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Reproductive senescence in a short-lived fish

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Abstract

1. Reproductive senescence is an age-associated decline in reproductive performance, which often arises as a trade-off between current and future reproduction. Given that mortality is inevitable, increased allocation into current reproduction is favoured despite costs paid later in life.
2. This assumption is violated in organisms with post-maturity growth whose reproductive output increases long after maturity. While reproductive senescence is frequently studied in animals with determinate growth at maturity, such as insects or mammals, we have very limited understanding of reproductive senescence in organisms with an extensive post-maturity growth period.
3. The fact that many post-maturity growers experience strong adult mortality leads to conflicting expectations for reproductive senescence. The aim of this study was to investigate how co-occurrence of rapid life history and post-maturity growth mould reproductive senescence in a short-lived killifish, *Nothobranchius furzeri*, using longitudinal data on laboratory and wild-type populations.
4. We followed the individual fecundity, fertility and fertilization of 132 singly housed fish from the perspectives of chronological and biological age. At the onset of senescence, the sex-specific contribution to decrease in fertilization capacity was investigated. Allocation trade-offs were estimated through the association between reproductive parameters and life span, and between early-life and late-life fecundity.
5. We demonstrate that female fecundity increased steadily after maturity and reproductive senescence occurred long after the growth asymptote. The prime age for fecundity coincided with 50% female survival and consequent decline in fecundity implies an association with somatic deterioration. Reproductive senescence in fertilization rate was stronger in females than in males. Females with high early fecundity experienced a long life span and high late-life fecundity, discounting the role of allocation trade-offs in reproductive senescence.
6. The present study reports a clear case of reproductive senescence in a fish with a long post-maturation growth period, unusually rapid development and short life span. The onset of reproductive senescence was postponed compared to animals that cease growing at sexual maturity. Fish and other animals with post-maturity growth have long been considered insusceptible to ageing but this conclusion may be related to the previous lack of longitudinal data rather than to the absence of reproductive senescence in such organisms.

KEYWORDS
annual killifish, indeterminate growth, life-history evolution, *Nothobranchius furzeri*, reproductive ageing, senescence

1 | INTRODUCTION

Reproductive senescence is an age-related decline in reproductive performance. This decline is predicted by evolutionary theories of ageing (Lemaître & Gaillard, 2017), either as an inevitable by-product of early investment into reproduction (Antagonistic Pleiotropy theory: Williams, 1957; Disposable Soma theory: Kirkwood, 1977) or persistence of late-acting detrimental mutations due to the weakened strength of natural selection after maturity (Medawar, 1952; Williams, 1957). Nevertheless, the predicted decrease in the strength of natural selection after maturity may not be universal (Finch, 1998; Vaupel et al., 2004).

In organisms with continuous post-maturity growth, the strength of natural selection is expected to persist long after maturity, since their fitness is closely linked to their body size (Finch, 1990; Vaupel et al., 2004). Consequently, these organisms are predicted to have evolved a delayed onset of senescence long after maturity (Medawar, 1952; Vaupel et al., 2004). Post-maturity growth is typical in molluscs, fish and reptiles (Barneche et al., 2018; Charnov et al., 2001; Heino & Kaitala, 1999) and, unlike birds, mammals or insects, their asymptotic size is not reached at the age of maturity but is postponed until long after sexual maturation (Blažek et al., 2013; Charnov et al., 2001; Heino & Kaitala, 1999). Some organisms with post-maturity growth are short lived (Eckhardt et al., 2017; Reichard & Polačik, 2019) and express rapid life history which predicts an accelerated rate of senescence (Jones et al., 2008). Hence, these organisms provide interesting models to examine the interplay between contrasting adaptations arising from post-maturity growth (postponed senescence) and fast life (rapid senescence).

