

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

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21 **Keywords:** desiccation, developmental temperature, drought, killifish, phenology

22

23 **Summary**

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

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

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

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

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

44 6. Annual fish demonstrated high developmental plasticity that buffered the negative
45 consequences of a severely altered seasonal climatic cycle. The capacity of these annual

- 46 fishes to cope with alternations to their life cycle will depend on the ability of egg banks
47 in the sediment to tolerate an increased unpredictability of precipitation.

48 1. INTRODUCTION

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

62 Changes in species phenology may also lead to changes in life history traits,
63 which determine the way an individual spreads its reproduction over its lifetime
64 (Forrest & Miller-Rushing, 2010). For example, delayed hatching time can lead to post-
65 hatching development under altered environmental conditions, shifting growth and
66 maturation patterns, with potential consequences for the viability of subsequent
67 generations (Varpe, 2017). In Atlantic salmon (*Salmo salar*), date of emergence of
68 offspring has consequences for juvenile body size, with a significant decrease in
69 juvenile body size at the end of the growing season in individuals that emerged one
70 week later than the first cohort. This outcome may affect size-selective events, such as
71 winter mortality, predation risk and reproduction (Einum & Fleming, 2000). Other
72 ecological challenges can alter key life-history events. Interspecific resource

73 competition can ultimately affect growth patterns and timing of sexual maturation
74 (Ferreira-Rodríguez *et al.*, 2018) to a degree that surpasses the effects of climate
75 (Rolinson *et al.*, 2016). Therefore, it is important to compare the relative importance of
76 one ecological challenge (climate change) with other potential modulators of life-
77 history events.

78 The effects of altered phenology are likely to be especially pronounced in
79 ephemeral environments. Temporary habitats are subject to shifts between alternative
80 habitat states, often predictably following annual cycles of precipitation and
81 temperature (Varpe, 2017). Temporary pools are extreme environments that depend on
82 natural seasonal cycles between aquatic and desiccated phases (Polačik & Podrabsky,
83 2015). Among the organisms exploiting these environments, many are exclusively
84 adapted to the periodic phases of the habitat. In some cases, selection favours adaptation
85 to the regular pattern of aquatic phases, such as annual spring flooding (Lytle & Poff,
86 2004), though other species appear adapted to the unpredictability of erratic events,
87 such as desert flooding (Polačik & Podrabsky, 2015). As climate change modifies
88 precipitation regimes and increases the incidence of temporarily abbreviated rainfall
89 and concomitant extended periods of drought and heavy rainfall (Witze, 2018),
90 temporary pools experience dramatic change in their seasonal patterns of aquatic and
91 desiccated phases (Brooks, 2009). How these changes might alter the viability of
92 populations occupying these environments has yet to be explored.

93 Annual killifishes are a unique group of vertebrates that is exclusively adapted
94 to live in temporary pools. In the Neotropics and Africa, killifishes
95 (Cyprinodontiformes) have repeatedly adapted to life in ephemeral freshwater habitats
96 (Furness, 2016), with three independent evolutionary transitions to adopt this life
97 history in the family Rivulidae (Furness *et al.*, 2015a), a Neotropical clade of

98 Cyprinodontiformes fishes (Loureiro *et al.*, 2018). Populations of annual fishes persist
99 in desiccated sediment in the form of embryos encased in eggs with a thickened chorion.
100 Development is halted in three diapause stages (Wourms, 1972) when the embryo is
101 particularly resilient to environmental stress (Podrabsky *et al.*, 2001). Fish hatch when
102 their natal pool is filled with water. After hatching they grow rapidly and reach sexual
103 maturity within a few weeks (Vrtílek *et al.*, 2018). Adults reproduce daily and the eggs
104 are deposited into the substrate where they survive until a subsequent aquatic phase.
105 The timing of embryo development is variable, with a marked differential propensity
106 of embryos to enter and leave diapause (Wourms, 1972; Podrabsky & Hand, 1999;
107 Furness *et al.*, 2015b; Polačik *et al.*, 2018), though it is broadly synchronised by
108 environmental cues (Domínguez-Castanedo *et al.*, 2017), including temperature
109 (Podrabsky *et al.*, 2010a), photoperiod (Levels & Denucé 1988) and relative humidity
110 (Podrabsky *et al.*, 2010b).

