Changing patterns of growth in a changing planet: how a shift in phenology affects critical life-history traits in annual fishes

Daniel García¹, Carl Smith²,³,⁴, Emanuel Machín¹, Marcelo Loureiro¹ and Martin Reichard²,*

¹Departamento de Ecología y Evolución, Instituto de Biología, Facultad de Ciencias, Universidad de la República, Iguá 4225, Montevideo, Uruguay
²The Czech Academy of Sciences, Institute of Vertebrate Biology, Květná 8, Brno, Czech Republic
³Department of Ecology & Vertebrate Zoology, University of Łódź, Banacha 12/16, 90-237 Łódź, Poland
⁴School of Biology and Bell-Pettigrew Museum of Natural History, University of St Andrews, St Andrews KY16 8LB, UK

ORCID: MR (0000-0002-9306-0074)

*Corresponding author: M. Reichard: The Czech Academy of Sciences, Institute of Vertebrate Biology, Květná 8, 603 65, Brno, Czech Republic, phone: +420 543 422 522, e-mail: reichard@ivb.cz

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Summary

1. Under current climate change trends, freshwater wetlands in the temperate Neotropics are predicted to undergo major alternations in precipitation regime.

2. We investigated consequences of altered seasonal patterns in precipitation on replicated populations of annual killifish, using a field survey. Annual killifish are endemic to ephemeral pools and highly endangered through disruptions to wetlands.

3. In 2015, the Pampa region of western Uruguay experienced an unprecedented mid-winter desiccation of temporary pools that naturally contain water from autumn to late spring and typically only desiccate in summer. In our study area, 72% of desiccated pools were re-inundated from later rains and annual killifish hatched a second cohort in some of them (53%).

4. Compared to the first cohort, the second cohort experienced lower water temperatures during hatching and juvenile development, but higher temperatures during the adult growth phase. The second cohort of fish developed more rapidly, with 40% earlier attainment of asymptotic body size (14 vs. 23 weeks) that was significantly smaller in one species (*Austrolebias bellottii*) but not in the other (*Austrolebias nigripinnis*).

5. Rapid juvenile development of second-cohort fish was associated with earlier maturation and greater investment in reproductive tissue. The abbreviated life history was adaptive because second-cohort fish lived shorter lives due to high summer temperatures desiccating pools and extirpating the annual populations.

6. Annual fish demonstrated high developmental plasticity that buffered the negative consequences of a severely altered seasonal climatic cycle. The capacity of these annual
fishes to cope with alternations to their life cycle will depend on the ability of egg banks in the sediment to tolerate an increased unpredictability of precipitation.
1. INTRODUCTION

Current climate change trends are rapidly driving modifications to the temporal and spatial distribution of temperature and precipitation across the planet (Trenberth, 2011; Walther et al., 2002). These changes can affect biodiversity at all levels, from individual populations to biomes (Parmesan, 2006). Some of the most striking effects of climate change are alterations in species phenologies; changes in timing of crucial life cycle events. This effect may lead to cascading effects at the community level (Edwards & Richardson, 2004; Gilman et al., 2010), through the responses of interacting species (Bellard et al., 2012). Changes in plant phenology, for example, can affect pollinator populations. Given their mutual dependency, altered plant phenology may ultimately lead to the extinction of both pollinators and plants (Rafferty & Ives, 2010). However, a range of other effects on the relationships between affiliate species can emerge (Rafferty et al., 2014; Hassall et al., 2019), with largely unknown capacity for resilience in some taxa (Hegg et al., 2013).

Changes in species phenology may also lead to changes in life history traits, which determine the way an individual spreads its reproduction over its lifetime (Forrest & Miller-Rushing, 2010). For example, delayed hatching time can lead to post-hatching development under altered environmental conditions, shifting growth and maturation patterns, with potential consequences for the viability of subsequent generations (Varpe, 2017). In Atlantic salmon (Salmo salar), date of emergence of offspring has consequences for juvenile body size, with a significant decrease in juvenile body size at the end of the growing season in individuals that emerged one week later than the first cohort. This outcome may affect size-selective events, such as winter mortality, predation risk and reproduction (Einum & Fleming, 2000). Other ecological challenges can alter key life-history events. Interspecific resource
competition can ultimately affect growth patterns and timing of sexual maturation (Ferreira-Rodríguez et al., 2018) to a degree that surpasses the effects of climate (Rolinson et al., 2016). Therefore, it is important to compare the relative importance of one ecological challenge (climate change) with other potential modulators of life-history events.

The effects of altered phenology are likely to be especially pronounced in ephemeral environments. Temporary habitats are subject to shifts between alternative habitat states, often predictably following annual cycles of precipitation and temperature (Varpe, 2017). Temporary pools are extreme environments that depend on natural seasonal cycles between aquatic and desiccated phases (Polačík & Podrabsky, 2015). Among the organisms exploiting these environments, many are exclusively adapted to the periodic phases of the habitat. In some cases, selection favours adaptation to the regular pattern of aquatic phases, such as annual spring flooding (Lytle & Poff, 2004), though other species appear adapted to the unpredictability of erratic events, such as desert flooding (Polačík & Podrabsky, 2015). As climate change modifies precipitation regimes and increases the incidence of temporarily abbreviated rainfall and concomitant extended periods of drought and heavy rainfall (Witze, 2018), temporary pools experience dramatic change in their seasonal patterns of aquatic and desiccated phases (Brooks, 2009). How these changes might alter the viability of populations occupying these environments has yet to be explored.

