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PRIMARY RESEARCH PAPER

Phenology of three coexisting annual fish species: seasonal patterns in hatching dates

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Abstract Annual fish are specialized freshwater fishes that are adapted to live in seasonal freshwater pools. Their life cycle is tightly adapted to seasonally predictable aquatic and desiccated phases in their habitat. We used daily increments in otoliths to test the hypothesis of the direct association between seasonal rains and hatching dates of three coexisting *Austrolebias* species across 14 temporary pools in the Uruguayan pampa. Hatching was relatively synchronous within and between species across a small but topographically diverse region. Hatching occurred over 1 month in midautumn and peaked between 15 and 20 April 2015. The prediction of earlier hatching of a large predatory annual fish species was not confirmed. Unexpectedly, an unusual desiccation event in the middle of the winter growing season (May–July) affected many pools. Some pools re-filled after extensive precipitation in August, followed by the hatching of a new cohort in some (but not all) of those pools. The first cohort survived throughout the year (until late spring) in the pools that did not desiccate. Our study demonstrates how annual fish can cope with unexpected seasonal rainfall patterns that may be a consequence of current climate change.

Keywords Birth date · Climate change · Intraguild predation · Killifish · Hatching synchrony · Otoliths

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Introduction

Temporary pools are extreme habitats that alternate between aquatic and dry phases, typically associated with cycles of precipitation and evaporation. Direct links with permanent waters and connections among adjacent pools may be established during major flooding events. In other cases, groundwater flow systems can affect the water level and desiccation of temporary pools. The relative importance of each factor in the seasonal dynamics of the aquatic phase and the connectivity of temporary pools depends primarily on their distance from water sources and the local topography (Winter, 1999; Williams, 2006).

Community structure in temporary pools is strongly associated with the duration of the aquatic phase (Schneider & Frost, 1996) and is highly resilient following a desiccation event (Brock, 1998). Organisms either recolonize the pools actively (e.g. colonization from permanent water bodies during flooding by many fishes or via active flight by insects) or hatch from dormant life stages that are present in the desiccated substrate (e.g. desiccation-resistant eggs of crustaceans or diapausing embryos of annual fishes) (Brock et al., 2003; Polačik & Podrabsky, 2015).

Species that inhabit temporary pools are often tightly adapted to their seasonal cycle. Fish may either exploit temporary pools seasonally by colonizing them from adjacent permanent habitats during floods or possess specific adaptations to complete their entire life cycle in temporary pools (Polačik & Podrabsky, 2015). Annual fishes are a specialized group of small freshwater fishes that are closely adapted to the life cycle of temporary pools. They all belong to the suborder Aplocheiloidei (Cyprinodontiformes) where similar adaptations in response to seasonal desiccation have evolved repeatedly in Africa (Nothobranchiidae) and in the Neotropics (Rivulidae) (Furness et al., 2015; Loureiro & de, 2016). The key adaptation involves resistant eggs that survive desiccation buried in the pool sediment, with embryos possessing special developmental adaptations to these harsh conditions, including a series of three diapauses (Wourms, 1972a,b, c; Podrabsky et al., 2001; Berois et al., 2012; Podrabsky et al., 2017). Although the existence of desiccation-resistant life stages is common in aquatic invertebrates (Williams, 1997; Brendock & De Meester, 2003; Brock et al., 2003), it is extremely rare in aquatic vertebrates (Burton, 1990; Polačik & Podrabsky, 2015).

One remarkable adaptation of annual fishes is their ability to time their hatching to the start of the aquatic phase of the pools. The timing of the hatching event is thought to be triggered by environmental cues (partial oxygen pressure, substrate humidity, temperature) (Watters, 2009), with sensitivity to threshold values being determined by genetic background at the interspecific level (Reichard et al., 2017) and varying individually as a consequence of maternal effects (Polačik et al., 2017). Such complex control of hatching is believed to distribute its timing, minimizing the risk of complete failure of any offspring to reach sexual maturity in the case of non-seasonal rains, extensive dry periods, or too rapid desiccation of newly filled pools (Podrabsky et al., 2016).

Annual fishes inhabit temporary pools in tropical, subtropical and temperate zones. Interestingly, annual fishes experience fundamentally different conditions during their embryonic and post-hatching periods under different climatic conditions. Most tropical annual fishes, such as *Nothobranchius* in Africa and *Austrofundulus* in the Neotropics, hatch in the summer, at the beginning of the rainy season, spending their post-hatching lifespan in high water temperatures and completing their embryonic development when the water from the highly seasonal rains evaporates from the pool (Podrabsky et al., 1998; Blažek et al., 2013; Reichard, 2016). In contrast, temperate annual fishes from the southern Neotropics inhabit regions with no distinct rainy and dry seasons and an even distribution of precipitation during the year. Instead, the seasonality of the aquatic phase is driven by high evaporation during the hot summer months. Hence, fish hatch in the autumn and live through the cold winter period (Lanés et al., 2014, 2016), with embryonic development completed over the following summer (Arezo et al., 2005). This difference suggests that the adaptations and environmental clues that control hatching may be quite distinct among temperate compared to tropical annual fishes.

A remarkable feature of annual fish populations is the lack of overlap among generations. After hatching, annual fishes express rapid growth and reach sexual maturity within a very short time; from less than 3 weeks in tropical species (Blažek et al., 2013) to 12 weeks in subtropical/temperate species (Berois et al., 2012). Adults reproduce daily, but their eggs do not hatch and embryos likely remain in the sediment in one of the developmental diapauses (Reichard, 2016; Podrabsky et al., 2017). Current evidence suggests that the next generation hatches during a subsequent aquatic phase when desiccated period had eliminated parental generation. In African *Nothobranchius* from regions with erratic precipitation, pools may complete several phases of desiccation and re-filling during a single rainy season (Polačik et al., 2014), always with a new cohort of fish (Reichard et al., 2017). While there is some indication of several age cohorts coexisting in subtropical *Cynopoecilus* fishes on the basis of size distribution of adult fish (Arenzon et al., 2001) and possibility of continuous hatching in *Cynopoecilus* fishes (Arenzon et al., 1999), no study has thus far focused on estimating hatching dates (and their variability) in Neotropical annual fishes. Hatching synchrony (or lack thereof) has been demonstrated to be a strongly selected adaptive strategy in several taxa (Howe, 1976; Findlay & Cooke, 1982; Flint et al., 1994; Hillström & Olsson, 1994; Huss et al., 2010);

yet no direct information is available for Neotropical annual fishes.

