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Avian predation mediates size-specific survival in a Neotropical annual fish: a field experiment

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Predation and population density have fundamental size- and sex-specific effects on individual survival and demographic parameters. Given the overlap and interactions between different age cohorts in natural populations, separating the factors related to differential survival and growth based on longitudinal field-collected data is problematic. Using a Neotropical annual fish (*Austrolebias minuano*) with a single age cohort per generation, we used replicated field enclosures to experimentally test the roles of avian predation and fish population density on survival and growth over adult lifespan. We found that mortality risk was higher in larger males and smaller females when predation was experimentally excluded. Exposure to avian predation eliminated this sex-specific effect of body size on survival. No overall sex difference in survival was found in the experiment, despite a female-biased sex ratio in natural populations. Individually based growth rates were highest in enclosures at low population density with no predation risk. Overall, we demonstrate that annual fish suffer high sex-dependent size-specific mortality that is more strongly related to predation than to density-dependent processes. This has important implications for our understanding of the evolution of senescence and other life history traits in annual fishes.

ADDITIONAL KEYWORDS: density-dependent growth – evo-demo – fish-eating birds – killifish – life history evolution – mortality.

INTRODUCTION

Predation rate and population density are key factors in determining both individual survival and demographic regulation at the population level (Forrester & Steele, 2000; Hixon & Jones, 2005; Einum, Sundt-Hansen & Nislow, 2006) and are linked through predation effects on population density (Ronget *et al.*, 2017). The impacts of predation and density-dependent regulation are frequently mediated by size- and age-specific effects, with particular size and age classes being affected disproportionally (Taylor, Trexler & Loftus, 2001; Lok *et al.*, 2013). These effects are important for the strength of natural selection on body condition, lifespan and other life history traits, as the theoretical predictions for the evolution of those traits diverge with respect to condition dependence in individual survival (Williams & Day, 2003; Chen & Maklakov, 2012). While differential survival in relation to predation and population density imposes particularly strong selection on body condition, both predation risk (Creel & Christianson, 2008; Ab Ghani, Herczeg & Merilä, 2016) and population density (Byström & García-Berthou, 1999) may mediate selection more subtly, by disproportionally affecting individual growth rates.

Multiple age cohorts typically overlap in natural populations, making it difficult to separate the effects of age, size and individual variation in growth from extrinsic, environmentally related factors on condition-dependent survival and growth (Ruehl & Trexler, 2015). The complexity of multiple causes affecting mortality rates in wild populations can be exemplified by a well-studied system of replicated populations of

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Trinidadian guppies (*Poecilia reticulata*). The primary factor considered to shape guppy mortality and population density is predation by cichlid fishes that varies predictably across guppy populations (Reznick & Endler, 1982). However, predation risk fully covaries with resource availability (Reznick, Bryant & Bashey, 2002), because predators live only in larger streams with higher primary productivity, complicating any straightforward interpretation of predation regime effects (Grether *et al.*, 2001). Populations with high and low predation pressure are also differentially sensitive to density-dependent regulation (Bassar *et al.*, 2013). Additionally, guppies breed year round and their age classes broadly overlap. This exposes co-occurring age cohorts to size- and age-specific mortality (Mattingly & Butler, 1994), with variable sensitivity to density dependence (Bassar *et al.*, 2013). Given that each individual may experience different environmental conditions that fluctuate seasonally (Arendt, Reznick & López-Sepulcre, 2014), disentangling the factors related to differential survival from longitudinal field-collected data is problematic.

The negative effect of high population density (i.e. individual crowding) on demographic parameters is typically ascribed to decreased fecundity and early-life survival (Einum *et al.*, 2006). However, population crowding leads to resource limitation manifested at the individual level by decreased growth rates (Murphy *et al.*, 2014) and increased mortality later in life (Hixon & Jones, 2005; Lobón-Cerviá, 2012). The effect of population density on survival is particularly noticeable in smaller individuals, as a consequence of their lower energy storage capacity, higher relative metabolic rate and lower success in competition for limiting resources (Byström & García-Berthou, 1999; Post, Parkinson & Johnston, 1999; Bassar *et al.*, 2013). This leads to population-density-mediated size-dependent survival (Sogard, 1997; Gaillard, Festa-Bianchet & Yoccoz, 1998).

Growth rate and survival may also vary between males and females. There are intersexual differences in the ability to maximize reproductive success, leading to a contrast in reproductive roles. Female reproductive effort is limited by the number of eggs they produce, setting a physiological limit to the number of their progeny. The variability in reproductive success among males is much larger than among females. Larger males are considered to be of superior intrinsic quality, have longer reproductive endurance and achieve greater reproductive success (Andersson, 1994; Guimarães *et al.*, 2017). However, their rapid initial growth (Lee, Monaghan & Metcalfe, 2013; Hooper *et al.*, 2017) and high signalling effort (Magnhagen, 1991) may limit their survival. This suggests a trade-off in reproductive success between rapid and slow male growth patterns. The role of growth on female condition-dependent survival is less clear, but a positive relationship between body mass and fecundity (Wootton and Smith, 2015), stronger predation risk of larger individuals (Trexler, Tempe & Travis, 1994) and the cost of rapid initial growth (Hooper *et al.*, 2017) are probably also applicable to relative survival and reproductive success of females.

