


ORIGINAL ARTICLE

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

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Abstract

1. Changes to the timing of key life-cycle events can alter selection on life-history traits and have the potential to drive a cascade of effects at the community level. We investigated how the growth rate and sexual maturation of two annual killifish species (*Austrolebias bellottii* and *Austrolebias nigripinnis*) were altered by a change in the pattern of precipitation. These are annual species, endemic to ephemeral pools, persist in desiccated sediment as partly developed embryos.
2. We sampled 18 pools supporting populations of both species in a Pampa region adjacent to the Rio Negro in western Uruguay throughout the austral winter of 2015. Fish growth and maturation were monitored from pool inundation to habitat desiccation, along with environmental variables.
3. The region experienced an unprecedented mid-winter desiccation of pools that usually contain water from autumn to late spring, typically only desiccating in summer. Many desiccated pools were re-inundated from later rains, generating a second cohort of killifish in some pools (53%) in response to atypical conditions.
4. The second cohort developed more rapidly than the first, with 40% earlier attainment of asymptotic body size. Rapid development of second-cohort fish was associated with earlier maturation and greater investment in reproductive tissue.
5. The study demonstrated the capacity of annual fish to express developmental plasticity that buffered the negative consequences of an atypical 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.
6. Overall, we demonstrate how phenotypic plasticity can mitigate the negative impacts of climate change and associated altered phenology.

KEYWORDS

desiccation, developmental temperature, drought, killifish, phenology

1 | INTRODUCTION

Current climate change trends are rapidly driving modifications to the temporal and spatial distribution of temperature and precipitation

(Trenberth, 2011; Walther et al., 2002). These changes can affect biodiversity from individual populations to biomes (Parmesan, 2006). Some of the most striking effects of climate change are alterations in species phenologies; changes in the timing of crucial life cycle

events. This effect may lead to cascading effects at the community level (Edwards & Richardson, 2004; Gilman, Urban, Tewksbury, Gilchrist, & Holt, 2010), through the responses of interacting species (Bellard, Bertelsmeier, Leadley, Thuiller, & Courchamp, 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, 2011). However, a range of other effects on the relationships between affiliate species can emerge (Hassall, Billington, & Sherratt, 2019; Rafferty, Caradonna, & Bronstein, 2015), with the capacity for resilience uncertain (Hegg, Kennedy, Chittaro, & Zabel, 2013).

Changes in species phenology may 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*, Salmonidae), date of emergence of offspring has consequences for juvenile body size, with a significant decrease in body size in individuals that emerged 1 week later. This outcome may affect size-selective events, such as winter mortality, predation risk, interspecific competition, and reproduction (Einum & Fleming, 2000). Interspecific resource competition can affect growth patterns and timing of sexual maturation to a degree that the effects can be higher than those of climate (Rollinson, Kaye, & Canham, 2016; Vrtílek, Žák, Polačik, Blažek, & Reichard, 2019).

The effects of altered phenology are especially pronounced in ephemeral environments. Temporary habitats experience shifts between alternative habitat states, often 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 (Polačik & Podrabsky, 2015). Many organisms exploiting these environments are exclusively adapted to the periodicity of the habitat. In some cases, selection favours adaptation to the regular pattern of aquatic phases, such as annual spring flooding (Lytle & Poff, 2004), although other species appear adapted to the unpredictability of erratic events, such as desert flooding (Polačik & Podrabsky, 2015). As climate change modifies precipitation regimes and increases the incidence of abbreviated rainfall and concomitant extended periods of drought and heavy rainfall (Witze, 2018), temporary pools will experience changed 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 adapted to live in temporary pools. In the Neotropics and Africa, killifishes (Cyprinodontiformes) have repeatedly adapted to life in ephemeral freshwater habitats (Furness, 2016). 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 when the embryo is particularly resilient to

environmental stress (Podrabsky, Carpenter, & Hand, 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, Žák, Pšenička, & Reichard, 2018). Adults reproduce daily and the eggs are deposited in 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 (Furness, Reznick, Springer, & Meredith, 2015; Polačik, Reichard, & Vrtílek, 2018), although it is broadly synchronised by environmental cues (Podrabsky, Garrett, & Kohl, 2010).