The genus *Austrolebias* (Cyprinodontiformes, Rivulidae) is a group of annual fishes with 39 currently recognized species that all have restricted geographic distributions throughout the subtropical and temperate zones of southeastern South America (Loureiro & de, 2016). Their high diversity has been attributed to short generation time (1 year), fragmented habitat that restricts dispersal and promotes population isolation (D'Anatro & Loureiro, 2005), and genomic instability (García et al., 2001). The post-hatching part of *Austrolebias'* life cycle is limited to the cold period of the year when evaporation is low. Under normal conditions, their habitat fills with water in the autumn (March–May) and the aquatic phase persists until the end of spring (October–November) (Lanés et al., 2016).

In the present study, we examined hatching synchrony within and among coexisting annual fish species across 14 temporary pools, clustered in five regional groups. Using precise daily age estimates of young fish obtained from otolith readings, frequent visits to study sites and information on precipitation and desiccation from local pluviometers and dataloggers, we tested whether the hatching period was brief and corresponded with seasonal inundation of the pools or lasted over a protracted period. We compared differences and variabilities in hatching dates among pools and among *Austrolebias* species within the pools. We also correlated individual age and body size to test whether size was a reliable predictor of fish age. We further supported our age estimates and inferences on potential cohort coexistence with regular seasonal sampling of fish in the study pools.

Materials and methods

Study area

The study was conducted in Villa Soriano (Soriano, Uruguay) during 2015. Villa Soriano is located in the lowlands of the lower Río Negro, close to the confluence with the Río Uruguay (33°23′56″S, 58°19′13″W) (Fig. 1). This area is a typical pampa biome and is characterized by low altitude (1–5 m above sea level) and the presence of wetlands within the active river floodplain and in its vicinity (Batallés et al., 2009; Brazeiro et al., 2014). The adjacent Río Negro river directly floods a fraction of the study area (region Rincón) during the highest river discharge. The terrestrial vegetation of Pampa Biome is characterized by subtropical grasslands, but including native trees such as *Acacia caven* and *Prosopis nigra* (Brazeiro et al., 2014).

The study area has a moist subtropical climate. The mean annual rainfall is 1,130 mm year^{-1}, varying from 224 mm in February (summer) to 42 mm in June (winter). The mean annual temperature is 17.3°C, ranging from 11.3°C in winter to 23.4°C in summer (https://es.climate-data.org/location/50536/). The year of the study (2015) had an annual rainfall of 1,099 mm, with particularly dry episodes (42 mm on February, 52 mm in May, and 36 mm in July; data obtained from local pluviometers, the National Meteorology Institute). Monthly precipitation totals and their interannual variation are given in Fig. 2A.

The study covered a region of 5.5 km (latitudinal distance) by 9.7 km (longitudinal distance) and contained many pools with annual fishes (Fig. 1B). Most of the pools were located in an area of extensive livestock ranging (cattle, horses), and some pools were influenced by adjacent agricultural development. Three species of annual fish were previously recorded from the study area: *Austrolebias bellottii* (Steindachner, 1881), *Austrolebias nigripinnis* (Regan, 1912) and *Austrolebias elongatus* (Steindachner, 1881). A total of 28 ponds were chosen for a long-term research project, of which 14 were sampled to obtain estimates of hatching dates from otoliths. The latter were selected to maximize the diversity of *Austrolebias* communities, habitat and local topography, and geography.

For the 14 selected pools, five local regions were characterized in the area (Fig. 1B; Table 1). The Rincón region contained five pools (1A–5A), with *A. bellottii* and *A. nigripinnis* coexisting in all of them. The Rincón was affected by the Río Negro flooding and used exclusively for cattle ranching. The Canaleta region included two pools (6B, 7B) that contained *A. bellottii* (both pools) and *A. elongatus* (single pool). The Ruta region included only a single pool (8C), located next to the main road, with an adjacent exotic *Eucalyptus* tree stand and soybean plantation. This pool was the most isolated. The Escuela region (two pools: 9D–10D) was located in an extensive livestock area with no agricultural development. Each Escuela

