

# The effect of population density on growth and survival of a Neotropical annual killifish

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## Abstract

We used a field experiment to test the effects of population density on the growth rate and survival of *Austrolebias bellottii*, a Neotropical annual killifish. Effects differed between the sexes: males at high densities achieved a smaller final size and experienced higher mortality while no such effects were observed in females. This sex-specific effect could be an indirect consequence of mate competition.

## KEYWORDS

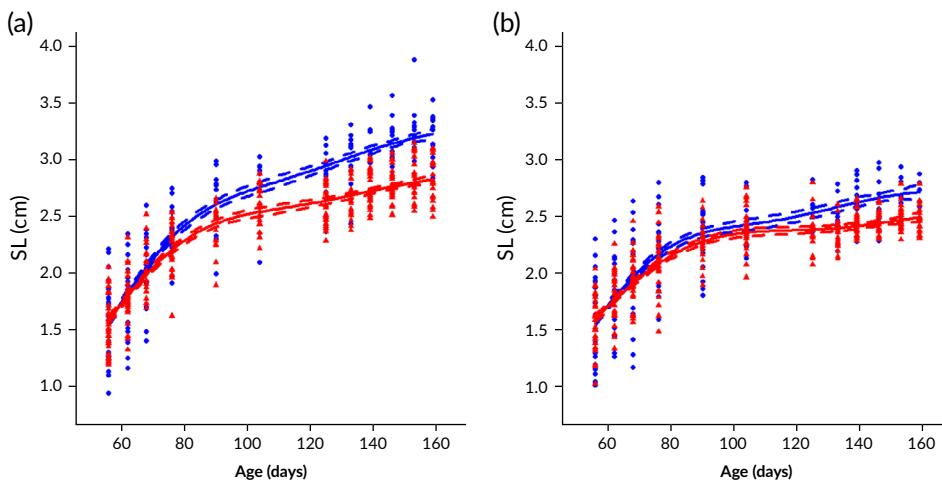
*Austrolebias bellottii*, density-dependence, differential mortality, sex differences

Body size is major factor affecting individual and population reproductive success across many fishes (Hildrew *et al.*, 2007). Realized via growth rate, body size is determined by the interplay between intrinsic factors (such as genetic background and developmental conditions) and external factors related to environmental and demographic parameters. High population density can lead to smaller body size (Peterman, 1984) and can also elevate mortality rates (Einum *et al.*, 2006; Reznick *et al.*, 2002). In resource-limited environments, organisms may invest in earlier reproduction at the expense of a smaller final size, given a relatively low probability of reaching a larger final size anyway. Survival is related to individual condition and modulated by age, sex and body size. High population density often precipitates individual-based survival, with larger individuals better able to cope with limited resources (Nussey *et al.*, 2011). On the other hand, a larger body requires more resources to simply maintain basic metabolic and physiological processes.

Annual killifishes are a group of cyprinodontiform taxa which has evolved specialized embryonic development that enables them to survive desiccation in ephemeral pools (Berois *et al.*, 2015; Furness, 2016). This life history has apparently evolved at least six times in Africa and the Neotropics (Furness *et al.*, 2015). In annual fishes, rapid growth is of enormous

importance as conditions for their existence may be transient and they need to reproduce before habitat desiccation (Vrtílek *et al.*, 2018). To achieve sexual maturity as early as possible, annual fishes display extremely rapid relative growth rates (Blažek *et al.*, 2013) allowing individual fish to reach sexual maturity in as little as 2 weeks when conditions are favourable for *Nothobranchius furzeri* (Vrtílek *et al.*, 2018). In contrast, when conditions are suboptimal, the same species may take more than 8 weeks to reach sexual maturity (Graf *et al.*, 2010).

The diet of annual fishes consists of zooplankton and larvae of aquatic arthropods (Polačik & Reichard, 2010; Laufer *et al.*, 2009). Prey availability may decline severely at high fish population density (Polačik & Reichard, 2010). Temporary pools hosting annual fishes represent highly variable habitats with major seasonal variation in size while population density is strongly related to the size of a particular pool (García *et al.*, 2019a). Annual fish are able to modify their growth rate and final body size (García *et al.*, 2019b). In a lineage of annual fishes from subtropical and temperate South American grasslands (genus *Austrolebias*), growth rates are primarily affected by water temperature (Fonseca *et al.*, 2013; Liu & Walford, 1966; Volcan *et al.*, 2012). A long-term field study confirmed the pivotal role of water temperature on *Austrolebias* growth and final body size under



**FIGURE 1** Growth trajectories of *A. bellottii* males (a) and females (b) in low-density treatments (blue diamonds) or high density treatments (red triangles). Individual body size measurements are indicated (standard length, in cm), along with GAMM-fitted curves with separate smoothers for high/low treatments

natural conditions (García *et al.*, 2019b), although the role of other factors on the remaining variation in growth remained unclear.

In this study, we used a field-based mesocosm experiment to test the effect of population density on growth rate and survival in the annual killifish *Austrolebias bellottii* (Steindachner 1881) across their entire lifespan. *Austrolebias bellottii* has a wide distribution in the Río Uruguay and Río Paraná-Paraguay basins (García *et al.*, 2012) and it is well established that its growth and sexual maturation responds to variation in environmental conditions (García *et al.*, 2019b). Growth, final size and mortality are also known to differ between the sexes (García *et al.*, 2019a,b). We constructed 12 enclosures (0.8 × 0.8 m, 2 mm mesh size) in a pool in the presence of a local *A. bellottii* population (33°28'55.37"S, 58°19'53.94"W). Enclosures were covered by a net (20 mm mesh size) on the top to prevent avian predation and were secured by four metal rods driven deep into the substrate (Supporting Information Figure S1). All fish within each enclosure were collected by dip net with repeated sampling to ensure no nonexperimental fish remained in the enclosure.