Differential survival can therefore be associated with age, size, sex and mating effort, causing selective disappearance of individuals from a population as they inherently vary in their condition. The role of individual condition for survival is especially pronounced under challenging circumstances such as environmental stress, high population density or high predation risk. Under condition-dependent mortality (i.e. selection for the survival of the individuals in the best condition), high-quality, larger individuals may cope better with limiting resources (Williams & Day, 2003; Nussey *et al.*, 2011). In contrast, smaller individuals may be better suited to survive particular challenges, such as being less favourable targets of predation attacks (Trexler *et al.*, 1994; Quinn, Hendry & Buck, 2001).

We experimentally studied the role of population density, predation, sex and individual condition on the growth and survival of a Neotropical annual fish, *Austrolebias minuano* Costa & Cheffe. Annual fishes are excellent model organisms to test the effect of biotic and abiotic factors on individual survival (Cellerino, Valenzano & Reichard, 2016; Blažek *et al.*, 2017). They are spatially confined to small water bodies with limited dispersal (Loureiro *et al.*, 2015). Their hatching is synchronous (García *et al.*, 2018), forming a single age cohort that can be followed over its lifespan and is subject to directly comparable environmental and biotic challenges across all individuals. Their brief lives facilitate monitoring of their entire lifespan.

In the present study, we used a field experiment to test the role of predation and population density on the survival and growth of *A. minuano* in their natural habitat. Using eight artificially enclosed areas in a natural pool (Supporting Information, Fig. S1), we manipulated access of bird predators to fish and varied fish population density in the enclosures. We used meshed enclosures that were either covered by a net as a protection from avian predation or not covered (predation treatment) (Fig. S1), and stocked with 20 or 60 fish per enclosure (low and high population density treatments). The mesh size allowed free access of planktonic food and benthic invertebrates but prevented the movement of fish. With all fish individually marked and measured for body size, we also tested sex-specific and size-dependent survival.

We predicted higher survival and greater growth rates in treatments without bird predation and with low population density. Between the sexes, we predicted lower survival among males in the predation treatment, as a consequence of conspicuous male signalling. We further predicted better survival of larger fish, i.e. condition-dependent survival in both sexes, and a stronger effect of condition dependence at a high population density. We also predicted predation would mitigate survival of the largest individuals as avian predators preferentially target larger fish. Ultimately, this was predicted to generate an interaction between treatments, with greater survival of large-bodied fish in the no predation treatment but not in the predation treatment.

MATERIAL AND METHODS

Study species

Austrolebias is the largest genus of Neotropical annual killifishes (Cynolebiidae: Cyprinodontiformes), distributed in ephemeral pools across the grassland floodplains of subtropical and temperate South America (García *et al.*, 2015). *Austrolebias minuano* is a typical member of the genus from the coastal plain of Rio Grande do Sul state in Brazil. Juveniles hatch in austral autumn (April and May) and sexually mature after 5–8 weeks (Lanés *et al.*, 2016). Adult coloration is sexually dimorphic. Males possess alternating dark and light vertical stripes and conspicuous bright spots on the median fins, while females are pale. *Austrolebias minuano* reproduce daily and males engage in visually elaborate courtship (García, Loureiro & Tassino, 2008; Passos *et al.*, 2015) that makes them potentially more visible to both avian and fish predators (Passos *et al.*, 2014). High water temperature causes increased fish mortality in summer, followed by habitat desiccation (when evaporation exceeds precipitation) that sets the upper limit of *A. minuano*'s potential natural lifespan at approximately 8 months (Lanés *et al.*, 2016). We have previously found that populations of *A. minuano* in the study area typically comprise a single age cohort and their abundance and density decline throughout the season until desiccation of the pools in the second half of November (late spring) (Lanés *et al.*, 2016).

Study area and experimental procedures

The study was conducted near the Lagoa do Peixe National Park in the Coastal Plain of Rio Grande do Sul, southern Brazil (31°16′31.8″S; 51°08′33.7″W; 20 m a.s.l.) where *A. minuano* are abundant and coexist with a community of annual and non-annual fishes (Lanés *et al.*, 2016). The study area has a flat topography and an abundance of ephemeral pools in a matrix of natural grassland (pampa) and wetland habitats. The climate is subtropical, with a mean monthly temperature range from 13 °C in winter (July) to 24 °C in summer (January) and mean annual rainfall of 1200–1500 mm, distributed equally over the year (Tagliani, 1995).