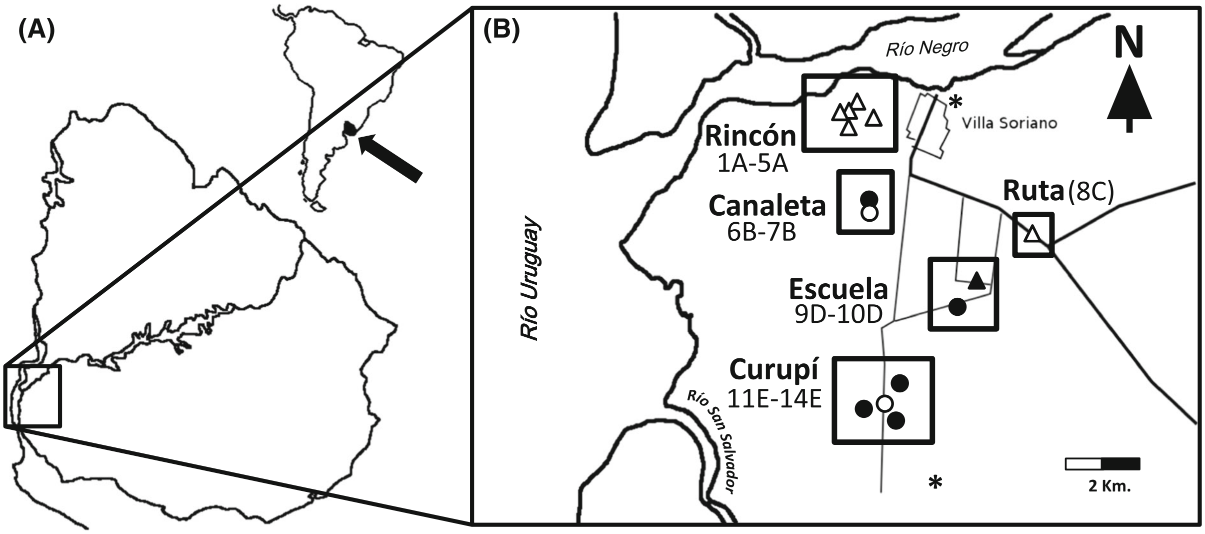

Fig. 1 Map of the study area. **A** Uruguay on the map of South America (marked by black arrow) and position of the study area within Uruguay. **B** Individual pools and their assignment to geographical clusters, indicating: pools that desiccated in winter and did not support a new cohort (open circles), pools that did not desiccated in winter and the first cohort persisted throughout the study (black circles), pools that desiccated, a second cohort established and did not coexist with the first cohort (open triangles), pools that partially desiccated and the second cohort coexisted with the first (black triangles). Asterisks indicate geographic position of two local pluviometers

pool had a variable connection with surrounding pools during the heaviest precipitation. The pools 8C–10D contained coexisting *A. bellottii* and *A. nigripinnis* populations. The Curupí region had four pools (11E–14E), with various combinations of all three study species (Table 1).

Fish collection

Fish sampled for otolith extraction were collected during 6 visits (16–18 May, 30 May–1 June, 28 June, 27–28 Aug, 11 Sept, 5 Nov) using a square dip net (20 × 15 cm, 2 mm mesh size) on a 150 cm long pole. Fish were immediately identified to species on the basis of their coloration and morphology (Teixeira de Mello et al., 2011). Upon fish collection, a subsample was chosen for the study, targeting the smallest and largest individuals in the sample to include as wide an age spectrum as possible for each species at each site. Sampled fish were euthanized with an overdose of clove oil and stored in 96% ethanol. This procedure maximized the chance of including the entire age range of fish present in the pool, in accordance with the aim of the study. A variable sample size of between 3 and 5 individuals per site was taken. In the laboratory, all fish were measured for standard length (from the tip of the snout to the end of caudal peduncle, i.e. excluding caudal fin) to the nearest 0.01 mm, sexed and individually labelled.

Other fish collected during the field trip were measured to the nearest 1 mm, with estimates of their density, sex ratio and relative abundance. This dataset (7,679 fish from 26 pools collected between 18 May 2015–1 Jan 2016) is analysed elsewhere (Daniel García, unpublished data) but we used that data as supporting evidence for an age cohort overlap.

Otolith reading

Fish otoliths were analysed at a commercial facility (Barcelona Otolith Reading Service (BORS), Spain, EU) for age estimates. The reading facility was blind to sample identity except for species identification. Reading was completed on sagittal otoliths. In the laboratory, sagittae were removed by dissection, mounted on microscope slides and polished until daily increments were visible as alternating dark and light circuli (Fig. 3; Fablet et al., 2011). Reading of each otolith was repeated three times; an additional set of

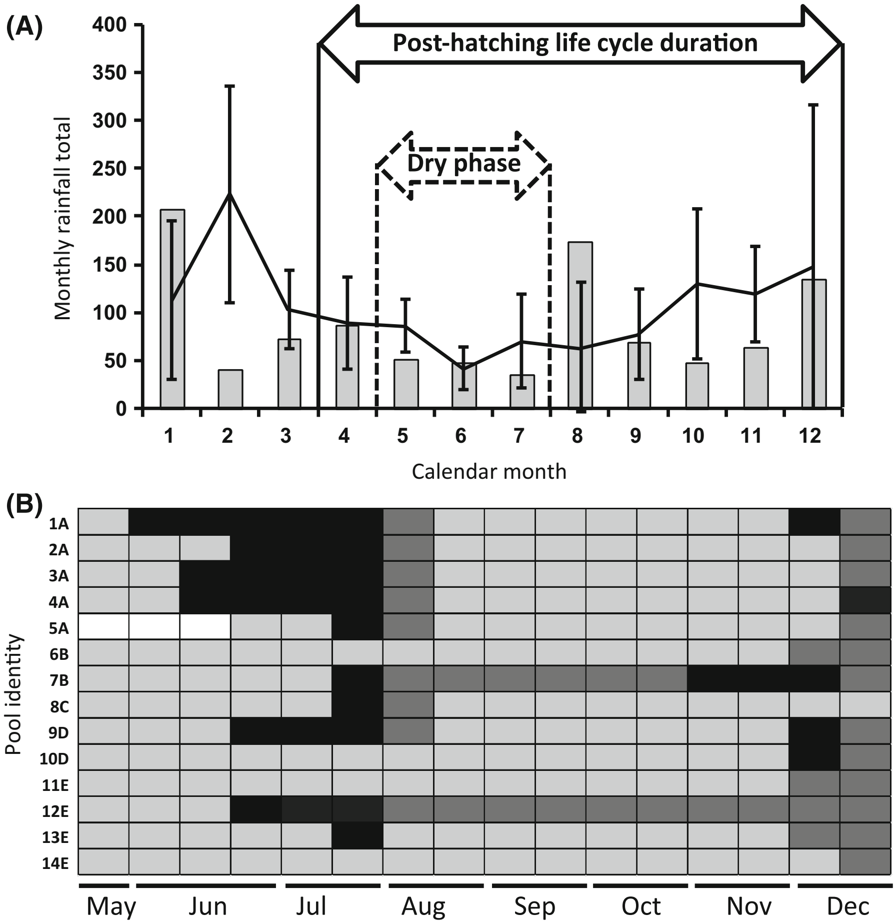

Fig. 2 **A** Monthly precipitation totals (grey bars, in mm) recorded in locally deposited pluviometer in 2015 and long-term (2009–2014) seasonal patterns in monthly precipitation totals (mean and standard deviation, data provided by the National Meteorology Institute (INUMET)). The duration of the complete life cycle (solid lines and arrow) and of the exceptional winter dry phase in the study year (broken line and arrow) are indicated. **B** Desiccation of each pool over the season. Black, dry pond; dark grey, pond with water and no fishes present; light grey, pond with water and fishes present; white, no data available

two or three readings was done in cases where the readings produced conflicting results. The full set of readings was provided to the authors by BORS.