Annual killifishes are a unique group of vertebrates that is exclusively adapted to live in temporary pools. In the Neotropics and Africa, killifishes (Cyprinodontiformes) have repeatedly adapted to life in ephemeral freshwater habitats (Furness, 2016), with three independent evolutionary transitions to adopt this life history in the family Rivulidae (Furness et al., 2015a), a Neotropical clade of
Cyprinodontiformes fishes (Loureiro et al., 2018). Populations of annual fishes persist in desiccated sediment in the form of embryos encased in eggs with a thickened chorion. Development is halted in three diapause stages (Wourms, 1972) when the embryo is particularly resilient to environmental stress (Podrabsky et al., 2001). Fish hatch when their natal pool is filled with water. After hatching they grow rapidly and reach sexual maturity within a few weeks (Vrtílek et al., 2018). Adults reproduce daily and the eggs are deposited into the substrate where they survive until a subsequent aquatic phase. The timing of embryo development is variable, with a marked differential propensity of embryos to enter and leave diapause (Wourms, 1972; Podrabsky & Hand, 1999; Furness et al., 2015b; Polačík et al., 2018), though it is broadly synchronised by environmental cues (Domínguez-Castanedo et al., 2017), including temperature (Podrabsky et al., 2010a), photoperiod (Levels & Denucé 1988) and relative humidity (Podrabsky et al., 2010b).

Neotropical annual killifish are highly endangered. The major recognized threat to their populations in southern South America (the Pampas biome) is habitat loss associated with extensive agriculture and floodplain regulation (Volcan & Lanés, 2018). In addition, annual fish depend on specific precipitation patterns and may be strictly adapted to local natural cycles of pool inundation and desiccation. In southern South America, the natural aquatic phase begins in the austral autumn (April), when evaporation decreases and pools retain water from precipitation. The dry phase begins in late spring (November), when evaporation increases and exceeds precipitation, and habitats desiccate. Hence, unlike in the tropical range of annual fishes in Africa (Reichard, 2016) and the Neotropics (Domínguez-Castanedo et al., 2013; Podrabsky et al., 1997), there are no distinct rainy and dry seasons, and precipitation (with substrate
wetting and short-term accumulation of water) is relevant throughout the dry season, though precipitation does not lead to an aquatic phase during the summer months.

With recent changes in climatic conditions, we have earlier reported (García et al., 2018) that the lower Río Negro floodplain (Villa Soriano district in western Uruguay, Pampa vegetation region) experienced an unusual desiccation of temporary pools in the middle of the aquatic phase (austral winter) in 2015, followed by an unusually heavy precipitation that re-filled most of the desiccated pools. This event triggered the hatching of a new cohort of annual fish in some pools (García et al., 2018).

Here, we tested how the alteration of seasonal patterns affected the growth, maturation and fecundity of these anomalous annual fish populations. The region of South American pampas, to which more than 40 species of annual killifish are endemic (Froese & Pauly, 2018), experienced a moderate increase in temperature and more than 30% decrease in precipitation between 1976 and 1990 (Walther et al., 2002). This area belongs to a region with the fastest estimated temperature increase (Loarie et al., 2009). Periods of heavy rains are predicted to increase in the region of between 20 - 50% in the next 90 years with a 3 °C increase in global temperature (Fisher & Knuti, 2015; Witze, 2018).

We investigated how the growth rate and sexual maturation of two annual killifish species, *Austrolebias bellottii* and *A. nigripinnis*, were affected by non-seasonal conditions (a second cohort that hatched in mid-winter rather than in autumn) and compared it with the effects of another ecological challenge, interspecific competition. Growth and maturation are crucial life history traits for individual fitness (Stearns, 1992) and population dynamics (Sæther & Bakke, 2000) and this is especially pronounced in short-lived annual fishes (Wootton & Smith, 2015; Vrtílek et al., 2018). We compared the growth and maturation of replicated *A. bellottii* populations...
coexisting with *A. nigripinnis* and replicated *A. bellottii* populations without intra-guild interspecific competition. Then, as the main goal of the study, we compared the growth and development between the two cohorts of *A. bellottii* and the timing and size at maturation of both cohorts and for each species. We predicted slower growth and later sexual maturation in populations of *A. bellottii* coexisting with *A. nigripinnis* as a result of interspecific competition between species occupying a similar ecological niche (Arim *et al.*, 2010). Based on life history theory, we further predicted that the second cohort (with a shorter expected lifespan) would trade somatic growth for a greater reproductive effort (Figueroedo *et al.*, 2006). Thus, individuals of the second cohort were predicted to reach a smaller final size but mature earlier than the first cohort and make a greater investment into reproduction than the first cohort, which was predicted to divert more resources to a longer growth phase resulting in higher lifetime fecundity.