The study pool surface area varied from 3229 to 7105 m^2 throughout the study (Table 1). Water conductivity ranged between 97 and 141 μS/cm^2, pH was 5.7–6.2 and turbidity was 22–42 NTU (nephelometric turbidity units). Water depth and water temperature fluctuations are presented in Table 1. The fish community included two annual (*A. minuano*, *Cynopoecilus fulgens* Costa) and 13 non-annual species (Supporting Information, Table S1). The site contained a rich invertebrate community and semi-aquatic and aquatic vegetation. From late August onward, floating *Azolla* sp. covered most of the water surface. The experimental enclosures were 2 × × 4 m in area, constructed from a net with 5 × 5 mm mesh size and supported by eight metal rods driven deep into the substrate alongside the circumference of the net (Fig. S1). The net was inserted into the sediment to a depth of 25 cm to prevent any fish movement between the enclosure and the pool. The enclosures were positioned around part of the pool perimeter, with the aim of keeping their topography similar. All enclosures were constructed on a slope; the

Table 1. Seasonal dynamics in water surface area, maximum water depth (range of maximum water depth across the enclosures), mean water temperature and water temperature range (based on continuous temperature recording at the pool bottom by Hobo UA-002-08 logger, Onset Ltd) measured in the experimental pool in the Lagoa de Peixe region in southern Brazil

	Surface area (m^2)	Maximum water depth (cm)	Mean (SD) temperature (°C)	Water temperature range (°C)
29 June 2015	7105	47–62	15.7 (2.5)*	9.9–23.7*
21 August 2015	6220	37–53	16.1 (2.6)	10.1–21.9
12 October 2015	4977	35–50	17.8 (2.5)	10.1–25.9
15 November 2015	3300	35–50	20.2 (1.3)	17.7–23.1
9 January 2016	72	5–28	24.0 (1.8)	20.0–29.4

*Mean temperature and its range from 25 May to 29 June.

shallow edge was 3–11 cm deep and the opposite edge was 19–32 cm deep during construction in May. Water depth fluctuated naturally over the seasonal cycle. The top of four enclosures was covered by a fine net (20 mm mesh size) that prevented bird predation on fish. The net provided no shade and could not affect the growth or composition of aquatic vegetation. The other four enclosures were left uncovered. Piscivorous birds (both wading and sit-and-wait predators) were frequently observed visiting the enclosures (Table S2). Cattle were excluded from the experimental area by a wire fence located at least 5 m from the enclosures.

On 29 June, the enclosures were stocked with fish collected in an adjacent pool. Fish were captured using a dip net and were sorted into three size categories for each sex. Prior to the stocking, all experimental fish were anaesthetized using clove oil, measured and individually marked with two VIE elastomer marks (Northwest Marine Research, USA) applied to four different body positions. The use of seven colours and a clear difference between males and females enabled unambiguous individual recognition of each fish. After marking, fish were left to recover in a tank with fresh cold water and strong aeration for at least 15 min.

Each enclosure was stocked with the same proportion of individuals from each size category. The high-density treatment had 58–59 fish (31–35 males, 24–27 females). The low-density treatment had 20–21 fish (12–15 males, 6–8 females). Initial fish size varied from 30 to 46 mm [mean (± SD) 37.5 (± 3.2) mm] in males and from 29 to 44 mm [mean (± SD) 37.1 (± 3.2) mm] in females. The spatial position of treatments (density, predation regime) in the pools was based on a predetermined random design. Before stocking the experimental fish, all fish that entered the enclosures during construction of the enclosures were collected using a dip net and removed. We fished all enclosures intensively, until no individual had been captured for at least 10 min. The same effort was repeated after a few hours to ensure no non-experimental fish remained in the enclosure.

On 21–22 August, fish from all enclosures were collected, individually identified and measured to the nearest 1 mm. Fish were then released back to their original enclosures. Twenty-two fish (8%, equally distributed across enclosures) lacked their marks. The marks may have been lost during the healing process; the immigration of adult *A. minuano* into the enclosures was unlikely. However, to account for our uncertainty concerning the origin of unmarked *A. minuano* in the enclosures, we constructed two alternative datasets. First, a conservative, individual-based dataset assumed that all unmarked fish had immigrated and missing marked fish had died. Second, a population-level dataset assumed that unmarked fish were originally stocked in the enclosure in June but had lost their marks. All reported analyses were conducted on the conservative, individual-based dataset; assigning unmarked fish to the missing marked fish with most likely matching body size had no effect on qualitative outcomes and interpretation. Despite high re-collection of marked fish in August (80.4%), the sum of marked and unmarked fish was not higher than the number of initially stocked fish for any enclosure. Therefore immigration, if present, was very rare. Several non-annual fishes, mainly small and juvenile Characidae and a poecilid *Phalloceros caudomaculatus*, were collected in enclosures (Supporting Information, Table S1). It is likely that they entered the enclosure through the mesh as small juveniles. The number of non-experimental fish in each enclosure was recorded; there was no bias toward a specific enclosure (or treatment level).

Fish were recovered again on 12 October and 15 November, using the same procedure. Fish were abundant (57.3% of originally stocked fish recovered) in October, but rare in November (2.2% recovered). The November sampling coincided with a major decrease in *A. minuano* abundance in natural habitats, associated with high water temperature and low water level (Lanés *et al.*, 2016). The sampling design is summarized in Figure 1.