From a total of 101 otoliths, 86 were read. Ten otoliths could not be read reliably due to deformations or breakage. The age of six otoliths was estimated as 65–84 days, with highly inconsistent reading replicates (difference of 20–62 days between consecutive estimates) and remarks from the commercial facility that readings were likely imprecise. We discarded those six otoliths and use only otoliths with the reading of age < 50 days. All analyses were completed on the final sample of 80 otoliths, originating from 14 pools.

We calculated mean values from multiple readings of each otolith and used them in all analyses. Repeated estimates were typically within the range of 0–3 days, and differed by 5 days at maximum (2 cases), in contrast to unrepeatable otolith readings from individuals with the mean estimated age of more than 50 days. The youngest fish were estimated to be 12 days old.

We assumed that each otolith increment corresponded to 1 day of age after hatching. The precision of daily increment counts in otoliths has previously been validated for *Nothobranchius furzeri*, an annual fish species from Africa (Polačik et al., 2011), and other fishes (Brothers et al., 1975; Campana & Nielson, 1982; Sogard, 1991; Carmeño et al., 2003) but not for *Austrolebias* fishes. Indirect evidence supports the assumption of daily deposition of increments (size at age analysis, the coincidence of estimated hatching dates with habitat inundation; see Results), but we acknowledge that estimates for our sample were not directly validated against individuals of known age.

Table 1 An overview of pools sampled, including location (GPS), surface area in m^2 (mean, minimum and maximum over entire study period, i.e., 18 May 2015–1 Jan 2016), the presence of annual fish species, incidence of midseason desiccation, the presence of a second cohort, and persistence of the first cohort throughout the entire year cycle

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	1,202	671	1,512	AB, AN	Yes	Yes	No
2A	Rincón	33°23′41.57″S	58°20′47.09″W	1,020	11	2,643	AB, AN	Yes	Yes	No
3A	Rincón	33°23′48.89″S	58°20′58.52″W	935	200	2,327	AB, AN	Yes	Yes	No
4A	Rincón	33°23′51.23″S	58°20′19.64″W	1,658	7	3,367	AB, AN	Yes	Yes	No
5A	Rincón	33°24′00.17″S	58°20′48.77″W	1 353	6	2,992	AB, AN	Yes	Yes	No
6B	Canaleta	33°24′59.32″S	58°20′18.60″W	161	4	702	AB	No	No	Yes
7B	Canaleta	33°25′15.75″S	58°20′20.20″W	407	4	1,203	AB, AE	Yes	No	No
8C	Ruta	33°25′40.21″S	58°17′12.73″W	52	9	160	AB, AN	Yes	Yes	No
9D	Escuela	33°26′17.63″S	58°18′14.72″W	81	6	259	AB, AN	No	Yes	Yes
10D	Escuela	33°26′33.78″S	58°18′37.51″W	138	44	218	AB, AN	No	No	Yes
11E	Curupí	33°28′01.48″S	58°19′46.85″W	212	11	533	AB, AN, AE	No	No	Yes
12E	Curupí	33°28′29.20″S	58°20′03.15″W	107	29	234	AB, AN	Yes	No	No
13E	Curupí	33°28′29.18″S	58°20′23.98″W	595	45	2,364	AB, AE	Yes	No	Yes
14E	Curupí	33°28′55.37″S	58°19′53.94″W	466	105	1,102	AB	No	No	Yes

AB *Austrolebias bellottii*, AN *Austrolebias nigripinnis*, AE *Austrolebias elongatus*

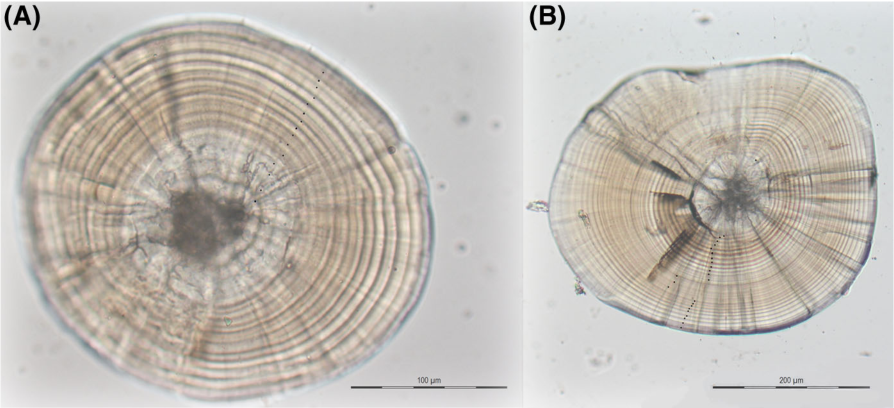

Fig. 3 Examples of otolith sections, with daily increments apparent as the alternation of dark and bright circuli (indicated by black dots). **A** Male *Austrolebias bellottii* (standard length 9.17 mm), collected on 8 May 2015, in pool 8C (Ruta), 15 days old. **B** Male *Austrolebias nigripinnis* (standard length 14.61 mm), collected on 19 May 2015, in pool 3A (Rincón), 26 days old. Note scale bars indicating age-related otolith size difference between **A** and **B**

Environmental data

We set data loggers (HOBO Onset UA-002-08) in 9 of the 14 pools that recorded ambient temperature at 3 h intervals throughout the study year. Data loggers were deployed on 18–21 May, i.e. after hatching of the first cohort. Loggers were attached to a metal rod driven deep to the substrate. Loggers recorded temperature at the pool bottom, with the depth of pools varying from 0 to 40 cm. The amplitude of daily fluctuations in ambient temperature is a good predictor of the presence of water; day:night fluctuations are minor during the wet phase but increase considerably when the logger is exposed to air (desiccated phase) (Reichard et al., 2017). We estimated the start and end of the aquatic phases by visual inspection of the records. This approach has been validated by direct observation during regular (fortnightly) visits to field sites. In addition, we measured pool size at every visit by walking around the pool shoreline with a portable GPS device with area calculation (Garmin, Etrex10). Environmental data are presented in Tables 1 and 2.