A captive environment provides protection from multiple environmental factors (Mason, 2010). Hence, animals in captivity often live longer and due to the absence of environmentally driven mortality, they can reach the senescent stage even when this is not commonly observed in the wild (Kirkwood & Austad, 2000; Tidière et al., 2016). By contrast, keeping populations for multiple generations under relaxed natural selection in a captive environment can affect organismal life histories including reproduction and senescence rate (Heath et al., 2003; Sgro & Partridge, 2000). Despite detrimental effects of captivity on reproduction, captive populations may outperform wild populations in reproductive parameters due to selection for high reproductive performance (Miller et al., 2002). There are contrasting assumptions from ageing theory of how captivity might shape organismal senescence. Williams (1957) suggested that a reduction in the environmentally driven mortality would lead to delayed maturity and life span extension while under Positive pleiotropy, the reduced purifying effect of condition-dependent mortality in captivity would contribute to low overall fitness in a captive-adapted population (Kimber & Chippindale, 2013; Maklakov et al., 2015). In addition, high allocation to reproduction may come with costs to survival, leading to a shorter life span of a more fecund population (Kirkwood, 1977). Thus captivity-adapted and wild populations differ in their history of senescence-shaping environmental mortality and comparison of senescence rate between such populations under common garden conditions may provide important insights into the evolution of senescence (Reznick et al., 2004; Sgro & Partridge, 2000).

Environmental mortality is a strong determinant of life span in small fish species (Blažek et al., 2017; Reznick et al., 2004), but little is known about reproductive senescence in fish. It is widely assumed that fish have evolved delayed senescence (Hamilton, 1966) or lack senescent deterioration at all (Finch, 1990; Vaupel et al., 2004). Given that fish experience a long period of post-maturity growth but often live at a fast pace (Winemiller & Rose, 1992), studies on ageing in fish can deliver new insights into the evolutionary mechanisms of reproductive senescence (Reznick et al., 2002, 2004). *Nothobranchius furzeri* are small fish (<7 cm) that express adaptions to a life cycle enabling them to occupy seasonally desiccating savanna pools in south-east Africa (Reichard & Polačik, 2019). Given their naturally short life span, they have become a vertebrate model for studies on ageing (Cellerino et al., 2016; Hu & Brunet, 2018). Constraints on their life span are predicted to produce strong selection for high allocation into early reproduction, irrespective of its potential future costs (Kirkwood, 1977). In conflict with this prediction, no functional reproductive senescence has been detected in captive (Blažek et al., 2017) or wild *N. furzeri* populations (Vrtílek, Žák, Blažek, et al., 2018), in line with expectations for organisms with a long period of post-maturity growth (i.e. *N. furzeri* mature at as little as 55% of their asymptotic body size, Blažek et al., 2013). Previous studies have used a cross-sectional design and, hence, are prone to be affected by selective disappearance (Nussey et al., 2008). Thus ideally a longitudinal study is required to test whether short-lived species, such as *N. furzeri*, are subject to functional reproductive senescence.

In the present study, we characterize reproductive senescence in two populations of *N. furzeri*, using detailed longitudinal data based on regular measurements from the same individuals. We predicted that despite post-maturity growth, the fast pace of life of *N. furzeri* and the protected environment of a laboratory setting would allow expression of reproductive senescence.

2 | MATERIALS AND METHODS

2.1 | Experimental fish and conditions

We used a common garden experiment with two populations of *N. furzeri*, laboratory (GRZ) and wild-type (WT), to compare their

demographic parameters in relation to reproductive senescence. The GRZ population has been held in captivity for >100 generations, and despite a broadly similar genetic background to the WT population (Bartáková et al., 2013), it is highly inbred (Cellerino et al., 2016; Reichwald et al., 2015). The GRZ population originated from the driest part of the natural range of *N. furzeri* in Zimbabwe and was collected in 1968 (Jubb, 1971). The WT strain is of recent wild origin (collected in 2011 in southern Mozambique (GPS 21°52′24.84″S, 32°48′2.34″E, approximately 100 km from the site of collection of the GRZ strain), relatively outbred (Cellerino et al., 2016) and was maintained in our laboratory (Institute of Vertebrate Biology [IVB], Czech Academy of Sciences) under the code MZCS NF 222 for six to eight generations prior to the experiment (permit 70084/2016-MZE-17214).