DATA ANALYSES

Survival data were analysed with a mixed-effect Cox proportional hazards model, using the *coxme* library in R (Therneau, 2012). The fixed factors were *Population density* (High, Low), *Predation regime* (Predation: bird predation not excluded, No predation: bird predation excluded by mesh covers), *Sex* (Males, Females) and *Initial body size* (measured during marking in June). *Enclosure identity* was modelled by allowing random intercepts for each enclosure to account for non-independence of data originating from the same enclosure.

In addition to the direct survival analysis, we validated the results using an additional analysis, binomial generalized linear mixed models (GLMMs) in the *lme4* library in R (Bates *et al.*, 2014; R Development Core Team, 2016). This supplemental analysis enabled us to test survival over most of the study period (29 Jun – 12 Oct) and to partition it into two temporal subsets (29 Jun – 21 Aug, 21 Aug – 12 Oct). Partitioning of the period allowed us to constrain the analysis of growth rate for the period when size-related mortality was not recorded (e.g. until 21 Aug, see Results). Extremely low survival until November restricted the last period from the GLMM analyses. The same fixed and random factors were used as in the survival analysis.

The analyses started with the full model, including all interactions, because they were biologically relevant. During model selection, we removed non-significant fixed terms sequentially, always starting with the highest hierarchical interactions. The removal of each term

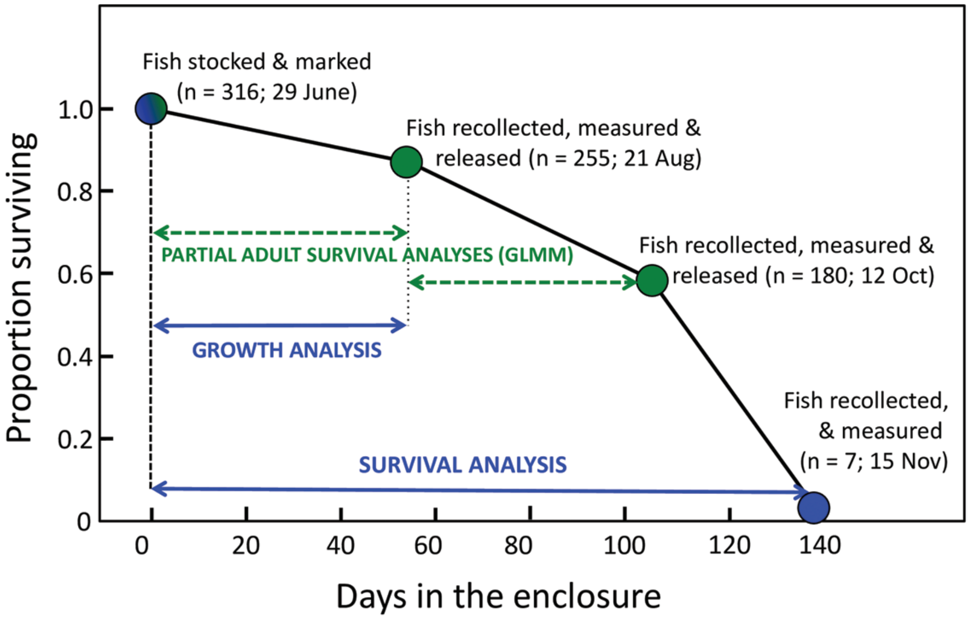

Figure 1. Schematic illustration of sampling design, showing the number of surviving fish and analytical periods.

was checked by comparing the reduced model with the full model using the log-likelihood test (using *drop1* function) and the change in Akaike information criterion (AIC) value. This procedure was repeated until the minimum adequate model (MAM) was reached (Zuur, Hilbe & Ieno, 2013). For the MAM, residuals were examined to ensure that model assumptions were met.

To analyse the growth rate, individual-based data from the period between 29 June and 21 August were used because survival was 80% during that period and was not related to initial body size (details in Results). Individual-based growth rate was modelled using a GLMM with Poisson error distribution and log link function. The same fixed and random effects were used as in the survival analyses. Body size (and hence growth increments) was measured to the nearest 1 mm and could not have been negative, qualifying the use of Poisson distribution. The Gaussian distribution was used initially but model fit produced overdispersed residuals. Four individuals (each in a different enclosure) were excluded from the individual-based dataset because they lacked a record of body size. Total sample size for the growth analysis was 254 individuals.

RESULTS

SURVIVAL

Mixed-effect Cox proportional hazards analysis revealed that the predation treatment mediated the sex-specific effects of body size on survival (three-way interaction between initial body size, predation and sex: $\chi^2 = 4.18$, d.f. = 1, $P = 0.041$; Table 2). Larger males and smaller females had a lower probability of survival in enclosures without predation. In contrast, no effect of body size on the survival of either sex was detected in the predation treatment (Fig. 2A). The same qualitative outcome was identified when unmarked fish were included in the dataset.