Neotropical annual killifish are highly endangered. The major recognised threat is habitat loss associated with agriculture and floodplain regulation (Volcan & Lanés, 2018). Annual fish rely on specific precipitation patterns and are adapted to natural cycles of pool inundation and desiccation. In southern South America, the aquatic phase begins in the austral autumn (April) and the dry phase in late spring (November). With recent changes in climatic conditions, the lower Río Negro floodplain experienced an unusual desiccation of temporary pools in the middle of the aquatic phase in 2015, followed by unusually heavy precipitation that triggered hatching of a new cohort of annual fish (García, Loureiro, Machín, & Reichard, 2018). Here, we tested how the alteration of seasonal patterns affected the growth, maturation, and fecundity of these anomalous cohorts.

We investigated how the growth rate and sexual maturation of two annual killifish species—*Austrolebias bellottii* and *Austrolebias nigripinnis* (Rivulidae)—were affected by non-seasonal conditions and compared them 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 (Vrtílek et al., 2018). We first 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 two (non-overlapping) 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. We further predicted that the second cohort, with a shorter expected lifespan, would trade-off somatic growth for greater reproductive effort (Figueredo 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 in reproduction.

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

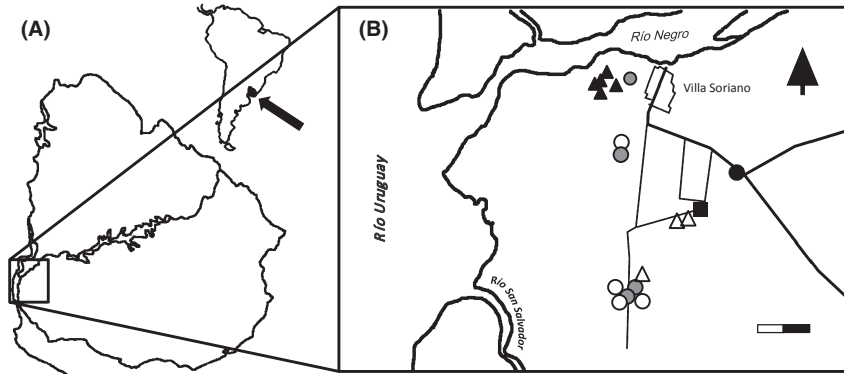


FIGURE 1 Location of the study area (a), with individual pools indicated (b). Symbols for individual pools designate presence of a single cohort that either survived the entire season (open symbol) or perished when the pool desiccated in mid-winter (grey symbol), and the presence of the first and second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

area of a typical Pampa biome. It is characterised 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 (Figure 1). The annual fish that inhabit the area are *A. bellottii*, *A. nigripinnis*, and *Austrolebias elongatus*. Thirteen pools (72%) were re-inundated but the second cohort of *A. bellottii* hatched only in six of 18 (33%) and *A. nigripinnis* in seven pools (39%). No second cohort hatched after re-inundation in the six pools. The first cohort persisted throughout winter in eight pools (*A. bellottii*) and three pools (*A. nigripinnis*), respectively. 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 2 weeks, except for a 20-day interval between trip 7 (9–10 August) and trip 8 (29 August–1 September). 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, which were only 0.1 km apart but clearly separate. Detailed descriptions of seasonal dynamics in population density and abundance are described in García, Loureiro,

Machín, and Reichard (2019) and García, Smith, Loureiro, Machín, and Reichard (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 (García et al., 2018; Lanés et al., 2016). Long-term meteorological data (10 years of precipitation and temperature data, provided by the National Meteorology Institute, INUMET) demonstrates unusually dry conditions from February to July 2015 (Figure S1).

Sampling was accomplished with a dip-net (200 × 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 × 80 mm) with a wetted surface and a reference scale. A total of 4,186 *A. bellottii* and 2,514 *A. nigripinnis* were measured. Sample size for each pool, species, sex, and sampling event is given in Table S1. A random sample of five females was taken at each sampling to estimate sexual maturation. Fish were euthanised 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 *tpsDig2* 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 five females was measured for TL, weighed (W, to the nearest of 0.001 g), dissected and gonad mass measured.