Data analyses

Hatching dates were obtained by subtracting estimated age in days from the date of sample collection. Dates were saved in Julian date format (score 1 was assigned to 1 January) and followed a Gaussian distribution. Linear mixed models (LMMs) were used to compare mean hatching date between coexisting species. Variation among pools was controlled by assigning random intercepts for each site. Models were checked for overdispersion of residuals. We also correlated estimated age and body size using Pearson correlations with body size data being log-transformed to standardize residuals. The correlations were performed for each species and sex separately. Data analysis was completed in the R statistical environment (R 3.2.4), using *lmer* library (Bates et al., 2014).

Results

Hatching dates and synchrony

Two clear cohorts were found in our study area within the single calendar year (Fig. 4). The first cohort contained all three species and hatched between 8 April and 7 May 2015 (Table 3). The second cohort

Table 2 Mean water temperature of study pools with dataloggers deposited on pool bottom recording ambient temperature every 3 h, with total range of temperature recorded over the particular period

Pool	18–31 May	1–30 Jun	1–31 Jul	1–31 Aug	1–30 Sep	1–31 Oct	1–30 Nov	1–31 Dec
1A*	16.1	12.0	11.2	15.9	16.1	18.1	20.5	NA
	9.9–25.4	− 3.7–30.8	− 2.8–33.6	9.1–28.6	7.0–27.2	8.3–32.2	12.2–34.5	NA
4A	16.5	12.5	11.8	15.5	13.6	16.3	19.6	25.4
	6.1–27.8	− 3.8–33.6	− 4.0–34.7	10.7–23.5	8.1–19.1	9.3–24.2	12.4–28.4	13.2–42.5
6B	16.3	12.2	11.8	15.3	13.7	16.5	21.0	25.7
	8.7–25.7	− 0.9–25.3	3.8–23.3	9.7–23.6	8.5–20.6	10.4–28.4	11.9–37.4	8.7–45.1
7B#	15.8	11.6	11.4	15.3	NA	NA	NA	NA
	10.6–22.0	0.7–22.0	0.01–29.6	10.7–18.1	NA	NA	NA	NA
8C	16.2	13.6	12.5	15.1	14.7	15.5	19.7	23.8
	9.9–23.1	2.3–27.6	1.1–38.1	10.2–22.9	8.7–20.4	10.0–24.0	12.3–30.6	14.0–36.6
9D	16.1	12.0	12.1	15.2	15.3	16.6	21.6	26.1
	8.3–25.1	− 1.6–25.9	0.5–29.0	9.4–23.5	8.6–22.9	10.5–27.1	11.2–38.9	6.2–45.0
10D	16.4	12.3	12.0	15.3	14.8	17.2	21.5	26.1
	8.5–24.9	− 1.6–28.9	4.6–23.0	8.6–23.0	8.7–19.1	11.7–24.3	12.6–40.3	9.8–45.7
11E	13.8	11.2	11.1	14.8	14.7	17.5	21.9	25.7
	9.3–19.3	1.1–23.4	5.0–18.7	11.4–19.6	10.3–18.5	10.4–25.1	14.3–36.1	10.0–48.4
12E§	15.2	12.2	11.9	15.7	15.4	15.7	NA	NA
	8.7–22.4	2.2–22.1	2.2–24.0	8.9–25.3	6.9–25.8	10.9–22.0	NA	NA

Subzero temperatures refer to the period when pools were desiccated (pools 1A and 4A) or nearly desiccated (pools 6B, 9D, 10D)

*Last read on 8 Nov 2015

#Last read on 11 Aug 2015

§Last read 1 Nov 2015

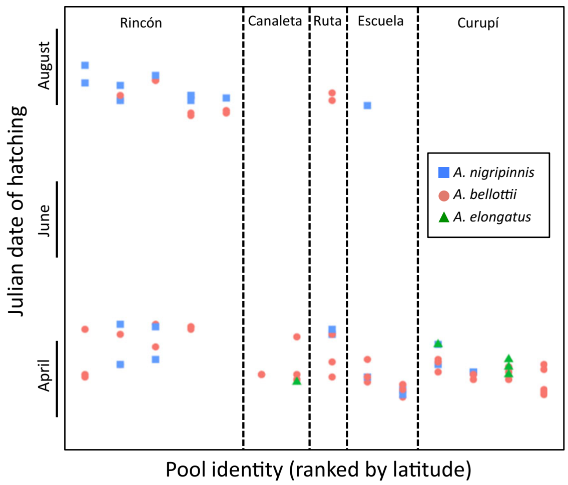

Fig. 4 Estimated individual hatching dates across study region and species. Populations are ranked along latitudinal gradient (north to south), with different local regions separated by broken vertical lines. Different species are represented by different symbols

was estimated to hatch in a subset of pools between 27 July and 16 August 2015. No specimens of *A. elongatus* were detected in the second cohort (Table 3).

The first cohort hatched in all 14 studied pools. The second cohort was recorded in seven of these pools (Table 1). Nine pools (64%) desiccated in the period between 1 June and 27 July (first dates recorded as dried) and remained dry until a spell of heavy rain between 30 July and 1 August (Table 1). Desiccation over that period was confirmed by datalogger estimates (Fig. 5). The two cohorts were typically separated by pool desiccation and did not coexist, except for a single pool (pool 9D, Escuela region) where the second cohort emerged without complete pool desiccation. In three pools (33% of those that desiccated), a second cohort was not found despite flooding of the habitat. In 5 pools (36%) that did not desiccate, the first cohort always survived throughout the study period (Table 1), until the end of spring (November–December).