The result was validated by modelling survival as a binomial factor. For the period between 29 June and 12 October (overall survival 57.3% from a total of 312 fish), the same three-way interaction between predation, sex and initial body size was statistically significant (Supporting Information, Table S3; Fig. 2).

Table 2. Adult survival of *Austrolebias minuano* in the experimental enclosures during the period from 29 June to 15 November 2015 analysed using mixed-effect Cox proportional hazards analysis of survival; note that population density had no effect on survival during that period and is not represented in the final model

Fixed factors	χ^2	*P*	
Predation	1.06	0.303	
Sex	0.04	0.841	
Initial body size	1.10	0.295	
Predation:Sex	1.84	0.174	
Predation:Initial body size	0.05	0.826	
Sex:Initial body size	1.30	0.254	
Predation:Sex:Initial body size	4.18	0.041	*

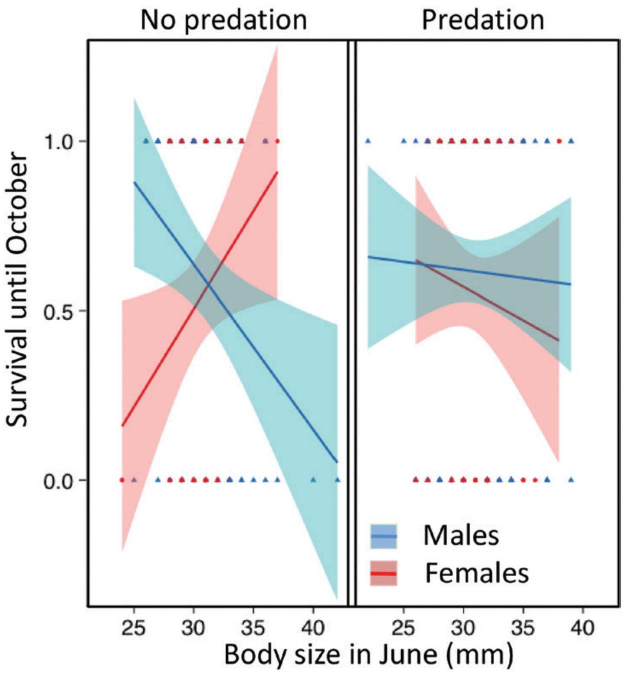

Figure 2. Predation on sex-specific effects of size-related survival for the period between 29 June and 12 October 2015. Survival estimates are visualized from the best linear fit to binomial data in the *ggplot* library. The shaded areas represent 95% confidence intervals.

Partitioning the study period demonstrated that over its first part (29 Jun – 21 Aug; overall survival 80.4% from 312 fish), male survival (87%) was higher than female survival (74%) ($z = 3.00$, $N = 312$, $P = 0.003$) but there was no effect of initial body size or treatment manipulation (Table S4). Over the second part of the study period (21 Aug – 12 Oct), the three-way interaction between predation, sex and initial body size was identical to that demonstrated for the entire study period (i.e. 29 Jun – 12 Oct; data not shown).

GROWTH RATE

Until 21 August (when survival was 80% and not related to initial body size, see above), surviving males grew faster than surviving females (GLMM with Poisson error: $z = 4.10$, $N = 254$ fish, $P < 0.001$). The mean increments over the period were 7.0 mm (model-based 95% confidence interval: 6.4–8.9 mm) in males and 5.7 (5.1–7.2) mm in females. Overall, fish grew faster in the low population density treatment ($z = 2.05$, $P = 0.041$), primarily due to a higher growth in the low-density treatment with no predation, although the interaction effect was not statistically significant (density by predation interaction: $z = 1.74$, $P = 0.081$; Fig. 3). Predation treatment had no overall effect on fish growth ($z = 0.41$, $P = 0.645$).