### 2. Methods

#### 2.1. Study area

The study was conducted in the surroundings of Villa Soriano in Uruguay (33°23'56"S, 58°19'13"W) in 2015. This is a wetland area of a typical Pampa biome. It is characterized by low altitude (1-7 m above sea level) and an abundance of shallow temporary freshwater pools. The pools are from 100 to over 2,000 m² in area, with a maximum depth of 0.4 m. Annual killifish (three species) are regularly encountered in the pools. Other fish species invade the pools sporadically, following flooding from the adjacent Rio Negro. The study area encompassed approximately 53 km². For a detailed description of the study area see García *et al.* (2018).

The study is based on sampling in 18 pools (Fig. 1). The annual fish that inhabit the area are *Austrolebias bellottii*, *A. nigripinnis* and *A. elongatus*. Thirteen pools (72%)
were re-inundated but the second cohort of *A. bellotti* hatched only in 6 of 18 (33%) and *A. nigripinnis* in 7 pools (39%). No second cohort hatched after re-inundation in the 6 pools. The first cohort persisted throughout winter in XXX pools (*A. bellottii*) and YYY pools (*A. nigripinnis*). Given the rare occurrence of *A. elongatus* (0.23% of sampled fish in a total of 17% of the pools), this study focused on the other two species.

2.2. Sampling and data collection

Sampling started on 18 May 2015 and lasted until no further fish could be collected or pools were dry (1 January 2016). Sampling was conducted every two weeks, except for a 20-day interval between trip 7 (9–10 Aug) and trip 8 (29 Aug–1 Sep). For all pools, fish hatching dates were estimated from otolith increments and the age of the fish was calculated by adding the number of days between sampling trips. The first cohort hatched in late April and the second cohort hatched in early August in some pools that experienced mid-season desiccation (García *et al.*, 2018). The pools were at least 0.3 km apart, except for pools 11D and 12D that were only 0.1 km apart but clearly separate. Detailed descriptions of seasonal dynamics in population density and abundance are described in García *et al.* (2019). In short, for the period of juvenile growth the pool area was larger in the second cohort, but fish density was comparable between the cohorts. Water temperature data were collected by data loggers (Hobo UA-002-08) deployed in 12 pools on the first day of sampling. The loggers also provided the timing of pool desiccation (Lanés *et al.*, 2016; García *et al.*, 2018).

Sampling was accomplished with a dip-net (200 x 150 mm, 2 mm mesh size) that was swept parallel to the bottom. All available habitats in the pool were explored. Sampling effort varied across sites due to wide variation in fish density and pool area. Sampling effort was recorded as 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 event by walking around the pool boundaries using a portable GPS unit to map the outline of the pool surface (Garmin Etrex10). After sampling, all annual fish collected were photographed on a tray (80 x 80 mm) with a wetted surface and a reference scale. A total of 4,804 *A. bellottii* and 2,592 *A. nigripinnis* were measured. Sample size for each pool, species, sex, and sampling event is given in Supporting Information (Table S1). A random sample of 5 females was taken at each sampling to estimate sexual maturation. Fish were euthanized with an overdose of clove oil and stored in 4% formaldehyde for later dissection in the laboratory. All other 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). Size of fish was measured from photographs using *tpsDig*2 software (v 2.1) (Rohlf, 2009) from the tip of the snout to the end of the caudal fin (Total Length, TL). In the laboratory, a sample of 5 females was measured for TL, weighed (W, to the nearest of 0.001g), dissected and gonad mass (Wg) measured.

### 2.3. Data analysis

All analyses were completed in the R statistical environment (R Core Team, 2018). First, we tested the effects of the presence of *A. nigripinnis* on the growth patterns of *A. bellottii*. *Austrolebias bellottii* was present in all pools but *A. nigripinnis* occurred only in 55% of pools (10 of 18). We used sex-specific Generalised Additive Mixed Models (GAMM) with a Gaussian error distribution, with Coexistence (factor with two levels) included as a fixed effect. The effect of age was modelled with a smoother term with 5 knots (k = 5), defined *a priori* based on the growth trajectory of *Austrolebias* (Volcan *et al.*, 2012) and allowing for potential selective disappearance of larger fish at the end of the season (Reichard *et al.*, 2018). The response variable was body size, measured
as TL. To account for dependency in the data from variation among pools, pool identity was included as random effect. Two models were constructed that differed in how the smoother term was modelled. First, a separate smoother was assigned for each treatment level (coexistence with *A. nigripinnis* or not). Second, the same smoother was used for both treatments. The fit of the models was compared using Akaike Information Criterion (AIC). An AIC difference of \( >10 \) is considered a substantial improvement in model fit. GAMM analysis was completed in the `gamm4` package (Wood & Scheipl, 2017).