2.3 | Data analysis

All analyses were completed in the R statistical environment (R Development 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 five knots ($k = 5$), defined a priori based on the growth trajectory of *Austrolebias* (Volcan, Fonseca, Figueiredo, Sampaio, & Robaldo, 2012) and allowing for potential selective disappearance of larger fish at the end of the season. 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 Integrated Nested Laplace Approximation (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. We modelled zero-truncated data using a Gaussian distribution. 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 six populations of *A. bellottii* without *A. nigripinnis* and five populations of *A. bellottii* competing with *A. nigripinnis*, with four populations dropped during data exploration due to the small number of females analysed (five or fewer).

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:

$$\text{SGR} = \left(\ln(\text{TL}_x) - \ln(\text{TL}_{x-1}) \right) \times \frac{100}{N_{\text{days}}}$$

where TL_x is the TL for the date x , TL_{x-1} is the TL for the previous date and N_{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 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 visualisation, 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 trend model in a Bayesian framework using INLA. Fish size was fitted for fish age following a γ distribution with a log link function. The model was fitted as:

$$\text{TL}_{ijk} \sim N(\mu_{ijk}, \tau)$$

$$E(\text{TL}_{ijk}) = \mu_{ijk} \text{ and } \text{var}(\text{TL}_{ijk}) = \frac{\mu_{ijk}^2}{\tau}$$

$$\log(\mu_{ijk}) = \eta_{ijk}$$

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

$$\text{Age}_j \sim N(0, \sigma_{\text{Age}}^2)$$

$$\text{Pool}_k \sim N(0, \sigma_{\text{Pool}}^2)$$

where TL_{ijk} is the size of fish i of age (Age) j . The variable Sex_{ijk} is a categorical covariate with two levels (males, females) and Coh_{ijk} is a categorical covariate with two levels (first or second cohort). The model also contained a linear effect for pool size (Area_{ijk}) as its extent varied over the season. The random intercept Pool was included in the model to introduce a correlation structure between observations for fish collected from the same pool with variance σ^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 fitted with a

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

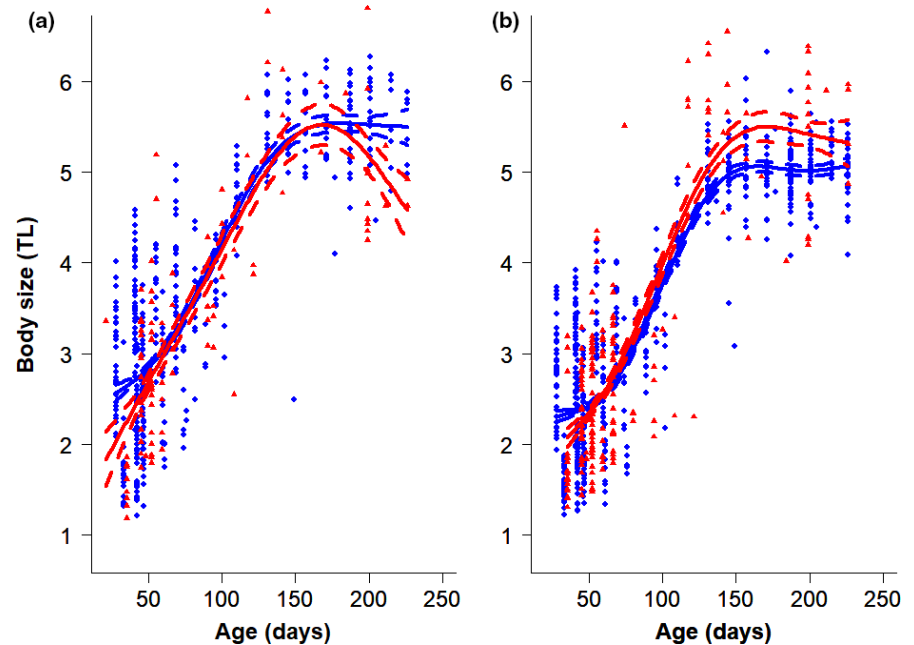


TABLE 1 Mean estimates, their standard errors (SE) and statistical significance for gonad mass in *Austrolebias bellottii* as a function of coexistence with *Austrolebias nigripinnis*, body size (total length [TL], log-transformed) and their interaction, modelled using a zero-altered model with pool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