There were regional differences in pool desiccation and the existence of a second cohort. Rincón and Ruta regions contained two consecutive age cohorts, with no temporal overlap. Temporal coexistence of both cohorts was detected in Escuela region. No second cohort was found in Canaleta and Curupí regions (Table 1; Fig. 2B).

Interspecific differences

There were no interspecific differences in hatching dates in the first seasonal cohort (LMM: first cohort: $F_{2,48.3} = 0.38$, $P = 0.683$) but *A. bellottii* hatched earlier than *A. nigripinnis* in the second cohort (LMM: $F_{1,13.0} = 11.22$, $P = 0.006$). The model estimated difference in hatching dates (taking into account variation among pools) suggested that *A. bellottii* hatched 1.65 (s.e. 1.93) days earlier than *A. nigripinnis* in the first cohort and 4.2 (s.e. 1.26) days in the second cohort. No difference in hatching date between *A. bellottii* and *A. nigripinnis* in the first cohort was detected even when the two species were compared without *A. elongatus* (LMM: $F_{1,44.2} = 0.92$, $P = 0.342$) and, therefore, the lack of correspondence

Table 3 Estimates of hatching dates (mean, standard deviation, median) for three study species, with sample size (N) and variability (earliest and latest recorded hatching and range in days)

Year 2015	*N*	Mean date	Mean julian date	SD	Median date	Min	Max	Range (in days)
(a) *A. bellottii*								
1st cohort	42	20 April	109.6	8.3	17 April	8 April	7 May	29
2nd cohort	10	2 Aug	215.1	5.2	2 Aug	27 July	10 Aug	14
(b) *A. nigripinnis*								
1st cohort	13	23 April	112.6	9.52	20 April	9 April	7 May	28
2nd cohort	10	6 Aug	219.0	5.1	4 Aug	31 July	16 Aug	16
(c) *A. elongatus*								
1st cohort	5	20 April	109.6	5.77	19 April	13 April	28 April	15

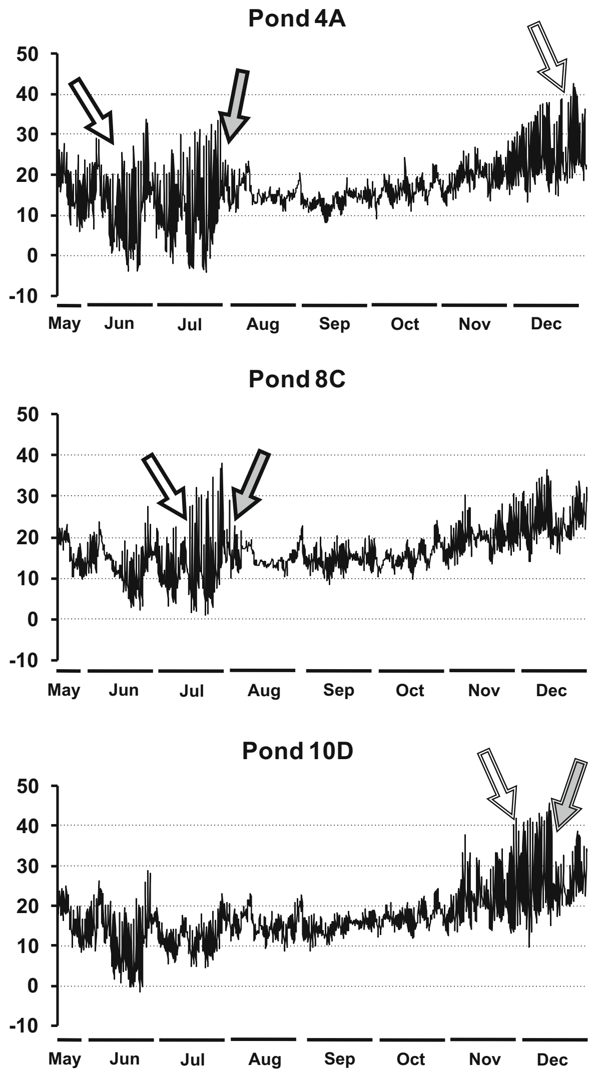

Fig. 5 Ambient temperature of six representative pools recorded directly by in-site loggers. Wet and dry phases are indicated by arrows (open arrow: start of the dry phase; grey arrow: start of the wet phase; simple border line: the first desiccation period, double border line: the second desiccation period)

between the first and second cohorts was not an analytical artefact. A detailed description of species- and cohort-specific hatching date summary is provided in Table 3.

Relationship between age and body size

Individual body size was positively correlated with the estimated age of male and female fish in *A. bellottii* and *A. nigripinnis* (Pearson correlation, body size log-transformed; Table 4; Fig. 6). The small sample size

Table 4 Pearson correlation between estimated age (days) and body size (standard length in mm)

Species	Sex	*R*	*P*	*N*
A. bellottii	Males	0.83	< 0.001	24
	Females	0.73	< 0.001	26
A. nigripinnis	Males	0.72	0.014	10
	Females	0.56	0.039	13