111 Neotropical annual killifish are highly endangered. The major recognized threat
112 to their populations in southern South America (the Pampas biome) is habitat loss
113 associated with extensive agriculture and floodplain regulation (Volcan & Lanés, 2018).
114 In addition, annual fish depend on specific precipitation patterns and may be strictly
115 adapted to local natural cycles of pool inundation and desiccation. In southern South
116 America, the natural aquatic phase begins in the austral autumn (April), when
117 evaporation decreases and pools retain water from precipitation. The dry phase begins
118 in late spring (November), when evaporation increases and exceeds precipitation, and
119 habitats desiccate. Hence, unlike in the tropical range of annual fishes in Africa
120 (Reichard, 2016) and the Neotropics (Domínguez-Castanedo *et al.*, 2013; Podrabsky *et*
121 *al.*, 1997), there are no distinct rainy and dry seasons, and precipitation (with substrate

122 wetting and short-term accumulation of water) is relevant throughout the dry season,
123 though precipitation does not lead to an aquatic phase during the summer months.

124 With recent changes in climatic conditions, we have earlier reported (García *et*
125 *al.*, 2018) that the lower Río Negro floodplain (Villa Soriano district in western
126 Uruguay, Pampa vegetation region) experienced an unusual desiccation of temporary
127 pools in the middle of the aquatic phase (austral winter) in 2015, followed by an
128 unusually heavy precipitation that re-filled most of the desiccated pools. This event
129 triggered the hatching of a new cohort of annual fish in some pools (García *et al.*, 2018).
130 Here, we tested how the alteration of seasonal patterns affected the growth, maturation
131 and fecundity of these anomalous annual fish populations. The region of South
132 American pampas, to which more than 40 species of annual killifish are endemic
133 (Froese & Pauly, 2018), experienced a moderate increase in temperature and more than
134 30% decrease in precipitation between 1976 and 1990 (Walther *et al.*, 2002). This area
135 belongs to a region with the fastest estimated temperature increase (Loarie *et al.*, 2009).
136 Periods of heavy rains are predicted to increase in the region of between 20 - 50% in
137 the next 90 years with a 3 °C increase in global temperature (Fisher & Knuti, 2015;
138 Witze, 2018).

139 We investigated how the growth rate and sexual maturation of two annual
140 killifish species, *Austrolebias bellottii* and *A. nigripinnis*, were affected by non-
141 seasonal conditions (a second cohort that hatched in mid-winter rather than in autumn)
142 and compared it with the effects of another ecological challenge, interspecific
143 competition. Growth and maturation are crucial life history traits for individual fitness
144 (Stearns, 1992) and population dynamics (Sæther & Bakke, 2000) and this is especially
145 pronounced in short-lived annual fishes (Wootton & Smith, 2015; Vrtílek *et al.*, 2018).
146 We compared the growth and maturation of replicated *A. bellottii* populations

147 coexisting with *A. nigripinnis* and replicated *A. bellottii* populations without intra-guild
148 interspecific competition. Then, as the main goal of the study, we compared the growth
149 and development between the two cohorts of *A. bellottii* and the timing and size at
150 maturation of both cohorts and for each species. We predicted slower growth and later
151 sexual maturation in populations of *A. bellottii* coexisting with *A. nigripinnis* as a result
152 of interspecific competition between species occupying a similar ecological niche
153 (Arim *et al.*, 2010). Based on life history theory, we further predicted that the second
154 cohort (with a shorter expected lifespan) would trade somatic growth for a greater
155 reproductive effort (Figueredo *et al.*, 2006). Thus, individuals of the second cohort were
156 predicted to reach a smaller final size but mature earlier than the first cohort and make
157 a greater investment into reproduction than the first cohort, which was predicted to
158 divert more resources to a longer growth phase resulting in higher lifetime fecundity.

159

160 **2. Methods**

161 **2.1. Study area**

162 The study was conducted in the surroundings of Villa Soriano in Uruguay (33°23'56"S,
163 58°19'13"W) in 2015. This is a wetland area of a typical Pampa biome. It is
164 characterized by low altitude (1-7 m above sea level) and an abundance of shallow
165 temporary freshwater pools. The pools are from 100 to over 2,000 m² in area, with a
166 maximum depth of 0.4 m. Annual killifish (three species) are regularly encountered in
167 the pools. Other fish species invade the pools sporadically, following flooding from the
168 adjacent Rio Negro. The study area encompassed approximately 53 km². For a detailed
169 description of the study area see García *et al.* (2018).