All experimental fish hatched in September 2017 at the IVB following a standard protocol (Polačik et al., 2016). Hatched fish were housed in communal tanks until the age of 5 weeks, when the sex of all fish could be determined (Polačik et al., 2016). Thereafter, 132 fish (65 GRZ and 67 WT, approximately 1:1 sex ratio) were placed in individual 2-L tanks in three separate recirculation systems (Aquamedic) where they were kept until natural death. For the analyses of reproductive senescence, only females which produced at least one clutch of eggs were used (i.e. we have not included seven GRZ females that died prior to producing their first clutch). Fish from each strain were assigned to alternate tanks in predetermined positions with equal proportions of WT and GRZ fish across recirculation units. Fish were fed twice each day (9:30, 17:00) ad libitum (amount consumed within 5 min) with frozen bloodworm. Prior to each feeding, the tank floor was cleaned by siphoning and the health status of every fish was visually inspected. Water temperature was maintained at 27°C (range 25–29°C), water conductivity was 250–300 μs and light regime was 14:10 L:D.

2.2 | Measurement of reproductive traits

Killifish have external fertilization, enabling us to separate and non-invasively estimate the production of ovulated oocytes and the fertilization rate. In our study, we estimated age-specific fecundity (number of eggs laid; estimate of female reproductive allocation), fertility (number of fertilized eggs laid; estimate of parental reproductive fitness), fertilization rate (proportion of eggs successfully fertilized; combination of male and female gamete quality), sexual maturation (age at first oviposition), lifetime reproductive output (sum of all eggs laid) and maximum clutch size (number of eggs in the most fecund oviposition over an individual's lifetime). Every week, pairs were placed in 2-L plastic containers prior to morning feeding and allowed to spawn on a sand substrate for 2 hr, which is sufficient for females to release all ovulated eggs representing their daily fecundity (Polačik et al., 2016). Spawned eggs were left in the plastic container for 24 hr (to allow egg-shell hardening) and then sieved from the sand (Polačik et al., 2016). Fertilized and unfertilized eggs were counted under a dissecting microscope (10× magnification). Fertilized eggs were identified by the presence of a perivitelline space (Polačik et al., 2016). After oviposition, the pair was photographed in a shallow plastic dish with a reference scale. Body size (length without caudal fin) was measured from photographs using ImageJ v 1.48 (USA, https://imagej.nih.gov).

A second, non-experimental oviposition was realized every week (3 days after the experimental oviposition) throughout the post-maturity life span. This was to prevent ovary malfunction (i.e. loss of capability to release ovulated eggs) which may arise when females are not allowed to spawn daily (Polačik et al., 2014) as is typical for *N. furzeri* in the wild (Žák et al., 2019). Despite this precaution, 6 WT (18%) female deaths were suspected to be the result of ovary malfunction, a substantial reduction from 82% observed in an earlier study during which females were not allowed to spawn over their life span (Polačik et al., 2014). Spawning pairs were stable, but in the case of death, a missing partner was replaced with a fish that had similarly lost its mating partner.

2.3 | Experimental pairing of young and old fish

At the age of 31 weeks, when fertilization rate was low (Figure 1d), 10 pairs of WT fish were combined with 10 pairs of young (8 weeks old) fish from the same population to determine the sex- and age-specific declines in reproductive function. Young fish originated from social tanks (10 males and 15 females in a 60-L tank). They were allowed to mate freely in their social home tanks, but isolated in two single-sex aquaria 4 days prior to first experimental spawning to standardize their reproductive state. Every fish (20 old and 20 young) spawned twice in the experiment, once with an old and once with a young partner, in a random order. This design resulted in four treatment combinations (old female–old male, old female–young male, young female–old male and young female–young male). The second spawning followed 3 days after the first. After the first oviposition, young fish were placed in single-sex trios (small, medium and large fish) in separate 35-L tanks to permit individual identification. Only the fertilization rate was compared between old and young fish, as fecundity of females from social tanks is typically higher than in individually housed fish, irrespective of age (J. Žák, pers. obs.).

2.4 | Life span and survival analysis

All statistical procedures were performed in the R environment v 3.6.1. (R Core Team, 2019).