Similarly, we compared whether coexistence with *A. nigripinnis* affected time to sexual maturity in *A. bellottii* females. Because a high proportion of fish were immature, data were modelled using a zero-altered (hurdle) model, using R-INLA (Rue et al., 2017). Zero-altered models are partitioned into two parts, with a binary process modelling zeros and positive counts, and a second process modelling only positive counts using a zero-truncated model (Hilbe, 2014). We modelled zero-truncated data using a Gaussian distribution (Zuur et al., 2014). This approach enabled us to separately identify the environmental variables that predicted maturity (binary part) and investment in gonad mass when gonads were present (zero-truncated part). Fixed factors were Coexistence (two levels), body size (TL, log-transformed, continuous predictor) and their interaction. In the model, the interaction between Coexistence and TL measured the contribution of species coexistence to the timing of sexual maturation relative to body size. This analysis was based on a contrast between 6 populations of *A. bellottii* without *A. nigripinnis* and 5 populations of *A. bellottii* competing with *A. nigripinnis*, with four populations dropped during data exploration due to the small number of females analysed (\( \leq 5 \)).
The main analysis compared growth trajectories and sexual maturation of fish from the first and second cohort, using the same approach as for testing the effect of coexistence. We predicted, *a priori*, that the two species and sexes within species, would differ in body size (Costa, 2006) and, consequently, we separated them before analysis. We fitted sex-specific GAMMs with a Gaussian error distribution that included the factor Cohort (two levels). Given that the coexistence of *A. bellottii* with *A. nigripinnis* had no effect on growth and sexual maturation, this factor was not included in the analysis. We modelled age either with separate smoothers for each cohort (model 1) or a common smoother for both cohorts (model 2), with \( k = 5 \) in both models. The fit of the two models was compared using AIC. The same analysis was performed for both fish species.

To formally test the differences in the growth rates (rather than size at age) among cohorts, we calculated Specific Growth Rates (SGR) using the function:

\[
SGR = \left( \ln(TL_x) - \ln(TL_{x-1}) \right) \times \frac{100}{N_{\text{days}}}
\]

Where \( TL_x \) is the Total Length for the date \( x \), \( TL_{x-1} \) is the Total Length for the previous date and \( N_{\text{days}} \) is the number of days between each date. SGR was estimated for each species and each pool separately. We tested whether SGR differed between cohorts by fitting a Gaussian Linear Model (LM) with age interval, cohort and their interaction as factors. The response variable was population-specific SGR. Given the shorter lifespan of the second cohort, we truncated age intervals for the first cohort to permit a full interaction between the two factors. This analysis was completed for *A. bellottii* only because we had insufficient estimates of population-specific SGR for the first cohort of *A. nigripinnis*. For visualization, we extended estimated SGR for the first cohort over their entire lifespan.
While growth curves are commonly used in analysing growth data, they suffer from temporal dependency. To formally accommodate temporal dependency in the data, we further modelled fish size using a random walk (RW2) trend model in a Bayesian framework using Integrated Nested Laplace Approximation (R-INLA; Rue et al., 2017). Fish size was fitted for fish age following a gamma distribution with a log link function. The model was fitted as:

\[ TL_{ijk} \sim N(\mu_{ijk}, \tau) \]

\[ E(TL_{ijk}) = \mu_{ijk} \quad \text{and} \quad \text{var}(TL_{ijk}) = \frac{\mu^{2}_{ijk}}{\tau} \]

\[ \log(\mu_{ijk}) = \eta_{ijk} \]

\[ \eta_{ijk} = \beta_1 + \beta_2 \times \text{Sex}_{ijk} \times \beta_3 \times \text{Coh}_{ijk} + \beta_4 \times \text{Area}_{ijk} + \text{Age}_i + \text{Pool}_k \]

\[ \text{Age}_i \sim N(0, \sigma^2_{\text{Age}}) \]

\[ \text{Pool}_k \sim N(0, \sigma^2_{\text{Pool}}) \]

Where \( TL_{ijk} \) is the size of fish \( i \) of age \( (\text{Age})_j \). The variable \( \text{Sex}_{ijk} \) is a categorical covariate with two levels (males, females) and \( \text{Coh}_{ijk} \) is a categorical covariate with two levels (first or second cohort). The model also contained a linear effect for pool size \( (\text{Area}_{ijk}) \) as its extent varied over the season. The random intercept \( \text{Pool} \) was included in the model to introduce a correlation structure between observations for fish collected from the same pool with variance \( \sigma^2 \), distributed normally and equal to 0. Uniform default priors were put on model parameters and fitted estimates of fish size matched observed data.

Time to sexual maturation and investment in reproduction were compared between cohorts using data on gonad mass (Wg) fitted with a zero-adjusted model with a gamma distribution (ZAG), like that used for interspecific competition contrasts. All...
final models were checked for overdispersion and model mis-specification following

the guidelines of Zuur & Ieno (2016).

