonal dynamics

Most pools desiccated between 1 June and 27 July (trips 2–6) - only five pools did not show signs of desiccation (Table 1: 7B, 11D, 12D, 13E, 17E), despite a major decline in pool surface area (Online Resource 1). However, there was a single cohort of *A. bellottii* that survived over the entire season in pools 10D, 15E and 18E. These three pools were recorded as dry during the mid-season dry period, but contained surviving adult fish after re-filling. *Austrolebias nigripinnis* was present in four of the pools that sustained *A. bellottii* populations over the entire season. Its populations in pools 11D and 12D survived over the entire season, while the first cohort of *A. nigripinnis* disappeared from the other two pools that sustained

Fig. 3 Seasonal dynamics in the ratio between *A. bellottii* (grey) and *A. nigripinnis* (black) in the pools where the two species coexisted. The codes for individual pools are shown on top of each panel



A. bellottii populations over the dry period, with a second cohort emerging in pool 10D but not in pool 13E. Pool-specific seasonal dynamics in fish abundance is visualised in Online Resource 2.

The second cohort hatched in six pools (1A, 3A–6A, 9C; Table 1). These pools were mainly located in the Río Negro floodplain where the co-occurrence of *A. bellottii* and *A. nigripinnis* was common. In one pool (10D), the early-season *A. bellottii* cohort initially co-occurred with the early-season *A. nigripinnis* cohort and later with a new, late-season *A. nigripinnis* cohort. No second cohort hatched in four pools (2A, 8B, 14E, 16E) despite a secondary flooding.

For the subset of pools that did not desiccate, there was no linear decline in pool surface area over the entire season (LMM, estimate: -0.009 ± 0.013 , $t_{65.5} = 0.73$, $p = 0.469$), but both fish density (-0.032 ± 0.006 , $t_{66.1} = -5.13$, $p < 0.001$) and fish abundance significantly declined over the season (-0.061 ± 0.014 , $t_{65.4} = -4.21$, $p < 0.001$) (Fig. 4a). Until the first desiccation, there were clear seasonal declines in pool surface area (LMM, estimate: -0.179 ± 0.037 , $t_{46.8} = -4.86$, $p < 0.001$), fish abundance (LMM, estimate: -0.364 ± 0.032 , $t_{46.5} = -11.47$, $p < 0.001$), and fish density (LMM, estimate: -0.074 ± 0.021 , $t_{48.8} = -3.58$, $p = 0.001$) (Fig. 4b). For the second cohort, there was a statistically significant decline in pool surface area (-0.070 ± 0.018 , $t_{37.1} = -3.91$, $p < 0.001$), but no decline in fish density (-0.004 ± 0.011 , $t_{38.7} = -0.37$, $p = 0.713$) and only a marginal decrease in fish abundance (-0.078 ± 0.027 , $t_{37.3} = -2.70$, $p = 0.011$) (Fig. 4c).

Sex ratio

Seasonal dynamics in adult sex ratio was concordant between *A. bellottii* and *A. nigripinnis*. In populations that persisted throughout the season, the proportion of males significantly decreased over the season in both species (dates 1–16, binomial GLMM; *A. bellottii*: -0.041 ± 0.015 , $z = 2.72$, $P = 0.007$, $n_{\text{OBS}} = 42$, $n_{\text{POP}} = 6$; *A. nigripinnis*: -0.133 ± 0.042 , $z = 3.18$, $P = 0.002$, $n_{\text{OBS}} = 16$, $n_{\text{POP}} = 7$). No significant seasonal decline in male proportion was detected before the mid-winter desiccation (dates 1–6, binomial GLMM; *A. bellottii*: 0.061 ± 0.053 , $z = 1.15$, $P = 0.251$, $n_{\text{OBS}} = 46$, $n_{\text{POP}} = 16$; *A. nigripinnis*: -0.107 ± 0.093 , $z = 1.14$, $P = 0.254$, $n_{\text{OBS}} = 10$, $n_{\text{POP}} = 7$). The overall sex ratio during that period was already female biased in *A. bellottii* (37.8% of males, 95% CI: 32.9–42.7%) but not in *A. nigripinnis*

(51.2% of males, 95% CI: 43.8–59.4%). In the second cohort, the proportion of males did decrease seasonally (dates 8–16, *A. bellottii*: -0.053 ± 0.022 , $z = 2.39$, $P = 0.017$, $n_{\text{OBS}} = 42$, $n_{\text{POP}} = 6$; *A. nigripinnis*: -0.193 ± 0.027 , $z = 7.09$, $P < 0.001$, $n_{\text{OBS}} = 39$, $n_{\text{POP}} = 6$) (Fig. 5).