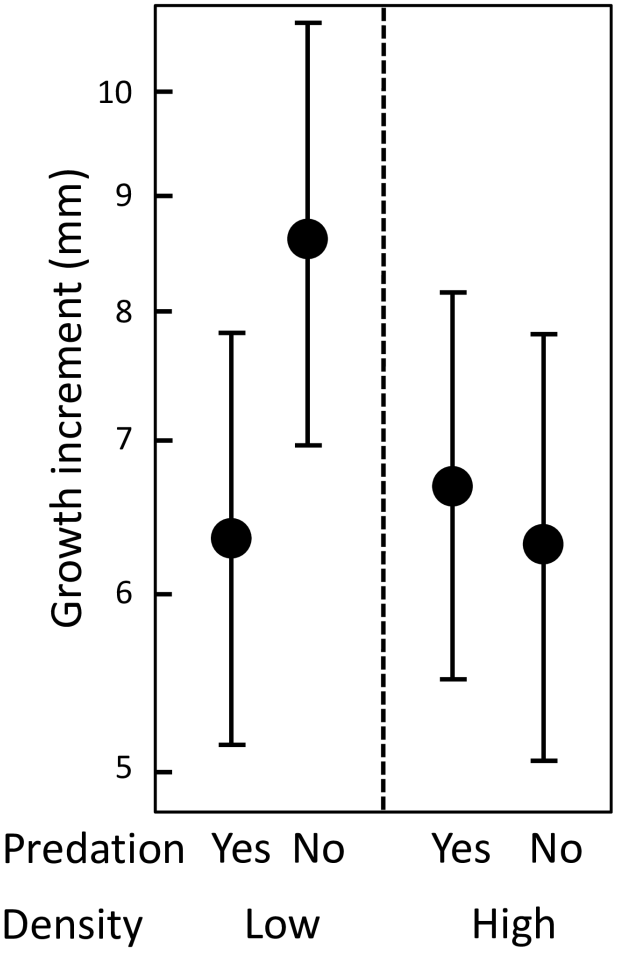

Figure 3. Mean model estimates (with 95% confidence intervals) of the effect of predation and population density on individual-based growth rate of adult *Austrolebias minuano* between 29 June and 21 August 2015.

Individual growth varied from 0 to 18 mm over that period. The details from the full model are presented in Supporting Information, Table S5. The experimental growth rates were biologically relevant – the body-size distribution in the adjacent natural population compared best to the low-density treatment with predation (Fig. 4).

DISCUSSION

Biotic and abiotic processes frequently influence demographic parameters through density-dependent and condition-dependent effects, with major impacts on the evolution of lifespan and related life history traits (Ronget *et al.*, 2017). Using a field experiment with a Neotropical annual fish, we manipulated population density and the potential for avian predation in replicated field enclosures, keeping abiotic parameters

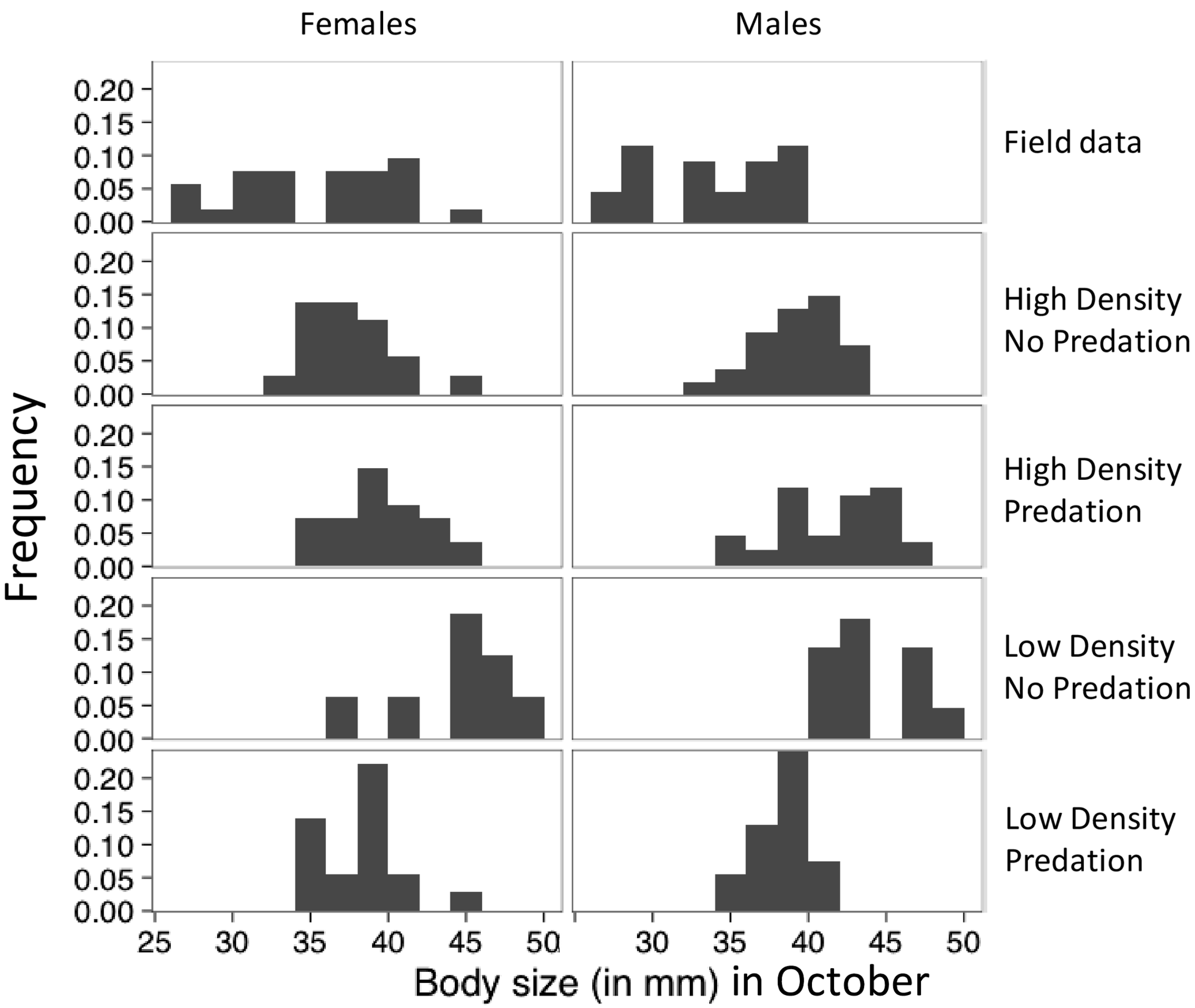

Figure 4. Body size distribution of male and female *Austrolebias minuano* on 12 October 2015 in four experimental treatments and in natural conditions [data from pools 1, 2 and 4 in Lanés *et al.* (2016) combined].