3. RESULTS

3.1. Effect of interspecific competition

There was no major effect of inter-specific competition on the growth of *A. bellottii* (GAMM, males: \( F = 0.12, P = 0.77 \); females: \( F = 1.27, P = 0.260 \)), with a clearly inferior fit of the separate-smoothers model for females (\( \Delta AIC = +256 \)). In males, the model with separate smoothers for males provided a better fit (\( \Delta AIC = -10.3 \)), though the principal difference was that the separate-smoothers model captured differences in size-selective mortality at the terminal phase of life rather than different growth rates among *A. bellottii* populations coexisting with *A. nigripinnis* (Fig. 2). There was no effect of coexistence with *A. nigripinnis* on the timing of sexual maturity of *A. bellottii* females (binary part of the model) or their allocation to gonads (zero-truncated part). Female *A. bellottii* maturation and allocation to reproduction depended on body size but the scaling of the relationship did not differ between coexistence treatment levels (Table 1).

3.2. Differences between the two cohorts

3.2.1. Water temperature

The first cohort experienced a temperature regime typical of temporary pools in the region. Water temperature decreased from warm (>20°C) to cold (<10°C) over the early life phase, remained relatively cold until the age of approximately 100 days and slowly climbed to 20°C again over the subsequent 100 days (Fig. 3). The second cohort
hatched in relatively colder water that steadily increased in temperature and never fell below 10°C (Fig. 3).

### 3.2.2. Growth trajectories

There were clear differences in growth trajectories of the first and second cohorts of both species, except for *A. nigripinnis* females. The models with separate smoothers for each cohort were clearly superior in *A. bellottii* males (ΔAIC = -118), *A. bellottii* females (ΔAIC = -246) and *A. nigripinnis* males (ΔAIC = -39), but only marginally in *A. nigripinnis* females (ΔAIC = -2.52). The growth curves were characterised by earlier attainment of the growth asymptote in the second cohort (approximately 100 days) than in the first cohort (approximately 150-170 days) (Fig. 4), resulting in a larger mean asymptotic size in *A. bellottii* (males: F = 19.79, P < 0.001, Fig. 4a; females: F = 16.16, P < 0.001; Fig. 4b) but not *A. nigripinnis* (Fig. 4c, 4d).

Comparing specific growth rates (SGR) confirmed a steeper growth trajectory of second cohort fish over age-matched categories (LM: F\(_{1,71} = 4.67, P = 0.024\)) and much stronger decline in the SGR with age in the second cohort fish (age by cohort interaction: F\(_{1,71} = 12.42, P = 0.001\); age: F\(_{1,71} = 3.28, P = 0.074\)) (Fig. 5). Overall, SGRs were initially higher in the second cohort but declined sharply with age. In contrast, the growth rates of the first cohort were relatively stable, though they varied among populations (Fig. 5).

### 3.2.3. Body size

Fitting a common smoother to growth data for *A. bellottii* using a gamma random walk trend model fitted using INLA demonstrated that sex differences in body size were higher in the second cohort (sex by cohort interaction in Table 2) and quantitatively confirmed that the second cohort grew more quickly (Cohort effect in Table 2).
3.2.4. Sexual maturation

Female maturation was strongly positively related to age (binomial occurrence model) with a steeper increase in the second-cohort females (Table 3a), implying that second-cohort fish reached sexual maturation at a younger age. In addition, the frequency model (magnitude of gonadal investment) demonstrated that the second-cohort females invested more heavily in gonad mass. Greater gonad mass was also recorded from pools with a larger area. As expected, gonad mass was positively associated with female age (Table 3b).

4. Discussion

The primary effects of climate change are alterations in precipitation and temperature dynamics which have profound implications for species phenologies (Parmesan, 2006; Ficetola & Maiorano, 2016). However, responses to climate change and its consequences potentially vary greatly among species (Both et al., 2009; Colchero et al., 2019) and among particular interspecific interactions (Gilman et al., 2010; Kordas et al., 2011; Hassall et al., 2019). We demonstrated previously that Austrolebias annual fishes are able to hatch a new cohort in response to a secondary inundation following an unexpected mid-winter (July) pool desiccation, though a second cohort hatched only in a subset of pools (García et al., 2018). In the present study, we tested the developmental consequences of late season hatching in an environment with a non-optimal temperature regime in A. bellottii. Overall, the changes associated with late hatching demonstrated the capacity of A. bellottii to cope with shifted seasonality and to adjust their life history to maximize reproductive success. Following non-seasonal desiccation and subsequent re-filling of pools, 46% (6 of 13) A. bellottii populations
hatched a second cohort. The second-cohort juveniles developed in colder water (until the age of approximately 50 days) but sustained accelerated growth and more rapid sexual maturation, along with a greater investment in fecundity. While this came at the cost of smaller final size, the response was adaptive since life expectancy (and actual lifespan) in the second cohort was shorter. The magnitude of response was substantial, with no effects on growth and sexual maturation in populations subjected to another ecological challenge, interspecific competition with *A. nigripinnis*, a related annual killifish species that coexisted in several pools with *A. bellottii*. *Austrolebias nigripinnis* also experienced accelerated growth in the second cohort, though the effect was marginal in *A. nigripinnis* females and their asymptotic size was not smaller.