170 The study is based on sampling in 18 pools (Fig. 1). The annual fish that inhabit
171 the area are *Austrolebias bellottii*, *A. nigripinnis* and *A. elongatus*. Thirteen pools (72%)

172 were re-inundated but the second cohort of *A. bellotti* hatched only in 6 of 18 (33%)
173 and *A. nigripinnis* in 7 pools (39%). No second cohort hatched after re-inundation in
174 the 6 pools. The first cohort persisted throughout winter in XXX pools (*A. bellottii*) and
175 YYY pools (*A. nigripinnis*). Given the rare occurrence of *A. elongatus* (0.23% of
176 sampled fish in a total of 17% of the pools), this study focused on the other two species.

177

178 **2.2. Sampling and data collection**

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

193 Sampling was accomplished with a dip-net (200 x 150 mm, 2 mm mesh size)
194 that was swept parallel to the bottom. All available habitats in the pool were explored.
195 Sampling effort varied across sites due to wide variation in fish density and pool area.
196 Sampling effort was recorded as time spent collecting fish (median = 26 min, mean =

197 32, standard deviation = 23.8) and pool surface area. Pool area was measured *in situ*
198 during each sampling event by walking around the pool boundaries using a portable
199 GPS unit to map the outline of the pool surface (Garmin Etrex10). After sampling, all
200 annual fish collected were photographed on a tray (80 x 80 mm) with a wetted surface
201 and a reference scale. A total of 4,804 *A. bellottii* and 2,592 *A. nigripinnis* were
202 measured. Sample size for each pool, species, sex, and sampling event is given in
203 Supporting Information (Table S1). A random sample of 5 females was taken at each
204 sampling to estimate sexual maturation. Fish were euthanized with an overdose of clove
205 oil and stored in 4% formaldehyde for later dissection in the laboratory. All other fish
206 were then returned to the pool. Species and sex were determined from photographs on
207 the basis of external characters (fin size, shape and colouration). Size of fish was
208 measured from photographs using *tpsDig2* software (v 2.1) (Rohlf, 2009) from the tip
209 of the snout to the end of the caudal fin (Total Length, TL). In the laboratory, a sample
210 of 5 females was measured for TL, weighed (W, to the nearest of 0.001g), dissected
211 and gonad mass (Wg) measured.

212 **2.3. Data analysis**

213 All analyses were completed in the R statistical environment (R Core Team, 2018).
214 First, we tested the effects of the presence of *A. nigripinnis* on the growth patterns of *A.*
215 *bellottii*. *Austrolebias bellottii* was present in all pools but *A. nigripinnis* occurred only
216 in 55% of pools (10 of 18). We used sex-specific Generalised Additive Mixed Models
217 (GAMM) with a Gaussian error distribution, with Coexistence (factor with two levels)
218 included as a fixed effect. The effect of age was modelled with a smoother term with 5
219 knots ($k = 5$), defined *a priori* based on the growth trajectory of *Austrolebias* (Volcan
220 *et al.*, 2012) and allowing for potential selective disappearance of larger fish at the end
221 of the season (Reichard *et al.*, 2018). The response variable was body size, measured

222 as TL. To account for dependency in the data from variation among pools, pool identity
223 was included as random effect. Two models were constructed that differed in how the
224 smoother term was modelled. First, a separate smoother was assigned for each
225 treatment level (coexistence with *A. nigripinnis* or not). Second, the same smoother was
226 used for both treatments. The fit of the models was compared using Akaike Information
227 Criterion (AIC). An AIC difference of >10 is considered a substantial improvement
228 in model fit. GAMM analysis was completed in the *gamm4* package (Wood & Scheipl,
229 2017).

230 Similarly, we compared whether coexistence with *A. nigripinnis* affected time
231 to sexual maturity in *A. bellottii* females. Because a high proportion of fish were
232 immature, data were modelled using a zero-altered (hurdle) model, using R-INLA
233 (Rue *et al.*, 2017). Zero-altered models are partitioned into two parts, with a binary
234 process modelling zeros and positive counts, and a second process modelling only
235 positive counts using a zero-truncated model (Hilbe, 2014). We modelled zero-
236 truncated data using a Gaussian distribution (Zuur *et al.*, 2014). This approach enabled
237 us to separately identify the environmental variables that predicted maturity (binary
238 part) and investment in gonad mass when gonads were present (zero-truncated part).
239 Fixed factors were Coexistence (two levels), body size (TL, log-transformed,
240 continuous predictor) and their interaction. In the model, the interaction between
241 Coexistence and TL measured the contribution of species coexistence to the timing of
242 sexual maturation relative to body size. This analysis was based on a contrast between
243 6 populations of *A. bellottii* without *A. nigripinnis* and 5 populations of *A. bellottii*
244 competing with *A. nigripinnis*, with four populations dropped during data exploration
245 due to the small number of females analysed (≤ 5).