equal across replicates while reflecting their natural seasonal dynamics. We found that exposure to avian predation did not decrease overall survival rate but eliminated the sex-specific effect of body size on the survival that was detected in enclosures without avian predation. When predation was excluded, mortality risk was higher in larger males and smaller females. Growth rates were highest at low population density with no predation. In broader terms, the effect of sex- and size-specific survival of annual fishes, and the roles of predation and population density on size-specific survival, are important for our understanding of the evolution of their unusual life history. As annual fish hatch simultaneously (Polačik, Donner & Reichard, 2011; García *et al.*, 2018), they may experience high initial population densities.

Our data corroborated our a priori prediction of the highest growth rates in a predator-free environment and low population density (Byström & García-Berthou, 1999; Creel & Christianson, 2008). In strongly territorial species such as salmonid fishes, density-dependent growth occurs at low densities, whereas density-dependent mortality occurs at high densities (Jenkins *et al.*, 1999). In less territorial species, such as *A. minuano*, the negative impact of density-dependent interference competition is probably smaller, but scramble competition effects may still be substantial (Hixon & Jones, 2005). Lower population density increases per-capita resource availability and a predator-free environment enables more time to be devoted to feeding (Fraser & Gilliam, 1992). In our experiment, we varied population density three-fold, which is well within the range of naturally occurring differences among populations (Lanés *et al.*, 2016), and demonstrated that the growth of *A. minuano*, measured on individually based longitudinal data, is density-dependent.

In relation to individual survival, we predicted size- and sex-specific mortality at high population density, especially in the no predation treatment (Forrester, 1995). However, we detected no population density effect on *A. minuano* survival. Density-dependent adult survival is suggested to occur in natural populations of the brown trout (*Salmo trutta*) (Lobón-Cerviá, 2012). Yet, experimental studies on other freshwater fish frequently fail to detect any density-dependent survival despite common clear evidence of density-dependent growth (Tonn, Holopainen & Paszkowski, 1994). One reason could stem from the relatively short timeframes to detect any density-dependent survival (Hixon & Jones, 2005). In long-term field experiments with short-lived reef goby fishes, density-dependent effects on survival were clearly observed, both unrelated to predation and directly inflicted by fish predators, with a difference among three related goby species (Forrester & Steele, 2000). We followed the survival of our short-lived species over their adult lifespan at naturally relevant densities but failed to detect any effect of experimental population density on overall or condition-dependent survival.

The mortality patterns of *A. minuano* were affected by avian predation, as commonly observed in other wetland fishes (e.g. Trexler *et al.*, 1994), including other annual killifish (Keppeler *et al.*, 2016). However, predation did not increase overall mortality, but instead modulated the effects of size- and sex-dependent mortality. Specifically, it mitigated the size- and sex-dependent effects on survival observed in the no predation treatment. This suggests that avian predation forms a considerable part of *A. minuano* mortality and, despite the fact that habitat desiccation ultimately eliminates annual fish populations (Lanés *et al.*, 2016), both sexes suffer significant mortality mediated by predation.

Larger females had better survival than smaller females when predation was prevented but not under avian predation. It is conceivable that bird predation was preferentially directed towards larger females (Trexler *et al.*, 1994), reducing their otherwise higher relative survival. Unexpectedly, the reverse pattern of size-specific mortality in males (i.e. the negative association between body size and survival in males at no predation but not at the predation treatment) was observed. We primarily predicted that the potentially higher intrinsic quality of larger males (Passos *et al.*, 2014) is reflected in their better survival. However, larger males may also be more fragile than smaller males. The increased susceptibility to mortality in larger males may be linked to trade-offs between their rapid early growth and survival (Lee *et al.*, 2013; Hooper *et al.*, 2017), costly courtship behaviour (Andersson, 1994; Passos *et al.*, 2015), male–male competition to maintain their position in the dominance hierarchy (Passos *et al.*, 2013) and generally higher cost of maintenance of their larger bodies (Blanckenhorn, 2000). Yet, this effect is predicted to dominate in the absence of predation and weaken with predation that typically represents a higher risk for larger (and not smaller) males (Trexler *et al.*, 1994; Quinn *et al.*, 2001). We speculate that smaller males were forced to occupy less favourable habitats farther from the vegetated shelters and in shallower water as an outcome of male–male competition for access to superior breeding territories (Passos *et al.*, 2013). Shallow and open microhabitats are more vulnerable to avian predation (Schlosser, 1987) and *A. minuano* (like all other annual killifish) breed daily (García *et al.*, 2008; Passos *et al.*, 2015), making this pressure persistent. Ultimately, higher intrinsic frailty of larger males and higher predation on smaller males in risky microhabitats might have produced the observed pattern of size-related male mortality. This unexpected pattern accentuates the fact that natural processes driving state-dependent mortality may be more complex than insights from simpler laboratory experiments can convey (Nussey *et al.*, 2011).