Differences in growth rate, final size and sexual maturation were most likely shaped by the different temperature dynamics experienced by juvenile and adult *A. bellottii*. The first cohort experienced typical seasonal dynamics in water temperature, with hatching and the juvenile period during warm-water conditions in the austral autumn (April), followed by a gradual decrease in temperature with adults living in a low temperature environment during winter and experiencing increased mortality in late spring (November) as the temperature again increased (*García et al.*, 2019). Those conditions are typical for most *Austrolebias* populations (*Lanés et al.*, 2016). The second cohort hatched in cold water with the temperature steadily increasing over the season and exposing young adults (5 months old) to ambient temperatures exceeding 25°C (Fig. 3), which is considered the upper limit for the long-term survival of *Austrolebias* (*Lanés et al.*, 2016). In addition, the cohorts experienced different photoperiods. While both cohorts hatched in a photoperiod of 10.5-11 hours of daylight, this period decreased by 1 hour over two months of juvenile growth for the first cohort, but increased by 2 hours for the second cohort, respectively (www.sunrise-sunset.org).
Increasing water temperature and photoperiod might have also increased productivity of the pools, further stimulating more rapid growth of the second cohort. Pools were generally larger after the second inundation, but killifish population densities were comparable (García et al., 2019).

Laboratory studies have demonstrated that annual fishes are sensitive to temperature regimes (Hsu & Chiu, 2009; Volcan et al., 2012; da Fonseca et al., 2013). *Austrolebias adloffi*, a related species from the same region, exhibited a decreased growth rate and shorter lifespan when a natural decrease in water temperature fluctuation was interrupted. After rearing juveniles at 22°C, a decrease in temperature (to 16°C) was associated with longer lifespan and sustained growth while control fish that remained at 22°C ceased growing (Liu & Walford, 1966). Our field data are consistent with laboratory studies of *Austrolebias* that reported earlier sexual maturity and more rapid growth at higher temperature (25°C compared to 15°C in *Austrolebias viarius*, and 22°C compared to 16°C in *Austrolebias nigrofasciatus*) (Errea & Danulat, 2001; Volcan et al., 2012). However, the positive correlation between water temperature and juvenile development is reversed when temperature exceeds an optimum of 21-24°C in *Austrolebias wolterstorffi* (Fonseca et al., 2013). High ambient temperature during the juvenile growth phase comes at the cost of shorter lifespan (Errea & Danulat, 2001). Thermal optima of *Austrolebias* are age-specific and persistent high ambient temperature is costly to adult fish (Walford & Liu, 1965; Liu & Walford, 1966).

We observed selective disappearance of larger individuals from populations during the terminal phases of growth (Fig. 4), though this may partly indicate selective predation (Reichard et al., 2018) or limited endurance of large males contingent upon their investment in courtship and reproductive activity (Passos et al., 2014) rather than
from a direct trade-off between rapid growth and longevity. Hence, while longevity of annual fishes is associated with lower ambient temperature (Walford & Liu, 1965; Valenzano et al., 2006; Hsu & Chiu, 2009), A. bellottii can cope with an altered seasonal trend in water temperature and appears able to respond with an adaptive increase in developmental rate and higher reproductive allocation. Second-cohort females invested in early reproduction with earlier sexual maturation and greater allocation to gonad mass, at the expense of a longer growth phase and, hence, greater investment in future reproduction. This outcome is concordant with predictions from life-history theory (Stearns, 1992), with cues indicating shorter life expectancy probably based on ambient temperature.

Populations of annual fishes survive drying of their environment in egg banks deposited in desiccated pool substrate (Polačík & Podrabsky, 2015). Propagule banks are essential for the long-term persistence of communities in temporary environments (Brock et al., 2003). In annual fishes, egg development is temporally staggered due to facultative diapause stages. After fertilization, the embryos initially develop for a few days and may be halted in Diapause I in the anoxic conditions of decaying pool substrate. Upon resumption of development, embryos may respond to desiccation by entering Diapause II and continue development if the substrate is moistened (Reichard & Polačík, 2019). Importantly, different embryos respond to external cues to a different degree (Furness, 2016; Polačík et al., 2017), resulting in the egg bank consisting of embryos at various stages of development. In the study pools, hatching of the second cohort was possible because the egg banks contained embryos ready to hatch at the time of secondary inundation. While we term that group a new cohort, we acknowledge that the fish may have hatched from eggs that were laid during the first inundation and may effectively represent a second “generation”. Interestingly, the length of embryonic
development has important consequences for post-hatching life. In African annual
killifish, rapidly developing embryos hatched to individuals that grew more quickly
(and were larger than individuals from slowly developing embryos from the age of 6
days), but lived shorter lives, and attained a smaller final size (Polačík et al., 2014).
While in that experiment, all fish experienced identical environmental conditions (i.e.,
temperature, food supply, density), the outcomes were consistent with our results from
the wild. This finding suggests that there is an interplay between environmental
conditions and intrinsic developmental (potentially epigenetic) processes that give rise
to the expressed phenotype.