Group-specific survival was visualized with Kaplan–Meier plots and analysed using a nonparametric Log-rank test from the SURVIVAL v 2.44.1.1 package (Therneau & Grambsch, 2000). To determine the best predictor of female life span, we included early-life reproductive effort (cumulative fecundity from the first three clutches), maximum fertilization rate (maximum value of fertilization rate for each individual

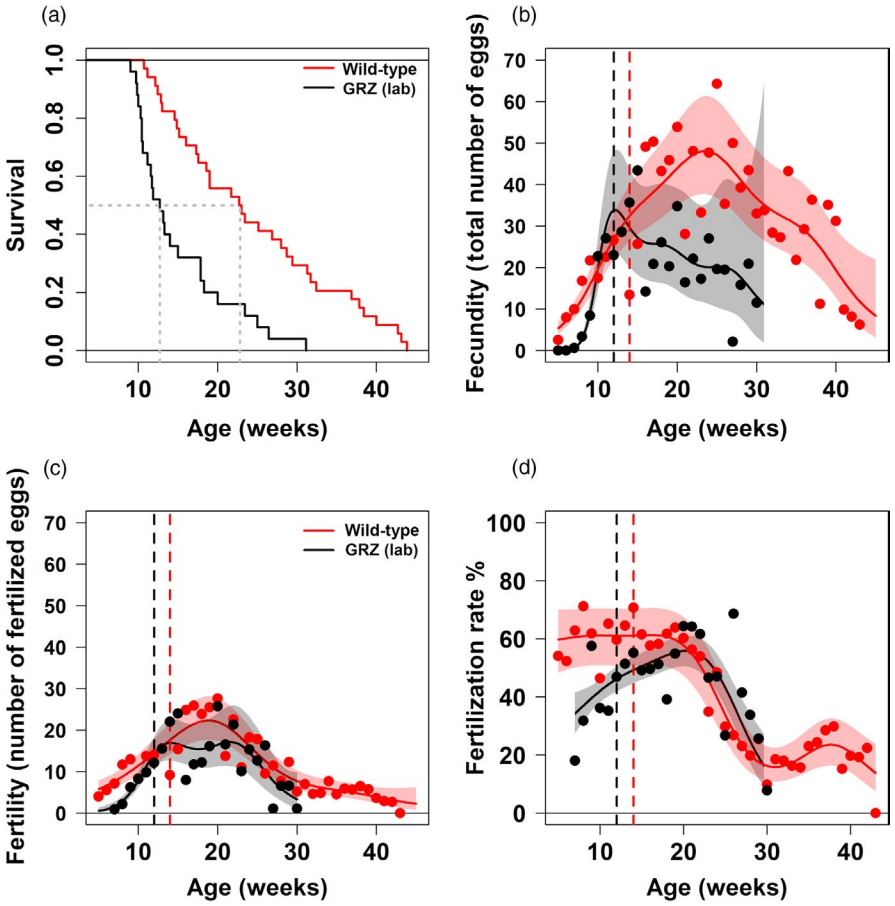

FIGURE 1 Survival and dynamics of reproductive parameters with chronological age in two populations of *Nothobranchius furzeri*. Age-specific female survival (Kaplan–Meier; a), fecundity (b), fertility (c) and fertilization rate (d) of the wild-type (WT; red) and laboratory GRZ (black) populations of the *N. furzeri*. Fecundity, fertility and fertilization rate curves are modelled with a GAM. Shading represents 95% confidence intervals. Points are produced by GLMM with age as a fixed factor and ID-female as a random factor to account for repeated measurements. Grey-pointed lines in (a) represent median survival. Black (GRZ) and red (WT) vertical-dashed lines in (b–d) are age when female-specific growth rate was not statistically different from 0 (see Figure S2 in Supporting Information). Male age-specific survival is presented in Figure S3

to provide an estimate of egg quality produced by each female), juvenile growth (size at 8 weeks) and population ID as explanatory variables (all continuous variables were zero-mean centred with standard deviation of 1, Schielzeth, 2010), and modelled as a Gamma GLM. We originally also included age at maturity as a predictor, but it was collinear with population ID (VIF > 3) and hence omitted from the final analysis. To account for the potential role of selective disappearance on life-history traits correlated with life span, we fitted another model with the same set of variables but added life span as a covariate (Hayward et al., 2013). To ensure that the observed effect was not related only to early-life fecundity, we fitted another model with the above-mentioned covariates but with the sum of three consecutive clutches at the age when fecundity peaked in each population (WT 22–25 weeks, GRZ 9–11 weeks) instead of the first three clutches.