246 The main analysis compared growth trajectories and sexual maturation of fish
247 from the first and second cohort, using the same approach as for testing the effect of
248 coexistence. We predicted, *a priori*, that the two species and sexes within species,
249 would differ in body size (Costa, 2006) and, consequently, we separated them before
250 analysis. We fitted sex-specific GAMMs with a Gaussian error distribution that
251 included the factor Cohort (two levels). Given that the coexistence of *A. bellottii* with
252 *A. nigripinnis* had no effect on growth and sexual maturation, this factor was not
253 included in the analysis. We modelled age either with separate smoothers for each
254 cohort (model 1) or a common smoother for both cohorts (model 2), with $k = 5$ in both
255 models. The fit of the two models was compared using AIC. The same analysis was
256 performed for both fish species.

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

$$259 \quad SGR = (\ln(TL_x) - \ln(TL_{x-1})) \times \frac{100}{N_{days}}$$

260 Where TL_x is the Total Length for the date x , TL_{x-1} is the Total Length for the previous
261 date and N_{days} is the number of days between each date. SGR was estimated for each
262 species and each pool separately. We tested whether SGR differed between cohorts by
263 fitting a Gaussian Linear Model (LM) with age interval, cohort and their interaction as
264 factors. The response variable was population-specific SGR. Given the shorter lifespan
265 of the second cohort, we truncated age intervals for the first cohort to permit a full
266 interaction between the two factors. This analysis was completed for *A. bellottii* only
267 because we had insufficient estimates of population-specific SGR for the first cohort of
268 *A. nigripinnis*. For visualization, we extended estimated SGR for the first cohort over
269 their entire lifespan.

270 While growth curves are commonly used in analysing growth data, they suffer
 271 from temporal dependency. To formally accommodate temporal dependency in the data,
 272 we further modelled fish size using a random walk (RW2) trend model in a Bayesian
 273 framework using Integrated Nested Laplace Approximation (R-INLA; Rue *et al.*, 2017).
 274 Fish size was fitted for fish age following a gamma distribution with a log link function.
 275 The model was fitted as:

$$276 \quad TL_{ijk} \sim N(\mu_{ijk}, \tau)$$

$$277 \quad E(TL_{ijk}) = \mu_{ijk} \text{ and } var(TL_{ijk}) = \frac{\mu_{ijk}^2}{\tau}$$

$$278 \quad \log(\mu_{ijk}) = \eta_{ijk}$$

$$279 \quad \eta_{ijk} = \beta_1 + \beta_2 \times Sex_{ijk} \times \beta_3 \times Coh_{ijk} + \beta_4 \times Area_{ijk} + Age_j + Pool_k$$

$$280 \quad Age_j \sim N(0, \sigma_{Age}^2)$$

$$281 \quad Pool_k \sim N(0, \sigma_{Pool}^2)$$

282 Where TL_{ijk} is the size of fish i of age (Age) j . The variable Sex_{ijk} is a categorical
 283 covariate with two levels (males, females) and Coh_{ijk} is a categorical covariate with two
 284 levels (first or second cohort). The model also contained a linear effect for pool size
 285 ($Area_{ijk}$) as its extent varied over the season. The random intercept $Pool$ was included
 286 in the model to introduce a correlation structure between observations for fish collected
 287 from the same pool with variance σ^2 , distributed normally and equal to 0. Uniform
 288 default priors were put on model parameters and fitted estimates of fish size matched
 289 observed data.

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

293 final models were checked for overdispersion and model mis-specification following
294 the guidelines of Zuur & Ieno (2016).

295

296 **3. RESULTS**

297 **3.1. Effect of interspecific competition**

298 There was no major effect of inter-specific competition on the growth of *A. bellottii*
299 (GAMM, males: $F = 0.12$, $P = 0.77$; females: $F = 1.27$, $P = 0.260$), with a clearly inferior
300 fit of the separate-smoothers model for females ($\Delta AIC = +256$). In males, the model
301 with separate smoothers for males provided a better fit ($\Delta AIC = -10.3$), though the
302 principal difference was that the separate-smoothers model captured differences in size-
303 selective mortality at the terminal phase of life rather than different growth rates among
304 *A. bellottii* populations coexisting with *A. nigripinnis* (Fig. 2). There was no effect of
305 coexistence with *A. nigripinnis* on the timing of sexual maturity of *A. bellottii* females
306 (binary part of the model) or their allocation to gonads (zero-truncated part). Female *A.*
307 *bellottii* maturation and allocation to reproduction depended on body size but the
308 scaling of the relationship did not differ between coexistence treatment levels (Table
309 1).