An outstanding question is how frequent the mid-season desiccation of
temporary pools in the pampas region was in the past, and how reliably the alternation
of flooding regime matches changes in the pattern of precipitation. While we have
shown that annual fish populations can respond to these changes with a secondary
hatching, a high frequency of mid-season desiccation could rapidly exhaust egg bank
reserves. Our dataset for the study region only encompasses 10 years of precipitation
and temperature data (data provided by National Meteorology Institute, INUMET).
This time series demonstrates unusually dry conditions from February to July 2015
(which resulted in desiccation) (Supporting Information, Fig. S1) even in the context
of the already less predictable rainfall pattern in the last decade (Ding et al., 2008). We
acknowledge that it is critical to understand whether the observed mid-season drought
was an exceptional and isolated event or represents a climatic trend and if so, how
repeated droughts will affect existing annual fish egg banks.

While climate change can alter the ecology of many species (Walther et al.,
2002; Evans & Wallenstein, 2014), potentially driving some towards extinction
(Thomas et al., 2004), we show that A. bellottii, a representative species of a highly
endangered group of annual fishes, appears capable of coping with a sporadic seasonal shift in rainfall patterns. This finding indicates that environmentally-triggered phenotypic plasticity of life-history traits expressed by *A. bellottii* permits maintenance of viable populations despite altered phenology (Nylin & Gottard, 1998, Colchero *et al.*, 2019). In fact, *A. bellottii* has a relatively wide distribution and some populations reach the Río Paraguay basin in northern Argentina (García *et al.*, 2012) where precipitation seasonality is different to the study region and fish hatch when the water temperature is warmer. Phenotypic plasticity is important for forecasting species distributions in a climate change scenario (Valladares *et al.*, 2014; Duputié *et al.*, 2015) and this is especially important for species with limited dispersal abilities such as annual fishes (García *et al.*, 2012).

*Austrolebias* species are the top predators in their communities (Laufer *et al.*, 2009) and their potential disappearance from ephemeral pools may lead to a cascade of effects (Paine, 1966; Baum & Worm, 2009) with unknown consequences for community structure. While the primary effects of climate change (alternations in precipitation and temperature dynamics) affect phenologies of certain species (Parmesan, 2006; Ficetola & Maiorano, 2016), downstream consequences vary greatly among communities (Both *et al.*, 2009; Gilman *et al.*, 2010; Kordas *et al.*, 2011; Hassell *et al.*, 2019). For example, in the plankton communities of the North Sea, phenology of primary producers (diatoms) is driven by photoperiod and hence unaffected by elevated temperature while phenology of secondary (zooplankton) and tertiary (fish larvae) consumers have moved forward by 10-45 days over a 45-year study period (Edwards & Richardson, 2004). The resulting mismatch in trophic cascade has been implicated in the decline of marine pelagic production, including commercially important fish species (Edwards & Richardson, 2004). In contrast, a shift in the peak abundance of
caterpillars was successfully compensated by modulation of egg-laying date and other life history characteristics in pied flycatchers (*Ficedula hypoleuca*) for which caterpillars represent the main food source (Both & Visser, 2005).

Freshwater wetlands inhabited by *Austrolebias* populations are important for ecosystem function, including water purification and carbon retention (Baron *et al.*, 2002). In our study region, freshwater wetlands inhabited by *Austrolebias* populations are utilized for cattle ranching, often in proximity to agricultural plots. While cattle ranching has no apparent negative effect on temporary pools, and *Austrolebias* populations may even benefit from cattle ranching (Volcan *et al.*, 2016), the major threat to *Austrolebias* fishes is an expansion of rice or soybean cultivation (Volcan *et al.*, 2016; Volcan & Lanés, 2018). In Uruguay, agricultural development is primarily associated with soybean, wheat and other crop, which have expanded as new technologies have become available (Arbeletche & Gutiérrez, 2010; Dellazoppa, 2014). The use of novel herbicides, for example, appears to alter the ability of *Austrolebias* to cope with high water temperature, decreases their fecundity and affects diapausing stages in the egg bank (Zebral *et al.*, 2018). In southern Brazil, rice cultivation alters the natural flooding regime, with consequences reported for anuran and invertebrate communities (Cuhna *et al.*, 2015; Ávila *et al.*, 2015) and with likely negative impacts on annual fish populations. At present, changes to land use appear to overshadow any negative consequences of climate change for *Austrolebias* fishes (Volcan & Lanés, 2018), though the interaction of these effects, which are difficult to predict, may represent their greatest threat.