To test for a trade-off between early and late fecundity within biological age, we used linear regression with residuals corrected for body size and repeated measurements from a Negative binomial GLMM. We fitted separate GLMMs for early fecundity (first two clutches) and late fecundity (last two clutches within individual female life span). Fecundity was the response variable and body size was the explanatory variable. Female ID was treated as a random factor. Because fecundity is very variable between two consecutive clutches from the same female, a single average value for each female (i.e. the average calculated from two residual values) was used for linear regression to test the relationship between early-life and late-life fecundity. We used the first and last two clutches (rather than three as in the other analyses), because many GRZ females did not produce six experimental clutches over their lifetimes and the use of three clutches would considerably reduce sample size in this strain. Females which produced less than four clutches within their life span ($N = 10$ GRZ, $N = 0$ WT) were excluded from this analysis.

2.5 | Demography

Because fish have a long period of post-maturity growth, body size is an important correlate of female fecundity (Barneche et al., 2018). Growth trajectories (from weekly measurements over the life span) were compared between populations using sex-specific Gaussian Generalized additive models (GAM) from the package MGCV v 1.8.28 (Wood, 2017). Body size was the response variable and age was the explanatory variable with basis dimension $k = 10$ (a method suitable for killifish growth analysis, García et al., 2019). Population was added as an explanatory factor and fish ID was treated as a random effect to account for repeated measurements. Only data to the age of death of the last GRZ (shorter-lived strain) fish were used for statistical tests, but the complete dataset was visualized in plots.

The population-specific age at maturity was modelled using a Gamma GLM, with individual age of sexual maturity (the age of first oviposition) for each female as the response variable and strain ID as an

explanatory variable. Size at the maturity was compared between populations with a Gaussian linear model. Fecundity and fertilization rate peaks were estimated from predicted GAM curves (Figure 1). Total reproductive output (sum of eggs over the entire life span) and individual maximum clutch size were modelled with a Gamma GLM. Population-specific maximum fertilization rate was modelled with a binomial GLM. An overview of all models and their structure is given in Table S1.

2.6 | Reproductive senescence from the perspective of chronological age

Using weekly measurements of individual fecundity, fertility and fertilization rate, strain-specific trajectories were analysed using a GAM. GAMs are not restricted to following a specific polynomial form and continuous variables are fitted with local smoothing functions (Wood, 2017). Fecundity and fertility age-dependent trajectories were modelled with a negative binomial GAM, with the basis dimension chosen by comparison of AIC and constrained to a maximum of $k = 7$ for a smoother fitted to age to retain a biologically meaningful trajectory. Female body size was not used as a covariate because we were specifically interested in the effects of age, and female size was not a population-specific trait (see Figure S1 in Supporting Information). A similar approach was used for fertilization rate (raw binomial data: fertilized and unfertilized eggs), using a binomial GAM (WT $k = 7$, GRZ, $k = 5$, selected on the basis of AIC). Individual female identity was added as a random factor (specified as *bs = 're'*) to account for repeated measurements in fecundity, fertility and fertilization rate models.

We investigated whether decline in fertilization rate was caused by senescence in egg quality (female effect), senescence in sperm quality (male effect) or their combination by pairing young (8 weeks) and old (31 weeks, when a consistently lower fertilization rate was detected) males and females of the WT strain. To analyse these data, a binomial Generalized mixed effect models from the package LME4 v 1.1.21 (Bates et al., 2015) were used. Raw binomial data on egg fertilization were the response variable and the interaction between female and male age (factors with two levels) was the explanatory variable. Individual female and male identity were used as random factors to account for repeated measurements (for the overview of model structures, see Table S1).

2.7 | Reproductive senescence from the perspective of biological age

Individuals age at different rates and, hence, senescence patterns are often more apparent at biological rather than chronological age (Froy et al., 2019; Hayward et al., 2013). In the absence of environmental sources of mortality in our setting and the lack of physiological markers of biological age, we standardized biological age by using the time to death (i.e. weeks to death, age at death = 0) of individuals as an explanatory variable. Separate models with biological age were fitted for each population due to population-specific longevity. Fecundity was fitted using a negative binomial GAM, weeks to death had basis dimension $k = 7$ for WT and $k = 5$ for GRZ. Fertilization rate was fitted using a binomial GAM (WT: $k = 6$, GRZ: $k = 7$). Female ID was treated as a random factor in the models. All basis dimensions (k) were selected by comparing the AIC with the upper limit restricted to $k = 7$ to retain a biologically meaningful trajectory. Finally, we compared models of chronological and biological age using AIC to test which measure of age has stronger explanatory power.