Parameter	(a) Occurrence model				(b) Frequency model			
	Estimate	SE	z	p	Estimate	SE	z	p
Intercept	-45.55	10.82	-4.21	<.001	-9.91	.44	-22.71	<.001
Coexistence	-51.95	33.75	-1.54	.124	-0.80	.69	-1.15	.254
logTL	32.45	7.68	4.23	<.001	5.44	.29	18.78	<.001
Coexist:TL	36.65	23.91	1.53	.125	0.51	.46	1.13	.264

zero-adjusted model with a γ distribution, like that used for inter-specific competition contrasts. All final models were checked for overdispersion and model mis-specification following the guidelines of Zuur and 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: $F = 0.12$, $p = .77$; females: $F = 1.27$, $p = .260$), with a clearly inferior fit of the separate-smoothers model for females ($\Delta\text{AIC} = +256$). In males, the model with separate smoothers for males provided a better fit ($\Delta\text{AIC} = -10.3$), although 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* (Figure 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 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 ($>20^\circ\text{C}$) to cold ($<10^\circ\text{C}$) 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 (Figure 3). The second cohort hatched in relatively colder water that steadily increased in temperature and never fell below 10°C (Figure 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 ($\Delta\text{AIC} = -118$), *A. bellottii* females ($\Delta\text{AIC} = -246$) and *A. nigripinnis* males ($\Delta\text{AIC} = -39$), but only marginally in *A. nigripinnis* females ($\Delta\text{AIC} = -2.52$). 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; Figure 4), resulting in a larger mean asymptotic size

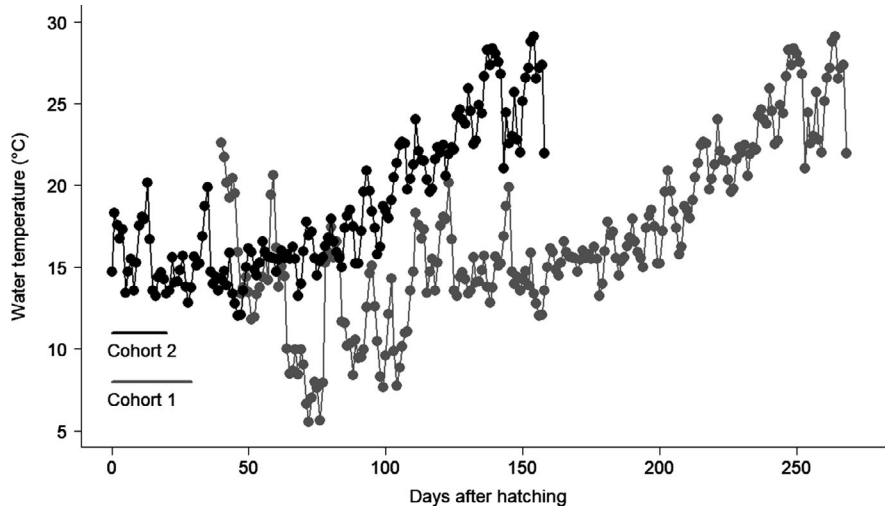


FIGURE 3 Water temperature experienced by the first (grey) and second (black) cohort of *Austrolebias bellottii* measured in situ using dataloggers. Daily mean values are presented. Bars indicate hatching period for each cohort. Note that values experienced by the first and second cohort overlap and 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