Discussion

Austrolebias bellottii was the only species present in all pools studied and its abundance was high in all pools. This suggests that *A. bellottii* is a generalist species that copes well with pools that vary in their flooding regime. The abundance of *A. nigripinnis* was highest in pools associated with active floodplain and regular flooding. The wide distribution of this species (reaching to the floodplain of the Río Paraná in Argentina) (Costa 2009) could indicate that *A. nigripinnis* has adapted to an environment subject to periodic flooding, though its populations also persist outside the active floodplain. Borthagaray et al. (2015) demonstrated that high pool connectivity was associated with lower annual fish diversity in the Atlantic coastal region, in contrast to diversity trends in vascular plants, odonates and anurans. It was hypothesised that this unexpected relationship was related to intraguild predation by large *Austrolebias* (Borthagaray et al. 2015). When pools are well connected, greater populations of predatory *Austrolebias* species may be supported at the expense of non-predatory *Austrolebias*, their common prey. In our study, *Austrolebias elongatus*, a member of the clade of large *Austrolebias* species (Costa 2009) was restricted to pools outside the active floodplain, but its low overall abundance and the rapid desiccation of many pools in July do not permit more general conclusions on its effect on *Austrolebias* assemblages.

Our record of *A. alexandri* represents the first documented observation of the species south of the Río Negro in the east coast of Río Uruguay. The range of *A. alexandri* (maximum size 50 mm) overlaps with *A. bellottii* and *A. nigripinnis* in the lower and middle Río Uruguay basin (Alonso et al. 2018b). The distribution of *A. alexandri* on both banks of the Río Uruguay is consistent with the limit of the paleo-sea (Cavallotto et al. 2004; Alonso et al. 2018b), with the species being restricted to the higher elevation outside the reach of river flooding. We registered four individual *A. alexandri* in two pools in the active flood plain of the river, which might indicate dispersal events rather