310 **3.2. Differences between the two cohorts**

311 **3.2.1. Water temperature**

312 The first cohort experienced a temperature regime typical of temporary pools in the
313 region. Water temperature decreased from warm ($>20^{\circ}\text{C}$) to cold ($<10^{\circ}\text{C}$) over the early
314 life phase, remained relatively cold until the age of approximately 100 days and slowly
315 climbed to 20°C again over the subsequent 100 days (Fig. 3). The second cohort

316 hatched in relatively colder water that steadily increased in temperature and never fell
317 below 10°C (Fig. 3).

318 **3.2.2. Growth trajectories**

319 There were clear differences in growth trajectories of the first and second cohorts of
320 both species, except for *A. nigripinnis* females. The models with separate smoothers for
321 each cohort were clearly superior in *A. bellottii* males ($\Delta\text{AIC} = -118$), *A. bellottii*
322 females ($\Delta\text{AIC} = -246$) and *A. nigripinnis* males ($\Delta\text{AIC} = -39$), but only marginally in
323 *A. nigripinnis* females ($\Delta\text{AIC} = -2.52$). The growth curves were characterised by earlier
324 attainment of the growth asymptote in the second cohort (approximately 100 days) than
325 in the first cohort (approximately 150-170 days) (Fig. 4), resulting in a larger mean
326 asymptotic size in *A. bellottii* (males: $F = 19.79$, $P < 0.001$, Fig. 4a; females: $F = 16.16$,
327 $P < 0.001$; Fig. 4b) but not *A. nigripinnis* (Fig. 4c, 4d).

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

335 **3.2.3. Body size**

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

340 **3.2.4. Sexual maturation**

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

348

349 **4. Discussion**

350 The primary effects of climate change are alterations in precipitation and temperature
351 dynamics which have profound implications for species phenologies (Parmesan, 2006;
352 Ficetola & Maiorano, 2016). However, responses to climate change and its
353 consequences potentially vary greatly among species (Both *et al.*, 2009; Colchero *et al.*,
354 2019) and among particular interspecific interactions (Gilman *et al.*, 2010; Kordas *et*
355 *al.*, 2011; Hassall *et al.*, 2019). We demonstrated previously that *Austrolebias* annual
356 fishes are able to hatch a new cohort in response to a secondary inundation following
357 an unexpected mid-winter (July) pool desiccation, though a second cohort hatched only
358 in a subset of pools (García *et al.*, 2018). In the present study, we tested the
359 developmental consequences of late season hatching in an environment with a non-
360 optimal temperature regime in *A. bellottii*. Overall, the changes associated with late
361 hatching demonstrated the capacity of *A. bellottii* to cope with shifted seasonality and
362 to adjust their life history to maximize reproductive success. Following non-seasonal
363 desiccation and subsequent re-filling of pools, 46% (6 of 13) *A. bellottii* populations

364 hatched a second cohort. The second-cohort juveniles developed in colder water (until
365 the age of approximately 50 days) but sustained accelerated growth and more rapid
366 sexual maturation, along with a greater investment in fecundity. While this came at the
367 cost of smaller final size, the response was adaptive since life expectancy (and actual
368 lifespan) in the second cohort was shorter. The magnitude of response was substantial,
369 with no effects on growth and sexual maturation in populations subjected to another
370 ecological challenge, interspecific competition with *A. nigripinnis*, a related annual
371 killifish species that coexisted in several pools with *A. bellottii*. *Austrolebias nigripinnis*
372 also experienced accelerated growth in the second cohort, though the effect was
373 marginal in *A. nigripinnis* females and their asymptotic size was not smaller.

374 Differences in growth rate, final size and sexual maturation were most likely
375 shaped by the different temperature dynamics experienced by juvenile and adult *A.*
376 *bellottii*. The first cohort experienced typical seasonal dynamics in water temperature,
377 with hatching and the juvenile period during warm-water conditions in the austral
378 autumn (April), followed by a gradual decrease in temperature with adults living in a
379 low temperature environment during winter and experiencing increased mortality in
380 late spring (November) as the temperature again increased (García *et al.*, 2019). Those
381 conditions are typical for most *Austrolebias* populations (Lanés *et al.*, 2016). The
382 second cohort hatched in cold water with the temperature steadily increasing over the
383 season and exposing young adults (5 months old) to ambient temperatures exceeding
384 25°C (Fig. 3), which is considered the upper limit for the long-term survival of
385 *Austrolebias* (Lanés *et al.*, 2016). In addition, the cohorts experienced different
386 photoperiods. While both cohorts hatched in a photoperiod of 10.5-11 hours of daylight,
387 this period decreased by 1 hour over two months of juvenile growth for the first cohort,
388 but increased by 2 hours for the second cohort, respectively (www.sunrise-sunset.org).