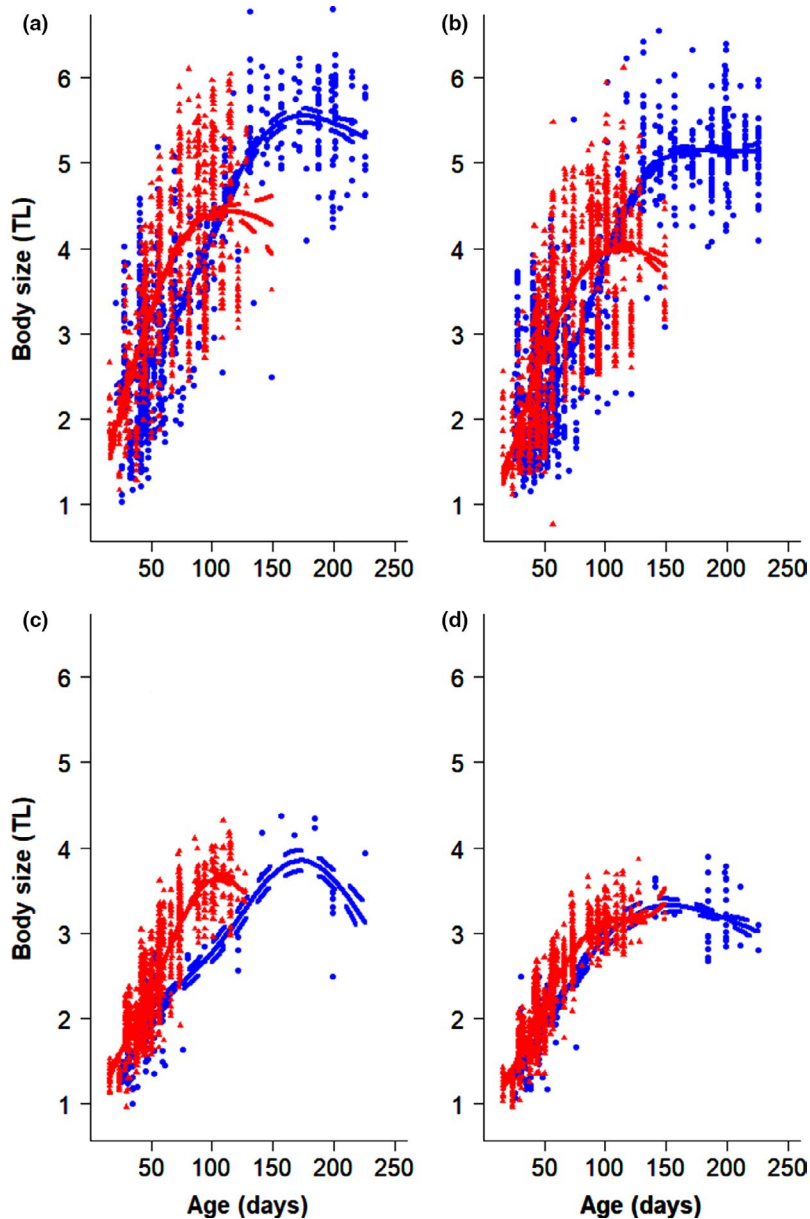


FIGURE 4 Growth trajectories of the first (blue circles) and second (red triangles) cohorts of *Austrolebias bellottii* males (a) and females (b) and *Austrolebias nigripinnis* males (c) and females (d). Individual body size measurements are indicated (total length [TL], in cm), along with GAMM-fitted curves with separate smoothers for each cohort

in *A. bellottii* (males: $F = 19.79$, $p < .001$, Figure 4a; females: $F = 16.16$, $p < .001$; Figure 4b) but not *A. nigripinnis* (Figure 4c,d).

Comparing SGR confirmed a steeper growth trajectory of second cohort fish over age-matched categories (linear model: $F_{1,71} = 4.67$, $p = .024$) and much stronger decline in the SGR with age in the second cohort fish (age by cohort interaction: $F_{1,71} = 12.42$, $p = .001$; age: $F_{1,71} = 3.28$, $p = .074$; Figure 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, although they varied among populations (Figure 5).

3.2.3 | Body size

Fitting a common smoother to growth data for *A. bellottii* using a γ 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

Using field-based monitoring of multiple populations, we show that a second cohort of annual killifish, generated in response to an

unprecedented mid-winter desiccation of pools that were re-inundated from later rains, developed more rapidly than the first cohort, with 40% earlier attainment of asymptotic body size. The rapid development of second-cohort fish was associated with earlier maturation and greater investment in reproductive tissue.