Body size data were log-transformed prior the analysis to normalize residuals

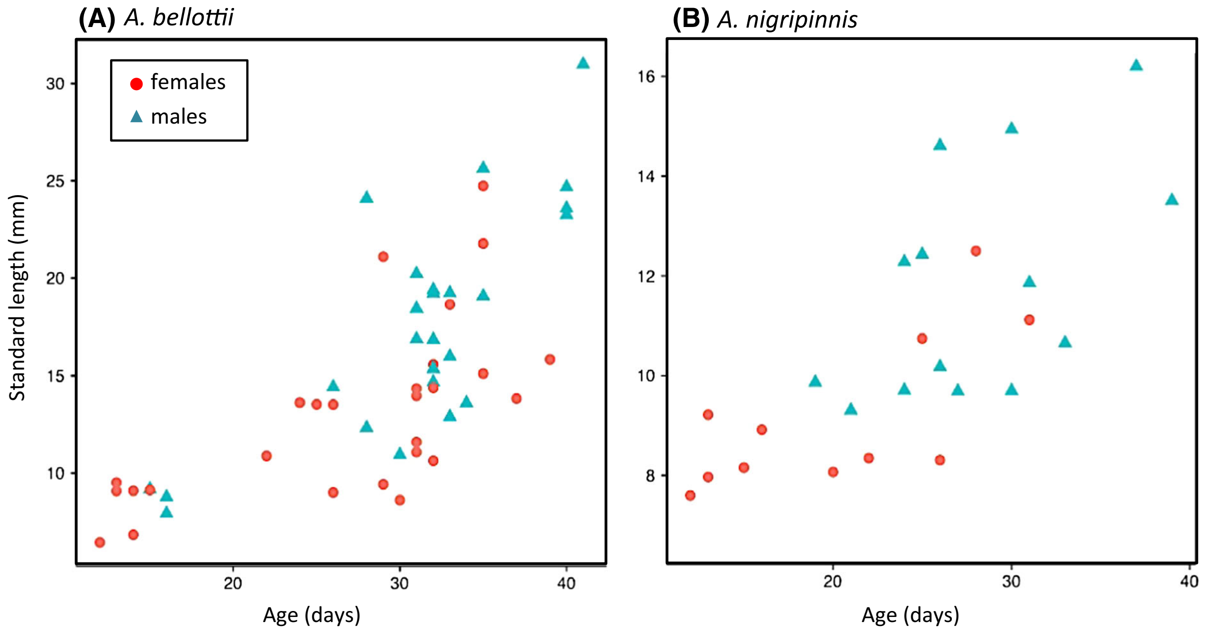

Fig. 6 Relationship between individual body size (standard length, in mm) and estimated age across all samples from all study pools with body size measurements for (**A**) *A. bellottii* and (**B**) *A. nigripinnis*. Males are denoted by blue triangles and females by red circle

of *A. elongatus* did not permit comparison of size at age.

Discussion

We have demonstrated that hatching of *Austrolebias* fishes in temporary pools of Uruguayan pampa was synchronous among individual pools and, partly, among sympatric species. It has been hypothesized that the large size of some *Austrolebias* species has evolved along with adaptation to a predatory trophic niche (Costa, 2009). In the annual fish community we studied, *A. elongatus* is the large species (Teixeira de Mello et al., 2011). By hatching earlier, *A. elongatus* offspring would be able to prey upon newly hatched *A. bellottii* and *A. nigripinnis*. This has not been observed, and all analysed otoliths of *A. elongatus* were within the age range of the oldest individuals of the two coexisting species. Indeed, preliminary studies of gut content did not confirm predation of *A. elongatus* on smaller congeners (E. Machín, personal observation). Greater size at hatching and rapid initial growth rates could instead explain the size advantage of *A. elongatus* over its syntopic congeners, but this possibility remains to be tested.

In dragonfly larvae, within-species hatching asynchrony is known to promote cannibalism (Hopper et al., 1996). In general, cannibalism among cohorts may influence cohort size structure via size-selective mortality, increased growth rates of the cannibals and of surviving younger cohorts as they benefit from relaxed competition for shared resources (Huss et al., 2010). Synchronizing hatching can, therefore, minimize cannibalism by older individuals. However, the ability to modulate the timing of hatching events depends critically on the cues used by pre-hatching stages as environmental hatching triggers. Environmental keys (i.e. sea tides) appear to determine hatching in crabs (Saigusa, 2000), but the start of the hatching appears to be based on a more complex set of cues in annual fishes (Reichard et al., 2017).

Hatching occurred between early April and early May, with a peak on 15–20 April. Unexpectedly, 9 (64%) of the pools desiccated during June and July, following their re-filling during a spell of high precipitation between 30 July and 1 August. In six of the nine re-filled pools, a new cohort hatched, developed normally and reached sexual maturity but no fish hatched in the other three. No new cohort hatched in pools that did not desiccate, with the exception of a single pool where the new cohort coexisted with a few older fish. The first cohort survived throughout the year (until late spring) in the pools that did not desiccate.

Hatching of the first cohort at the beginning of autumn is typical for *Austrolebias* (Berois et al., 2014). Heavy precipitation in summer (December–February) coincides with high evaporation, providing no opportunity for the pools to persist for a longer time. There is no evidence that *Austrolebias* hatch during this period of heavy rains, although we note that February precipitation was unusually low in the study year (Fig. 2A). In contrast, relatively light precipitation in autumn (March–May) leads to inundation and stable aquatic conditions in the pools and subsequent rains continue to maintain the aquatic phase until the end of spring (October–November), before evaporation exceeds precipitation. As a result, only a single cohort survives and completes its entire life cycle (Lanés et al., 2016). This scenario was valid for four pools that contained only a single cohort. In three of these pools, the population size dramatically decreased after the unusually dry period in midwinter (when other pools desiccated), and only a few adults survived until the end of the year (D. García, unpublished data). Only in a single pond (14E), the volume of the pool did not decrease dramatically, and the *Austrolebias* population did not experience major mortality.

A second cohort hatched in seven pools in early August, within 2 weeks after re-filling. Contrary to the case among African *Nothobranchius* (Polačik et al., 2014; Reichard, 2016; Reichard et al. 2017), the succession of multiple cohorts over a single year was not documented for *Austrolebias*. The new cohort experienced very different environmental conditions to the first cohort, with larger pool area, higher water temperature (Table 2) and more abundant food. The presence of *Austrolebias* is related to colder ambient temperatures, and it is assumed that longer exposure to high water temperature is not compatible with its survival (Walford & Liu, 1965). High temperature retards *Austrolebias* growth and lifespan (Liu & Walford, 1966; Volcan et al., 2012). Despite this, we recorded adult *Austrolebias* in pool 8C until summer (31 December) when water temperature reached 36°C. This population was strongly female-biased (15 females, 1 male) and fish possessed clear symptoms of physical deterioration (D. García, personal observation). This pool was surrounded by extensive stands of *Eucalyptus* trees that shaded the water surface. In other pools, the water temperature reached in excess of 40°C (Table 2), and no fish were found. Such environmental conditions are common for tropical annual fishes (Podrabsky et al., 1998; Reichard et al., 2009; Shalk et al., 2014; Reichard 2016) but are documented here for *Austrolebias* for the first time.