389 Increasing water temperature and photoperiod might have also increased productivity
390 of the pools, further stimulating more rapid growth of the second cohort. Pools were
391 generally larger after the second inundation, but killifish population densities were
392 comparable (García *et al.*, 2019).

393 Laboratory studies have demonstrated that annual fishes are sensitive to
394 temperature regimes (Hsu & Chiu, 2009; Volcan *et al.*, 2012; da Fonseca *et al.*, 2013).
395 *Austrolebias adloffii*, a related species from the same region, exhibited a decreased
396 growth rate and shorter lifespan when a natural decrease in water temperature
397 fluctuation was interrupted. After rearing juveniles at 22°C, a decrease in temperature
398 (to 16°C) was associated with longer lifespan and sustained growth while control fish
399 that remained at 22°C ceased growing (Liu & Walford, 1966). Our field data are
400 consistent with laboratory studies of *Austrolebias* that reported earlier sexual maturity
401 and more rapid growth at higher temperature (25°C compared to 15°C in *Austrolebias*
402 *viarius*, and 22°C compared to 16°C in *Austrolebias nigrofasciatus*) (Errea & Danulat,
403 2001; Volcan *et al.*, 2012). However, the positive correlation between water
404 temperature and juvenile development is reversed when temperature exceeds an
405 optimum of 21-24°C in *Austrolebias wolterstorffi* (Fonseca *et al.*, 2013). High ambient
406 temperature during the juvenile growth phase comes at the cost of shorter lifespan
407 (Errea & Danulat, 2001). Thermal optima of *Austrolebias* are age-specific and
408 persistent high ambient temperature is costly to adult fish (Walford & Liu, 1965; Liu &
409 Walford, 1966).

410 We observed selective disappearance of larger individuals from populations
411 during the terminal phases of growth (Fig. 4), though this may partly indicate selective
412 predation (Reichard *et al.*, 2018) or limited endurance of large males contingent upon
413 their investment in courtship and reproductive activity (Passos *et al.*, 2014) rather than

414 from a direct trade-off between rapid growth and longevity. Hence, while longevity of
415 annual fishes is associated with lower ambient temperature (Walford & Liu, 1965;
416 Valenzano *et al.*, 2006; Hsu & Chiu, 2009), *A. bellottii* can cope with an altered
417 seasonal trend in water temperature and appears able to respond with an adaptive
418 increase in developmental rate and higher reproductive allocation. Second-cohort
419 females invested in early reproduction with earlier sexual maturation and greater
420 allocation to gonad mass, at the expense of a longer growth phase and, hence, greater
421 investment in future reproduction. This outcome is concordant with predictions from
422 life-history theory (Stearns, 1992), with cues indicating shorter life expectancy
423 probably based on ambient temperature.

424 Populations of annual fishes survive drying of their environment in egg banks
425 deposited in desiccated pool substrate (Polačik & Podrabsky, 2015). Propagule banks
426 are essential for the long-term persistence of communities in temporary environments
427 (Brock *et al.*, 2003). In annual fishes, egg development is temporally staggered due to
428 facultative diapause stages. After fertilization, the embryos initially develop for a few
429 days and may be halted in Diapause I in the anoxic conditions of decaying pool
430 substrate. Upon resumption of development, embryos may respond to desiccation by
431 entering Diapause II and continue development if the substrate is moistened (Reichard
432 & Polačik, 2019). Importantly, different embryos respond to external cues to a different
433 degree (Furness, 2016; Polačik *et al.*, 2017), resulting in the egg bank consisting of
434 embryos at various stages of development. In the study pools, hatching of the second
435 cohort was possible because the egg banks contained embryos ready to hatch at the time
436 of secondary inundation. While we term that group a new cohort, we acknowledge that
437 the fish may have hatched from eggs that were laid during the first inundation and may
438 effectively represent a second “generation”. Interestingly, the length of embryonic

439 development has important consequences for post-hatching life. In African annual
440 killifish, rapidly developing embryos hatched to individuals that grew more quickly
441 (and were larger than individuals from slowly developing embryos from the age of 6
442 days), but lived shorter lives, and attained a smaller final size (Polačik *et al.*, 2014).
443 While in that experiment, all fish experienced identical environmental conditions (i.e.,
444 temperature, food supply, density), the outcomes were consistent with our results from
445 the wild. This finding suggests that there is an interplay between environmental
446 conditions and intrinsic developmental (potentially epigenetic) processes that give rise
447 to the expressed phenotype.