To determine if change in terminal (i.e. 30 days prior death) reproductive function differed between long-lived and short-lived females, we split our data by those with life spans above and those with life spans below the population-specific median. The last 30 days were chosen arbitrarily because this period provided a roughly equal sample size (4–5 bouts per female) for short- and long-lived individuals of both populations. Terminal fecundity was modelled using a negative binomial GAM with a population- and longevity-specific relationship (i.e. factor with four levels) to days to death and basis dimension $k = 3$. Body size was used as a covariate to account for differences in body sizes of females that died earlier and later. Female ID was a random factor. Terminal fertilization rate was modelled using a binomial GAM with $k = 5$ with a population longevity-specific relationship to days to death. Female ID was used as a random factor. The basis dimension was always chosen by AIC. An overview of all models related to biological age and their structure is presented in Table S2.

3 | RESULTS

3.1 | Demography

Weekly measurements of body size demonstrated that outbred WT and inbred GRZ females did not differed significantly in their body size (Gaussian GAM, $F_{1,847} = 3.14$, $p = 0.077$, Figure S1). Growth was asymptotic and females of both strains effectively ceased growth at an age of 12–14 weeks (Figure S2), several weeks after sexual maturation (Table 1). Despite matching body size, WT females greatly outperformed GRZ females in all fitness-related characteristics—longer life span (Figure 1a), earlier maturity, smaller body size at maturity, higher fecundity and higher fertilization rate (Table 1; Tables S3–S5).

3.2 | Reproductive senescence appeared long after cessation of growth

Fecundity increased from sexual maturity until a prime age and then declined (Figure 1b). Peak fecundity was achieved 9 weeks after growth cessation in WT females (at the age of 23 weeks), but occurred earlier and corresponded with growth cessation in GRZ females (age 13 weeks). These ages correspond to 50% survival of females in both populations (Figure 1a). Age-dependent fertility was high since cessation of growth until the age of 25 weeks when fertility declined in both population (Figure 1c). Fertilization

TABLE 1 Life-history traits of females from two populations of *Nothobranchius furzeri* differing in number of captive generations. Detailed test statistics are presented in Supporting Information (Tables S1–S3)

Life-history trait	Wild-type (WT)	Laboratory (GRZ)	Test statistics
Median life span (weeks)	23	13	log-rank test, $\chi^2_1 = 19.3$, $p < 0.001$
Age at maturity (weeks)	5.9 ± 0.13	8.3 ± 0.21	Gamma GLM, $\chi^2_1 = 108.9$, $p < 0.001$
Body size at maturity (mm)	28 ± 0.4	32 ± 0.5	Gaussian LM, $F_{1,57} = 35.29$, $p < 0.001$
Most fecund clutch size (*N* eggs)	92 ± 13	34 ± 6	Gamma GLM, $\chi^2_1 = 18.93$, $p < 0.001$
Lifetime fecundity (*N* eggs)	668 ± 146	197 ± 50	Gamma GLM, $\chi^2_1 = 11.92$, $p < 0.001$
Max. fertilization rate (%)	90 ± 5.2	64 ± 9.9	Binomial GLM, $\chi^2_1 = 5.76$, $p < 0.001$