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Data Availability Statement

Primary data are deposited at Figshare (doi: xxxx).
References


Table 1. Mean estimates, their standard errors (SE) and statistical significance for gonad mass in *A. bellottii* as a function of coexistence with *A. nigripinnis*, body size (TL, log-transformed) and their interaction, modelled using a zero-altered model with pool identity included as a random term.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>SE</th>
<th>z</th>
<th>P</th>
<th>Estimate</th>
<th>SE</th>
<th>z</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>-45.55</td>
<td>10.82</td>
<td>-4.21</td>
<td>&lt;0.001</td>
<td>-9.91</td>
<td>0.44</td>
<td>-22.71</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Coexistence</td>
<td>-51.95</td>
<td>33.75</td>
<td>-1.54</td>
<td>0.124</td>
<td>-0.80</td>
<td>0.69</td>
<td>-1.15</td>
<td>0.254</td>
</tr>
<tr>
<td>logTL</td>
<td>32.45</td>
<td>7.68</td>
<td>4.23</td>
<td>&lt;0.001</td>
<td>5.44</td>
<td>0.29</td>
<td>18.78</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Coexist:TL</td>
<td>36.65</td>
<td>23.91</td>
<td>1.53</td>
<td>0.125</td>
<td>0.51</td>
<td>0.46</td>
<td>1.13</td>
<td>0.264</td>
</tr>
</tbody>
</table>
Table 2. Posterior mean estimates of *A. bellottii* TL modelled using a gamma random walk trend model fitted using INLA. CrI is the 95% Bayesian credible interval. Credible intervals that do not contain zero indicate a statistically important difference. *Sex* is mature fish sex. *Cohort* is the cohort of fish to which an individual belonged. *Area* is the surface area of the pool in which fish were captured.

<table>
<thead>
<tr>
<th>Model parameter</th>
<th>Posterior mean</th>
<th>Lower CrI</th>
<th>Upper CrI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>1.077</td>
<td>0.989</td>
<td>1.164</td>
</tr>
<tr>
<td>Sex (male)</td>
<td>0.119</td>
<td>0.106</td>
<td>0.132</td>
</tr>
<tr>
<td>Cohort (second)</td>
<td>0.236</td>
<td>0.201</td>
<td>0.271</td>
</tr>
<tr>
<td>Area</td>
<td>-0.008</td>
<td>-0.024</td>
<td>0.007</td>
</tr>
</tbody>
</table>
Table 3. Posterior mean estimates for gonad mass in *A. bellottii* as a function of cohort, age and pool area, modelled using a zero-altered gamma (ZAG) model with pool included as a random term. CrI is the 95% Bayesian credible interval. Credible intervals that do not contain zero in bold to indicate statistical importance.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>(a) Occurrence model</th>
<th>(b) Frequency model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Posterior mean</td>
<td>Lower CrI</td>
</tr>
<tr>
<td>Intercept</td>
<td>1.47</td>
<td>0.15</td>
</tr>
<tr>
<td>Cohort(\text{second})</td>
<td>2.11</td>
<td>0.43</td>
</tr>
<tr>
<td>Age</td>
<td>3.83</td>
<td>2.83</td>
</tr>
<tr>
<td>Area</td>
<td>-0.51</td>
<td>-1.29</td>
</tr>
</tbody>
</table>
Figure Captions

Figure 1. Location of the study area (A), with individual pools indicated (B).
Symbols for individual pools designate presence of a single cohort that either survived
the entire season (open symbol) or perished when the pool desiccated in mid-winter
(grey symbol), and the presence of the first and second cohort (black symbol). Pools
with *A. bellottii* and *A. nigripinnis* are marked by triangles and pools without *A.*
*nigripinnis* are indicated by circles. Note that in two pools, the first cohort survived
despite apparent desiccation.

Figure 2. Growth trajectories of *A. bellottii* males (A) and females (B) in pools
without *A. nigripinnis* (blue diamonds) or coexisting with *A. nigripinnis* (red
triangles). Individual body size measurements are indicated (TL, in cm), along with
GAMM-fitted curves with separate smoothers for presence/absence of *A. nigripinnis*.

Figure 3. Water temperature experienced by the first (grey) and second (black) cohort
of *A. bellottii* measured *in situ* using dataloggers. Daily mean values are presented.
Bars indicate hatching period for each cohort. Note that values experienced by the
first and second cohort overlap and values experienced by adults from the first cohort
from the age of 110 days are the same as those experienced by the second cohort from
day 0.

Figure 4. Growth trajectories of the first (blue circles) and second (red triangles)
cohorts of *A. bellottii* males (A) and females (B) and *A. nigripinnis* males (C) and
females (D). Individual body size measurements are indicated (TL, in cm), along with GAMM-fitted curves with separate smoothers for each cohort.

Figure 5. Specific growth rates (SGR) of the first (blue circles) and second (red triangles) cohort of *A. bellottii* (both sexes pooled). Note that formal analysis compared only SGRs until Time 7 to avoid bias from unequal lifespan between the two cohorts.

Supporting Information Table S1. Sample size for each pool, species, sex, and sampling event.

Supporting Information Figure S1. Precipitation and temperature data for the study area (Villa Soriano, Uruguay) comparing the year 2015 with 10 years database. Data were provided by National Meteorology Institute, INUMET.