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

461 While climate change can alter the ecology of many species (Walther *et al.*,
462 2002; Evans & Wallenstein, 2014), potentially driving some towards extinction
463 (Thomas *et al.*, 2004), we show that *A. bellottii*, a representative species of a highly

464 endangered group of annual fishes, appears capable of coping with a sporadic seasonal
465 shift in rainfall patterns. This finding indicates that environmentally-triggered
466 phenotypic plasticity of life-history traits expressed by *A. bellottii* permits maintenance
467 of viable populations despite altered phenology (Nylin & Gottard, 1998, Colchero *et*
468 *al.*, 2019). In fact, *A. bellottii* has a relatively wide distribution and some populations
469 reach the Río Paraguay basin in northern Argentina (García *et al.*, 2012) where
470 precipitation seasonality is different to the study region and fish hatch when the water
471 temperature is warmer. Phenotypic plasticity is important for forecasting species
472 distributions in a climate change scenario (Valladares *et al.*, 2014; Duputié *et al.*, 2015)
473 and this is especially important for species with limited dispersal abilities such as annual
474 fishes (García *et al.*, 2012).

475 *Austrolebias* species are the top predators in their communities (Laufer *et al.*,
476 2009) and their potential disappearance from ephemeral pools may lead to a cascade of
477 effects (Paine, 1966; Baum & Worm, 2009) with unknown consequences for
478 community structure. While the primary effects of climate change (alternations in
479 precipitation and temperature dynamics) affect phenologies of certain species
480 (Parmesan, 2006; Ficetola & Maiorano, 2016), downstream consequences vary greatly
481 among communities (Both *et al.*, 2009; Gilman *et al.*, 2010; Kordas *et al.*, 2011; Hassell
482 *et al.*, 2019). For example, in the plankton communities of the North Sea, phenology of
483 primary producers (diatoms) is driven by photoperiod and hence unaffected by elevated
484 temperature while phenology of secondary (zooplankton) and tertiary (fish larvae)
485 consumers have moved forward by 10-45 days over a 45-year study period (Edwards
486 & Richardson, 2004). The resulting mismatch in trophic cascade has been implicated
487 in the decline of marine pelagic production, including commercially important fish
488 species (Edwards & Richardson, 2004). In contrast, a shift in the peak abundance of

489 caterpillars was successfully compensated by modulation of egg-laying date and other
490 life history characteristics in pied flycatchers (*Ficedula hypoleuca*) for which
491 caterpillars represent the main food source (Both & Visser, 2005).

492 Freshwater wetlands inhabited by *Austrolebias* populations are important for
493 ecosystem function, including water purification and carbon retention (Baron *et al.*,
494 2002). In our study region, freshwater wetlands inhabited by *Austrolebias* populations
495 are utilized for cattle ranching, often in proximity to agricultural plots. While cattle
496 ranching has no apparent negative effect on temporary pools, and *Austrolebias*
497 populations may even benefit from cattle ranching (Volcan *et al.*, 2016), the major
498 threat to *Austrolebias* fishes is an expansion of rice or soybean cultivation (Volcan *et*
499 *al.*, 2016; Volcan & Lanés, 2018). In Uruguay, agricultural development is primarily
500 associated with soybean, wheat and other crop, which have expanded as new
501 technologies have become available (Arbeletche & Gutiérrez, 2010; Dellazoppa, 2014).
502 The use of novel herbicides, for example, appears to alter the ability of *Austrolebias* to
503 cope with high water temperature, decreases their fecundity and affects diapausing
504 stages in the egg bank (Zebral *et al.*, 2018). In southern Brazil, rice cultivation alters
505 the natural flooding regime, with consequences reported for anuran and invertebrate
506 communities (Cuhna *et al.*, 2015; Ávila *et al.*, 2015) and with likely negative impacts
507 on annual fish populations. At present, changes to land use appear to overshadow any
508 negative consequences of climate change for *Austrolebias* fishes (Volcan & Lanés,
509 2018), though the interaction of these effects, which are difficult to predict, may
510 represent their greatest threat.