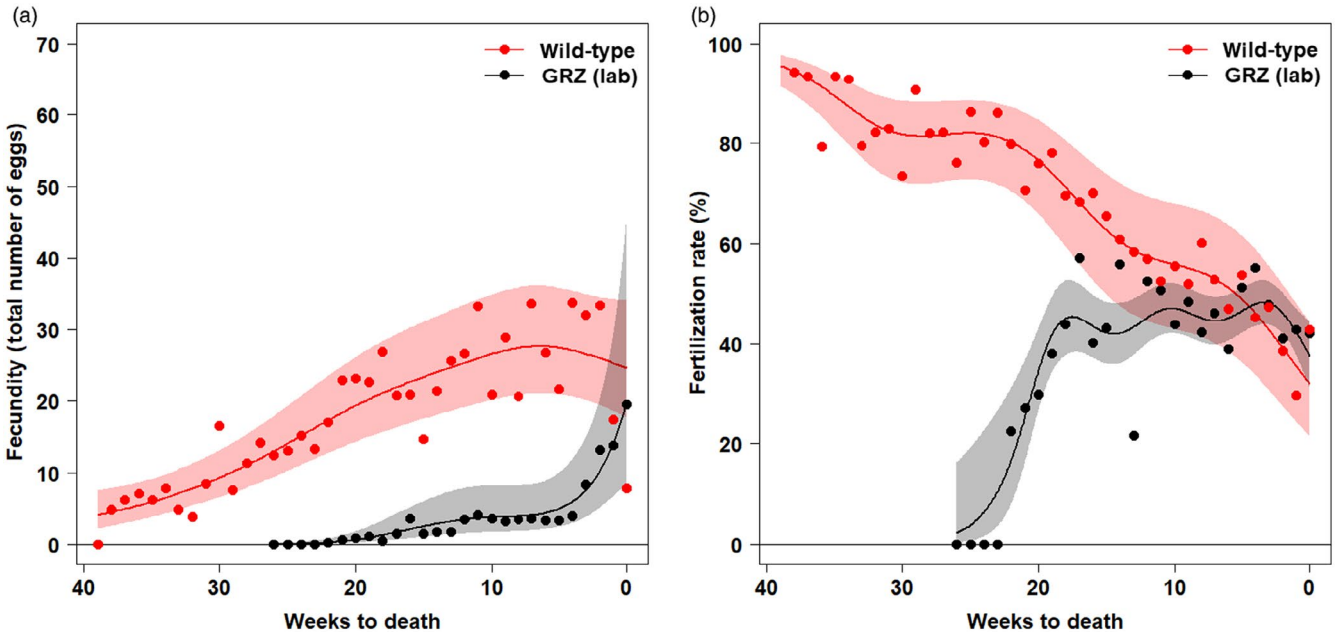

FIGURE 2 Fecundity (a) and fertilization rate (b) assessed on biological age (weeks to death) of individual survival in wild-type (WT) and laboratory (GRZ) populations. WT population is red and GRZ population is black. Data are modelled with a negative binomial GAM for fecundity and binomial GAM for fertilization rate. Shaded area represents 95% confidence interval. Points are means generated from GLMM (negative binomial for fecundity and binomial for fertilization rate) accounted for repeated measures of each individual and with weeks before death as a factor

rate peaked later than fecundity in the GRZ population (21 weeks) but at a similar age as peak in fecundity in WT (Figure 1d) and its decline coincided with the decline in fecundity in WT females (Figure 1d).

3.3 | Reproductive senescence from the perspective of biological age

In contrast to chronological age, there was only weak senescence in fecundity for biological age in WT (NegBin GAM, *edf* [effective degrees of freedom] = 3.189, $\chi^2 = 77.78$, $p < 0.001$, Figure 2a) and increase in fecundity was apparent in GRZ (*edf* = 3.325, $\chi^2 = 59.7$, $p < 0.001$, Figure 2a). However, the fecundity–age relationship had a clearly superior fit when chronological age was used (WT: ΔAIC = 118, GRZ: ΔAIC = 232, Table S6). Biological-age senescence in fertilization rate was apparent in WT population where it declined prior to death (*edf* = 4.96, $\chi^2 = 868.6$, $p < 0.001$, Figure 2b) but was relatively stable after reaching an asymptote in GRZ (*edf* = 5.938, $\chi^2 = 1{,}142.0$, $p < 0.001$, Figure 2b). Again, models with chronological-age senescence in fertilization rate had a superior fit over the biological-age senescence model (WT: ΔAIC = 368, GRZ: ΔAIC = 50, Table S6).

After splitting females into long- and short-lived groups, there was a statistically significant terminal decrease in fecundity only in long-lived WT individuals (*edf* = 1.235, *F* = 17.208, $p < 0.001$,

Figure S4) and a non-significant tendency towards a decrease in fertilization rate prior to death in long-lived WT females ($p = 0.082$) but no detectable terminal decrease in fecundity and fertilization rate in any other group ($p > 0.271$, Figure S4).