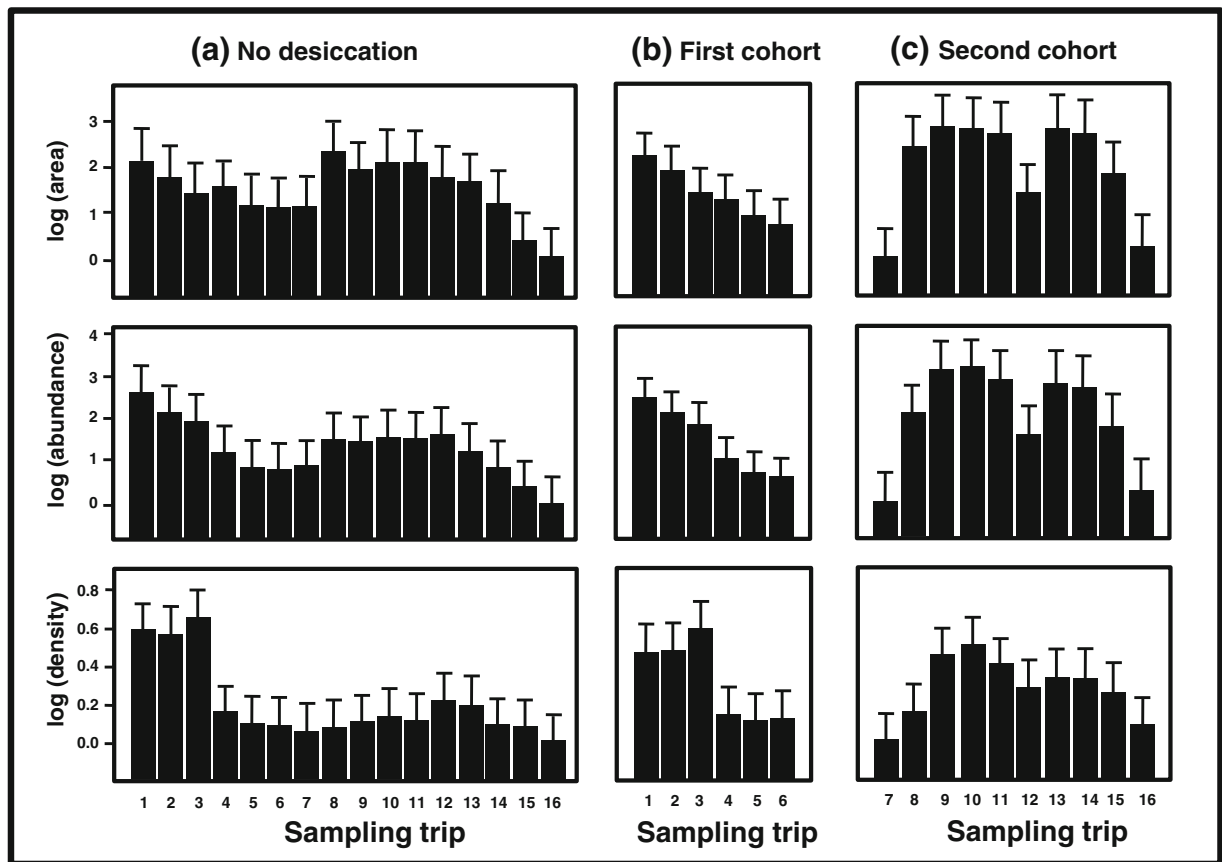


Fig. 4 Model-based estimates of mean pool area, fish abundance and fish density pooled across all sites with no mid-season desiccation (a), all sites prior to mid-season desiccation (b) and for second cohort (c). All response variables are log-transformed.

Mean values with one standard error are shown. To aid visual inspection of the seasonal dynamics, sampling trip was coded as a categorical (rather than continuous) variable

than stable populations. Large rivers do not represent a dispersal barrier to *Austrolebias* species, which are often present on both sides of the river (Alonso and Calviño 2018), in contrast to African *Nothobranchius* annual fishes where major rivers form sharp boundaries in species distributions (Bartáková et al. 2015).

The seasonal dynamics of previously studied *Austrolebias* species demonstrated that a single cohort persists over the entire wet season, with a continuous decline in abundance over the season (Lanés et al. 2014, 2016). We show that *Austrolebias* can respond to periods of unexpected mid-season drought by hatching a second cohort. The presence of several cohorts has occasionally been recorded in some African annual fishes of the genus *Nothobranchius* (Reichard et al. 2017), but our study documents it for the first time in *Austrolebias*. In the pools where some adults survived the apparent desiccation, no second cohort hatched, despite a large inundation of the secondary pool. This could be due to the

presence of a chemical cue (yet unidentified) of adult presence in the water that prevents embryos from hatching (Inglima et al. 1981). The single exception to hatching prevention by the presence of adults in the pool 10D might suggest that such a chemical cue may be species specific; only old adult (but no juvenile) *A. bellottii* and only juvenile (but no first-cohort adult) *A. nigripinnis* were found after the second filling of the pool 10D. The role of chemical cues and their specificity calls for an experimental study. We acknowledge that it is also possible that recently hatched second cohort fish were extirpated through predation by adult fish from the first cohort before we sampled those pools. However, the density of adult fish in recently inundated pools was too low to consider them extirpating the second-generation fish through predation. A continuous seasonal decline in fish abundance was apparent in common species, *A. bellottii* and *A. nigripinnis*, similar to that documented in the Atlantic coast *Austrolebias* species (Lanés et al. 2014).