511

512 **Acknowledgements**

513 Financial support came from Agencia Nacional de Investigación e Innovación (ANII,
514 FCE_2013_100380) to DG and Comisión Sectorial de Investigación Científica (CSIC
515 I+D 2014_7) to ML. DG holds a PhD scholarship from Agencia Nacional de
516 Investigación e Innovación (ANII, POS_NAC_2014_102280) and and from Comisión
517 Académica de Posgrado (CAP, Udelar, BFPD_2018_1#26027733). DG and ML
518 belongs to Sistema Nacional de Investigadores (SNI) of Uruguay. MR was funded
519 through CSF 18-26284S. All local and national legal and ethical guidelines were
520 followed. The research was approved under reference number 240011-001885-13. We
521 thank to Patricia Magariños, Diego Díaz, Esteban Ortiz, Matej Polačik, Radim Blažek
522 and Milan Vrtilek for the help in the field. We also thank to local land owners for
523 access on their properties, and local authorities of Villa Soriano for generous support
524 for the project. The authors declare no conflict of interest.

525

526 **Data Availability Statement**

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

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785

786 **Table 1.** Mean estimates, their standard errors (SE) and statistical significance for787 gonad mass in *A. bellottii* as a function of coexistence with *A. nigripinnis*, body size

788 (TL, log-transformed) and their interaction, modelled using a zero-altered model with

789 pool identity included as a random term.

| Parameter | (a) Occurrence model | | | | (b) Frequency model | | | |
|--------------------|----------------------|-------|-------|--------|---------------------|------|--------|--------|
| | Estimate | SE | z | P | Estimate | SE | z | P |
| <i>Intercept</i> | -45.55 | 10.82 | -4.21 | <0.001 | -9.91 | 0.44 | -22.71 | <0.001 |
| <i>Coexistence</i> | -51.95 | 33.75 | -1.54 | 0.124 | -0.80 | 0.69 | -1.15 | 0.254 |
| <i>logTL</i> | 32.45 | 7.68 | 4.23 | <0.001 | 5.44 | 0.29 | 18.78 | <0.001 |
| <i>Coexist:TL</i> | 36.65 | 23.91 | 1.53 | 0.125 | 0.51 | 0.46 | 1.13 | 0.264 |

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791

792 **Table 2.** Posterior mean estimates of *A. bellottii* TL modelled using a gamma random
 793 walk trend model fitted using INLA. CrI is the 95% Bayesian credible interval.
 794 Credible intervals that do not contain zero indicate a statistically important difference.
 795 *Sex* is mature fish sex. *Cohort* is the cohort of fish to which an individual belonged.
 796 *Area* is the surface area of the pool in which fish were captured.

797

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

798

799

800 **Table 3.** Posterior mean estimates for gonad mass in *A. bellottii* as a function of cohort,
 801 age and pool area, modelled using a zero-altered gamma (ZAG) model with pool
 802 included as a random term. CrI is the 95% Bayesian credible interval. Credible intervals
 803 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 |
| <i>Intercept</i> | 1.47 | 0.15 | 2.80 | 3.08 | 2.70 | 3.43 |
| <i>Cohort_(second)</i> | 2.11 | 0.43 | 3.97 | 1.37 | 0.84 | 1.93 |
| <i>Age</i> | 3.83 | 2.83 | 4.95 | 0.86 | 0.75 | 0.99 |
| <i>Area</i> | -0.51 | -1.29 | 0.21 | 0.27 | 0.11 | 0.44 |

804

805

806 **Figure Captions**

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

814
815 **Figure 2.** Growth trajectories of *A. bellottii* males (A) and females (B) in pools
816 without *A. nigripinnis* (blue diamonds) or coexisting with *A. nigripinnis* (red
817 triangles). Individual body size measurements are indicated (TL, in cm), along with
818 GAMM-fitted curves with separate smoothers for presence/absence of *A. nigripinnis*.

819
820 **Figure 3.** Water temperature experienced by the first (grey) and second (black) cohort
821 of *A. bellottii* measured *in situ* using dataloggers. Daily mean values are presented.
822 Bars indicate hatching period for each cohort. Note that values experienced by the
823 first and second cohort overlap and values experienced by adults from the first cohort
824 from the age of 110 days are the same as those experienced by the second cohort from
825 day 0.

826
827 **Figure 4.** Growth trajectories of the first (blue circles) and second (red triangles)
828 cohorts of *A. bellottii* males (A) and females (B) and *A. nigripinnis* males (C) and

829 females (D). Individual body size measurements are indicated (TL, in cm), along with
830 GAMM-fitted curves with separate smoothers for each cohort.

831

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

836

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

839

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