The higher mortality of larger males, and its decrease in the predation treatment, indicates a greater effect of intrinsic processes in shaping size-selective male survival compared to predation-related mortality. Larger males were individuals that invested in rapid initial growth; annual fishes usually hatch synchronously (Polačik *et al.*, 2011; García *et al.*, 2018) and all fish in our experiment formed a single age cohort. While steeper initial growth possibly compromised late-life survival, it probably also improved reproductive success in the larger males due to their superior access to mating opportunities (Andersson, 1994; Passos *et al.*, 2013). Male mortality was negligible between June and August and increased only during the final third of *A. minuano*'s lifespan (Fig. 2). Hence, larger males, despite living relatively shorter lives than smaller males, had abundant opportunities to reproduce throughout a substantial part of their lifespan. From the perspective of lifetime reproductive success, this may have compensated for their earlier mortality. Ultimately, rapid and slow growth trajectories may represent alternative routes to reproductive success in annual fishes, where habitat duration is unpredictable (Polačik *et al.*, 2014), and decrease the strength of selection on body size in males (Reichard, Smith & Bryja, 2008).

Overall sex-specific adult survival was not detected in our study, despite evidence of female-biased sex ratios in natural populations (Lanés *et al.*, 2016). Male *A. minuano* tend to dominate populations early in the season and decline in abundance later (Lanés *et al.*, 2016). A major decrease in male *A. minuano* abundance is apparent later in the season, typically in October (Lanés *et al.*, 2016), and populations of a related species, *Austrolebias reicherti* from Uruguay, were also female-biased in October but not in August (Passos *et al.*, 2014). This late decline in the proportion of males in a population contrasts with African annual killifishes where a significant decline in the proportion of males starts soon after sexual maturity (Reichard *et al.*, 2014). The lack of sex-specific survival in our study may have been related to the exclusion of predatory fish. Under experimental conditions, the direct presence of a fish predator in the enclosure (where prey escape is severely limited) would result in elimination of entire experimental stock. Fish predators (e.g. *Hoplias malabaricus* Bloch, *Synbranchus marmoratus* Bloch) naturally co-occur with *A. minuano* in the study area (Lanés *et al.*, 2016; see Supporting Information, Table S1 for the full list). Predation by non-annual fishes invading ephemeral pools during major flooding apparently increased annual fish mortality in the Orinoco basin (Nico & Thomerson, 1989). It is possible that fish (rather than avian) predators cause higher male-specific mortality in adult annual killifish (Passos *et al.*, 2014; Reichard *et al.*, 2014; Lanés *et al.*, 2016) which could not be detected in our experiment. Further research, perhaps a diet analysis of predatory fish from natural populations, may illuminate this possibility. Finally, we acknowledge that our results should be taken with caution, as the experiment replicates were all located within a single pool and used a single population. Ultimately, only a meta-replicated design with more locations and more study species can provide broader insight into the role of predation and population density on populations of annual fishes.

The maximum lifespan of all annual fishes is determined by disappearance of their ephemeral habitat and it has been argued that pool desiccation is the main source of mortality in annual fishes (Tozzini *et al.*, 2013). However, there were suggestions that annual fishes often suffer high mortality throughout their lives and that abundance in their natural populations declines over the season (Passos *et al.*, 2014; Reichard *et al.*, 2014; Lanés *et al.*, 2016). Our experimental data demonstrate that annual fishes suffer high sex- and size-specific mortality long before habitat desiccation.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Fig. S1. Photographs of the experimental setting. Top: overview of the experimental pool. Middle: detail of enclosures with avian predation excluded (meshed top) and not excluded (open top). Bottom: male of the study species, *A. minuano*.
Table S1. List of non-annual fish species collected in the study pool.
Table S2. List of piscivorous bird species observed in the vicinity of the experimental pool.
Table S3. Effects of predation, population density, sex, initial body size and their interactions on the survival of *A. minuano* in experimental enclosures from 29 June to 12 October.
Table S4. Effects of predation, population density, sex, initial body size and their interactions on the survival of *A. minuano* in experimental enclosures from 29 June to 21 August 2015.
Table S5. Effects of experimental treatments, sex and their interactions on the growth of *A. minuano* in experimental enclosures between 29 June and 21 August 2015.

SHARED DATA

Primary data are deposited at Figshare (doi: 10.6084/m9.figshare.5845143).