The midwinter dry period in the year 2015 was very unusual compared to previous years and it may be one of the signs of climate change. The current global climate change is likely causing changes in precipitation patterns (Trenberth, 2011). This affects species phenology (timing of life cycle events) (Bellard et al., 2012). While those changes are more apparent in plants (Cleland et al., 2007), effects on breeding and migratory phenology in birds, insect and aquatic taxa have been widely observed (Visser & Both, 2005; Manhard et al., 2017). In general, changes in phenology could disrupt ecosystem function as the level of response to climate change likely varies across taxonomic and functional groups and trophic levels (Edwards & Richardson, 2004). While *Austrolebias* fishes are adapted to variable environments and seem to cope with changes in seasonality in rainfall patterns by the production of multiple cohorts, a single-season study cannot document any longer-term consequences of midwinter hatching and spring juvenile development on maturation and egg production. Unlike African *Nothobranchius* (Polačik et al., 2014, 2017), *Austrolebias* annual fishes are not adapted to erratic and unpredictable precipitation. In this sense, longer-term research is needed to understand the population effects of altered rainfall seasonality on *Austrolebias* fishes.

The synchrony in hatching dates was stronger in the second cohort than in the first cohort. The pools were filled by relatively light rains in April and hatching of the first cohort may have been more protracted as the pool sediment had been flooded over an extended period, resulting in gradual filling. In contrast, the second cohort hatched after heavy precipitation that flooded pools to their full extent within a few hours. This was most pronounced in the Rincón area that was flooded from the adjacent river. Therefore, all eggs were flooded synchronously, resulting in a narrower range of hatching dates. Within each pool, all fish hatched within 2 weeks in the first cohort and within a single week in the second cohort (Fig. 4). This range is considerably narrower than reports from African

Nothobranchius where hatching can be distributed over 3-4 weeks despite similarly rapid flooding (Reichard et al., 2017). A particularly dry late summer period (February) in the study year may have contributed to the relatively more protracted hatching period.

In pools within the river alluvium or close to a permanent water body (pools 2A, 3A, 5A, 12E, 13E), the presence of non-annual fishes was recorded following flooding events. Typical stream fishes (Characiformes, Siluriformes, Cichlidformes and non-annual Cyprinodontiformes) were recorded in those ponds. In addition, synbranchid eels were recorded rarely in more isolated pools (7B, 14E), where no other stream fishes were detected. Non-annual fishes are frequently reported to coexist with annual fishes in many annual fish habitats (e.g. Vaz Ferreira et al., 1966; Nico & Thomerson, 1989; Reichard, 2016) and seem to have no major effect on *Austrolebias* populations (Lanés et al., 2016).

Standard length was a relatively good predictor of individual *Austrolebias* age. This is in contrast with African *Nothobranchius* where body size is strongly affected by density-dependent effects (Vrtílek & Reichard, 2016) and body size is not correlated with age (Polačik et al., 2011; Reichard et al., 2017). It is likely that the association is especially strong in younger fish (as included in our study) and levels off later in the season as fish approach their asymptotic body size.

Two cohorts coexisted in a single pool (9D). One explanation could be that the new cohort hatched in the presence of adult conspecifics when the water level rose after heavy precipitation and flooded the desiccated substrate near pool margins. Local coexistence of multiple age cohorts in *Austrolebias* is extremely rare (Lanés et al., 2016). There is also experimental evidence that the presence of adult fish prevents the completion of embryo development in African *Nothobranchius* (Inglima et al., 1981). The response is produced by the presence of chemical signals from adult conspecifics (Inglima et al., 1981), an important component of *Austrolebias* communication (Passos et al., 2013). However, the evidence is related to the development of embryos that have their development halted in a diapause. In pool 9D, the embryos may have completed their development in the desiccated sediment, with no cues from adult fish that were constrained in a small residual pool. When the pool increased following extensive precipitation, the new cohort may have hatched from the recently flooded sediment. Indeed, in embryos that are developmentally ready to hatch (pre-hatching stage sensu Wourms, 1971), the presence of conspecifics (or other fishes) does not prevent hatching (Polačik & Janáč, 2017). In addition, it is possible that the new cohort originated in a different pool and dispersed to pool 9D during high water level. The Escuela region is an area with many small pools scattered in close proximity and pool 9D could have received water from several different sources during the heavy rains. Hence, the coexistence of two cohorts may arise from a secondary source.

It is notable that no new cohort hatched in three of the pools despite abundant water persisting over a period of at least 3 months and the presence of adult fish in the preceding months. It is possible that in those pools, embryos were not in the pre-hatching stage and had not completed their development before the next inundation. Whatever the exact cause (e.g. too short a time to complete embryo development, lack of sufficiently high ambient temperature for embryo development over the summer), the absence of a second cohort may indicate a potential threat of climate change to the long-term persistence of *Austrolebias* populations, especially in regions with lower pool connectivity. It is also possible that some adults persisted in a wet substrate of the desiccated pool but were not recorded in any later sampling due to their very low numbers. While this could have prevented embryos from hatching (Inglima et al., 1981), as discussed above, this is not a very likely scenario.

Conclusions

We have demonstrated relatively synchronous hatching among three coexisting species of *Austrolebias* fishes in a series of topographically diverse temporary pools in an Uruguayan pampa region. The earlier hatching of large predatory species has not been confirmed. An unusual desiccation event in the middle of the winter growing season affected many pools. While pools that did not desiccate sustained *Austrolebias* populations throughout the study year, some desiccated pools were refilled later in the year and supported new *Austrolebias* cohorts. However, some refilled pools did not support a new cohort. Whether a level of stochasticity in *Austrolebias* presence is a typical feature at an intra- and inter-annual scale is currently unknown, but it may represent a recent consequence of current climate change. Only long-term research can address this question.

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