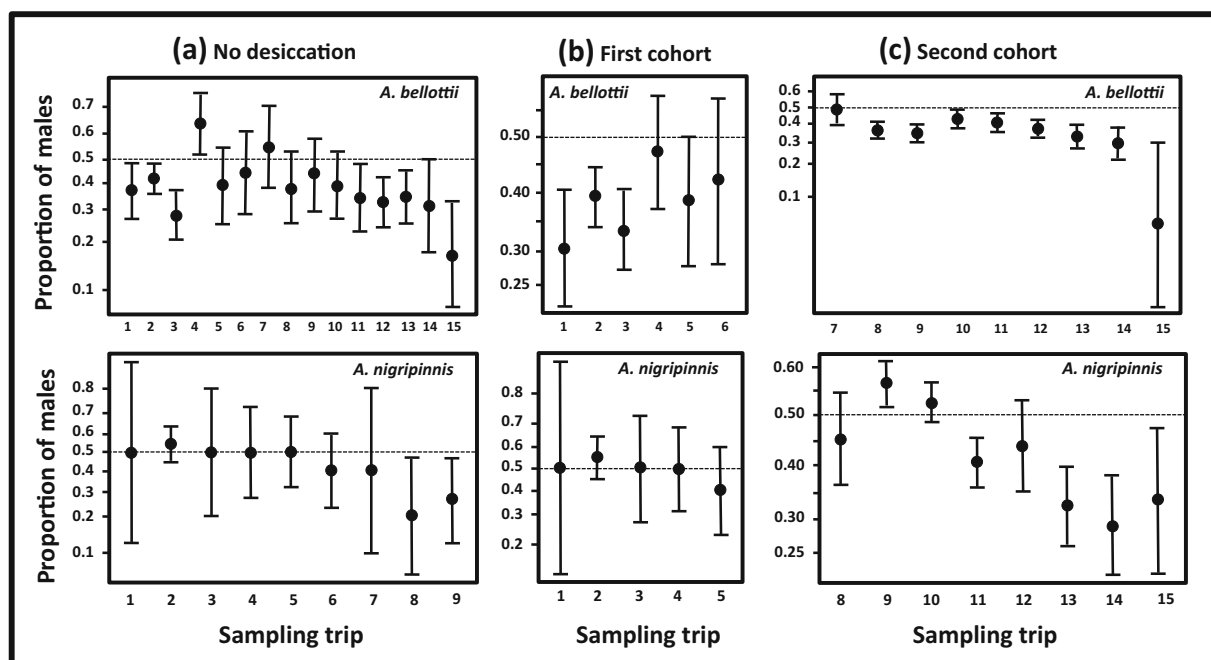


Fig. 5 Model-based estimates of sex ratio (proportion of males in the population) in *A. bellottii* (upper panels) and *A. nigripinnis* (lower panels) pooled across all sites with no mid-season desiccation (a), all sites prior to mid-season desiccation (b) and for second

cohort (c). Mean values with one standard error are shown. To aid visual inspection of the seasonal dynamics, sampling trip was coded as categorical (rather than continuous) variable

This pattern is commonly ascribed to the increase in mortality rate with age as a consequence of habitat deterioration, food limitation, rapid juvenile growth and intense reproductive activity (Arenzon et al. 2001; Volcán et al. 2012).

Population sex ratios showed a decline in the proportion of males over the season, resulting in a female bias by the end of the season. This has already been widely documented in other *Austrolebias* species (Passos et al. 2014; Lanés et al. 2014, 2016), as well as other annual (Reichard et al. 2014; Lanés et al. 2014, 2016; Vrtilík et al. 2018b) and non-annual kilifishes (Lanés et al. 2012). Higher male mortality is likely related to the high energetic demands of male reproductive activities, male-male aggression (Passos et al. 2014) or selective predation on larger and more conspicuous males (Haas 1976; Reichard et al. 2018). To this end, there was also a shift from *A. bellottii*-dominated populations to *A. nigripinnis* dominated populations, except when the first cohort of *A. bellottii* coexisted with the second cohort of *A. nigripinnis*. *A. nigripinnis* disappeared from that pool (10D, Fig. 3), being likely predated by large *A. bellottii*. The tendency for larger species to be more abundant at the beginning of the season while smaller species dominate at the end has also been documented in other annual

fishes (Lanés et al. 2016). Our observational data cannot establish whether this is the result of selective bird predation on larger fish (Reichard et al. 2018) or a higher life history trade-off between initial growth rate and rate of aging (Metcalf and Monaghan 2003).

One interesting finding of the present study is that three pools were recorded as desiccated during the mid-season drought, but harboured adults after re-filling. We believe that this indicates ability of the annual fish (especially *A. bellottii*) to survive extreme habitat conditions. During explorations of the dry pools, we detected some live adult *A. bellottii* buried in the moist mud in an almost dry pool (D. García, pers. obs.) and it is possible that some individuals survived in the mud over the short period of desiccation. An alternative explanation is that the heavy rains (lasting 3 days) might have connected the desiccated pools to other nearby pools enabling colonisation by dispersal of adult fish. It should be noted, however, that such dispersal must have been completed early in the flooding in order to prevent the hatching of a second cohort.

In conclusion, we demonstrate that the seasonal dynamics of the *Austrolebias* populations in the Río Uruguay basin are similar to those of the Atlantic region, except that they suffered an unusual mid-season

drought. The drought caused a bout of harsh environmental conditions that eliminated most *Austrolebias* populations, while some *A. bellottii* populations apparently survived the desiccation in the pool substrate. We found *A. bellottii* to be a generalist species whereas *A. nigrispinnis* was more common in the active floodplain where the pools are periodically flooded from an adjacent river. The two species had populations surviving for up to 7 months from hatching (April to November) or two age cohorts (April to July, August to November). Predatory *A. elongatus* were rare and disappeared relatively early in the season (June). Male-biased mortality led to increasingly female-biased populations from the age of 3–4 months, with a steeper male decline in warmer conditions later in the year. This study collects fundamental information on the life-history of the annual fish of this region, contributing to the interpretation of its ecological dynamics and the variation that arises from climate oscillations.

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