Experimental fish were laboratory-reared fish to standardize their early developmental conditions. Their parents ( $N = 8$ ) originated from the same pool where the experiment was conducted. Fish were stocked to experimental enclosures at the age of 56 days and mean standard length ( $L_S$ ) of 1.5 cm (S.D.  $\pm 0.3$ ). All fish were measured before their introduction to the enclosures and allocated haphazardly to particular enclosures. There were two treatments, each with six replicates. The low-density enclosures contained four males and four females and the high-density enclosures were stocked with eight males and eight females. The field site was visited every 6–8 days, except between visits 4 and 5, and 5 and 6 (14 days) and visits 6 and 7 (21 days). During each visit, all enclosures were sampled by repeated dip netting until 1 min of sampling yielded no fish. All captured fish were stored in a bucket, counted, individually measured (for  $L_S$ ) and then released back to their enclosure. The experiment was terminated on 12 November 2016, when fish were 159 days old and the pool approached desiccation. The care and use of experimental animals complied with Uruguay animal welfare laws, guidelines and policies as approved by the ethical commission (CHEA) of the university

(approval number 240011-000546-17). No fish were harmed or killed during this research.

To analyse differential survival of fish between treatments, we used a mixed-effect Cox proportional hazards model (library *coxme*) in R (Therneau, 2019), with treatment (high and low density) and sex (male, female) as fixed effects. Mortality was defined as inverse to survival, that is, absolute change in the number of live individuals in the enclosure. Enclosure identity was included as a random factor to account for dependency of fish survival within the same enclosure. To analyse growth, we used generalized additive models (GAM) and generalized additive mixed models (GAMM) with Gaussian error distribution. Given the major difference in growth rates between the sexes (García *et al.*, 2019b), we analysed male and female growth rates separately. We modelled growth as a function of body size (response variable) and treatment (high and low density) across age (12 sampling dates) with enclosure identity as a random factor. We constructed two models based on previous information on the growth rate of *A. bellottii* (García *et al.*, 2019b). First, the model contained a common smoother function for both treatments. Second, we constructed a model that contained treatment-specific smoothers, hence separating growth curves between high- and low-density treatments. The smoother terms in both models contained five knots ( $k = 5$ ), which was defined *a priori* based on the growth trajectory of *Austrolebias* (García *et al.*, 2019b; Volcan *et al.*, 2012). We then compared the fit of the GAM models using the Akaike information criterion (AIC). The GAMM final models were checked for overdispersion and model misspecification following the guidelines of Zuur and Ieno (2016).

There was no significant difference in overall survival between fish in high- and low-density treatments ( $0.483 \pm 0.558$ ,  $z = 0.87$ ,  $P = 0.390$ ) or between males and females ( $0.251 \pm 0.334$ ,  $z = 0.75$ ,  $P = 0.450$ ). However, a significant interaction ( $z = -2.23$ ,  $P = 0.026$ ) indicated that males (but not females) suffered higher mortality in the high-density treatment (Supporting Information Figure S2). Indeed 83% of males survived until the end of experiment in the low-density treatment but only 60% in the high-density treatment. Survival for females was 54% and 63% in the low- and high-density treatments, respectively.

Population density significantly decreased male body size; the fit of the model with separate smoothers was clearly superior ( $\Delta\text{AIC} = 16.5$ ). There was high mortality in two high-density enclosures that resulted in a decrease in fish abundance to the level <8 fish (*i.e.*, actually below the low-density treatment values) from sampling dates 3 and 5, respectively. We therefore repeated the analysis with a dataset excluding these two enclosures and this provided even stronger support ( $\Delta\text{AIC} = 20.1$ ) for our conclusions. There was negligible difference between the treatments until sampling 4 (age 76 days), followed by a major difference in growth patterns between samplings 5 and 7 (90–125 days) (Figure 1a). Details on model parameters are provided in Supporting Information Tables S1 and S2).

There was no significant difference in female body size between the treatments (slightly superior fit of the model with a single smoother ( $\Delta\text{AIC} = -5.3$ )). The same conclusion can be derived for the reduced dataset, where the model fit with two smoothers was superior, but only by  $\Delta\text{AIC} 5.2$ . In females, there was no difference between the treatments during the period of 90–125 days (Figure 1b). Details on model parameters are provided in Supporting Information Tables S1 and S2.

We experimentally demonstrated that growth and survival of annual fishes are affected by population density. Importantly, the impact differed between the sexes. High population density treatment affected male but not female survival and growth; males suffered higher mortality and lower growth rates at high densities. We suggest this could be an indirect effect of mating competition. Male *Austrolebias* compete strongly for mating opportunities (Passos *et al.*, 2013) and elevated competition for partners likely requires substantial time and energy that is diverted from other activities, including feeding. This is perhaps intensified at high population densities, in addition to conceivably lower food availability when population density doubles. While elevated male–male competition can also favour large final size over evolutionary time, density-dependent regulation (Reznick *et al.*, 2002) in our high-density treatments likely limited the final size of males via ecological and developmental processes, resulting in smaller male body size at high density. Given that neither female body size nor survival were affected by treatment density, we speculate that social stress from competitive interactions rather than simple food depletion was involved in the lower growth of males in the high-density treatment. Future research should investigate the proximate causes of this sex-specific effect, possibly using hormonal assays to test the role of stress hormones on growth and survival at different densities.

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## AUTHOR CONTRIBUTIONS

DG developed the experiments, collected the data, analysed the data and wrote the paper. MR designed the experiment, analysed the data and wrote the paper.

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#### SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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