The primary effects of climate change are alterations in precipitation and temperature dynamics, which have profound implications for species' phenologies (Ficetola & Maiorano, 2016; Parmesan, 2006). However, responses to climate change and its consequences potentially vary among species (Both, Van Asch, Bijlsma, Van Den Burg, & Visser, 2009; Colchero et al., 2019) and particular interspecific interactions (Gilman et al., 2010; Hassall et al., 2019). The changes associated with late hatching that we observed demonstrated the capacity of *A. bellottii* to cope with a shift in seasonality and to adjust their life history to maximise reproductive success. Following non-seasonal desiccation and subsequent re-filling of pools, 46% (6 of 13) of *A. bellottii* populations hatched a second cohort. The second-cohort juveniles developed in colder water (until age approximately 50 days) but sustained accelerated growth and more rapid sexual maturation, along with a greater investment in fecundity. While these responses came at the cost of smaller final size, the response appears adaptive since life expectancy in the second cohort was shorter. The magnitude of response was substantial, with no effects on growth and sexual maturation in populations subjected to 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, although the effect was marginal and their asymptotic size was not smaller.

Observed differences in growth, size and maturation were probably shaped by the different temperature dynamics experienced by juvenile and adult *A. bellottii*. The first cohort experienced typical seasonal dynamics in water temperature. Fish hatched and completed their juvenile period in warm conditions in the austral autumn. Adults experienced a gradual decrease in temperature during winter and

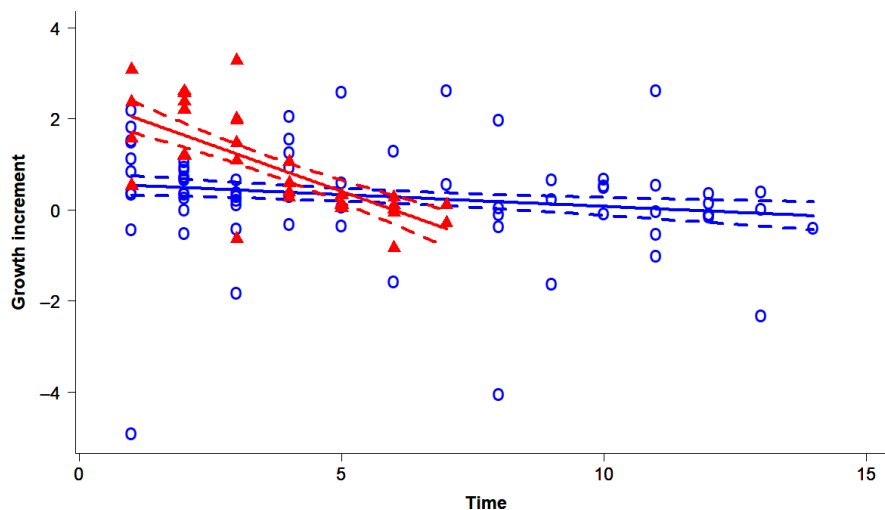


FIGURE 5 Specific growth rates (SGR) of the first (blue circles) and second (red triangles) cohort of *Austrolebias bellottii* (both sexes pooled). Note that formal analysis compared only SGRs until Time 7 to avoid bias from unequal lifespan between the two cohorts

their mortality increased in late spring as the temperature rose again (García, Loureiro, et al., 2019; García, Smith, et al., 2019). The second cohort hatched in cold water with the temperature steadily increasing over the season and exposing young adults (age 5 months) to ambient temperatures exceeding 25°C, which is considered the upper limit for the long-term survival of *Austrolebias* (Lanés et al., 2016). We acknowledge that other environmental factors could have affected growth; i.e. different photoperiods. While both cohorts hatched in a photoperiod of 10.5–11 hr of daylight, this period decreased by 1 hr over 2 months of juvenile growth for the first cohort, but increased by 2 hr 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, Loureiro, et al., 2019; García, Smith, et al., 2019).

Laboratory studies have demonstrated that annual fishes are sensitive to temperature (Fonseca, Volcan, Sampaio, Romano, & Robaldo, 2013; Volcan et al., 2012). *Austrolebias adloffii*, 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 that have reported earlier sexual maturity and more rapid growth at higher temperature for *Austrolebias* spp. (Volcan et al., 2012). However, the positive relationship between water temperature and

juvenile development is reversed when temperatures exceed an optimum of 21–24°C (Fonseca et al., 2013), and high ambient temperatures during juvenile growth comes at the cost of shorter lifespan.

We observed selective disappearance of larger individuals from populations during the terminal phase of growth, although this effect may partly indicate selective predation or limited endurance of large males contingent upon investment in courtship and reproduction (Passos, Tassinio, Reyes, & Rosenthal, 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 (Valenzano, Terzibas, Cattaneo, Domenici, & Cellerino, 2006), *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 (Polačik & Podrabsky, 2015). Propagule banks are essential for the long-term persistence of communities in temporary environments (Brock, Nielsen, Shiel, Green, & Langley, 2003). In annual fishes, egg development is temporally staggered due to facultative diapause stages. After fertilisation, 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 & Polačik, 2019). There are individual differences in response to external cues (Furness, 2016; Polačik, Smith, & Reichard, 2017), resulting in the egg bank comprising embryos at various stages of development. In the studied pools, hatching of the second cohort was possible because the egg banks contained embryos ready to hatch. 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. 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

TABLE 2 Posterior mean estimates of *Austrolebias bellottii* TL modelled using a γ 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

Model parameter	Posterior mean	Lower CrI	Upper CrI
Intercept	1.077	0.989	1.164
Sex _(male)	0.119	0.106	0.132
Cohort _(second)	0.236	0.201	0.271
Area	-0.008	-0.024	0.007

TABLE 3 Posterior mean estimates for gonad mass in *Austrolebias bellottii* as a function of cohort, age and pool area, modelled using a zero-altered γ 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

Parameter	(a) Occurrence model			(b) Frequency model		
	Posterior mean	Lower CrI	Upper CrI	Posterior mean	Lower CrI	Upper CrI
Intercept	1.47	0.15	2.80	3.08	2.70	3.43
Cohort _(second)	2.11	0.43	3.97	1.37	0.84	1.93
Age	3.83	2.83	4.95	0.86	0.75	0.99
Area	-0.51	-1.29	0.21	0.27	0.11	0.44

lived shorter lives, and attained a smaller final size (Polačik et al., 2014). This finding suggests that there is an interplay between environmental conditions and intrinsic developmental (potentially epigenetic) processes that give rise to the expressed phenotype. Our study cannot discern the magnitude of their relative contribution and should be the focus of future research.

An outstanding question is how frequent mid-season desiccation of temporary pools in the pampas region was in the past, and how reliably the alternation of flooding matches 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 long-term dataset, which only encompasses 10 years of precipitation and temperature data, demonstrates unusually dry conditions from February to July 2015 (resulting in desiccation), demonstrating a less predictable rainfall pattern over the last decade (Ding, Li, & Ha, 2008). 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), potentially driving some towards extinction (Thomas et al., 2004), we show that *A. bellottii* appears able to cope with sporadic shifts in rainfall patterns. This finding indicates that environmentally triggered phenotypic plasticity of life-history traits expressed by *A. bellottii* can maintain viable populations despite altered phenology (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) and is important for species with limited dispersal abilities such as annual fishes (García et al., 2012).

In conclusion, we investigated how the growth rate and sexual maturation of two annual killifish species were altered by a change in the pattern of precipitation. A second cohort that was generated in response to changed conditions developed more rapidly than the first, with 40% earlier attainment of asymptotic body size. Rapid development of second-cohort fish was associated with earlier maturation and greater investment in reproductive tissue. The study demonstrated that annual fish can express adaptive developmental plasticity, which can buffer the negative consequences of an unusual climatic cycle. The ability of annual fishes to respond adaptively to alternations to their life cycle will depend on the capacity of egg banks to tolerate increased unpredictability of precipitation. In general, we demonstrate that phenotypic plasticity can significantly mitigate the negative impacts of altered environmental conditions and represents a powerful mechanism enabling organisms to cope with climate change.

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DATA AVAILABILITY

Primary data are available from Figshare (<https://doi.org/10.6084/m9.figshare.8089148>).

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