3.4 | Faster functional reproductive senescence in females

Senescence in fertilization rate depended on both sexes (binomial GLMM, female.age:male.age interaction, $\chi^2_1 = 3.97$, $p = 0.0463$, Figure 3) but there was a much stronger effect of female age ($\chi^2_1 = 22.72$, $p < 0.001$) than male age ($\chi^2_1 = 2.22$, $p = 0.136$). The fertilization rate in old females was low irrespective of male partner age (12.1 ± 5.0% [mean ± *SE*] with old males; 12.4 ± 4.9% with young males) but young female success was higher with young males (69 ± 8.7%) than with old males (52.7 ± 10.1%, Figure 3).

3.5 | Positive association between reproduction and life span

Female life span was positively associated with early-life fecundity (calculated as the sum of eggs in the first three clutches, $p = 0.002$, Table 2) and there was no association between life span and juvenile growth (measured as body size at the age of 8 weeks: $p = 0.707$, Table 2) or life span and maximum fertilization rate ($p = 0.540$, Table 2). Similar relationships were observed when the model was fitted to the population-specific age of peak fecundity (i.e. mid-adulthood, peak fecundity: $p = 0.006$, Table S7) and when it accounted for selective disappearance (early fecundity: $p = 0.001$, Table S8). Contrary to the assumption of reproduction trade-off, there was a tendency towards a positive relationship between early-life and late-life fecundity (linear regression of residuals from GLMM corrected on body size and repeated measures, $F_{1,48} = 3.08$, $p = 0.085$, Figure S5).

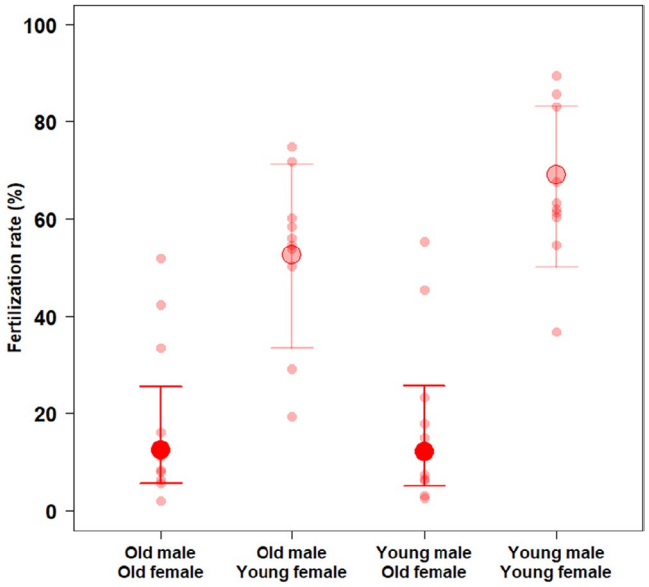

FIGURE 3 Decrease of fertilization rate of 31-week females but not old males of the wild-type population. Large points are means, error bars are 95% confidence intervals. Dark-red points are means for old female combinations. Light-red point means are for young female combinations

TABLE 2 Summary table of the Gamma GLM for predictors of female life span in *Nothobranchius furzeri*. Life span data were square-root transformed. All explanatory variables (except 'Population') were zero-centred with *SD* = 1 prior to analysis

	Estimate (*SE*)	*t*-value	*p*
Intercept	5.15 (0.071)	72.4	**<0.001**
Number of eggs in the first three clutches	0.16 (0.050)	3.2	**0.002**
Maximum fertilization rate	0.05 (0.059)	0.8	0.429
Body size at 8 weeks	−0.03 (0.053)	−0.5	0.625
Population (laboratory)	−0.50 (0.124)	−4.3	**<0.001**

4 | DISCUSSION

Reproductive senescence has long been considered absent in fish, given their post-maturity growth and strong increase in fecundity as a function of increasing body mass (Barneche et al., 2018; Bidder, 1932; Finch, 1990). We report a clear case of reproductive senescence in a fish with a long post-maturation growth period, unusually rapid development and short life span. We show that the pattern of reproductive senescence was sex-dependent, varied between populations with a different history of captive breeding and genetic diversity. Reproductive senescence was stronger in females than in males. Individual life span was positively associated with fecundity and unrelated to juvenile growth, not supporting the role of allocation trade-offs in reproductive senescence.

Our longitudinal study had greater power to detect reproductive declines than previous cross-sectional studies on senescence in killifish (Blažek et al., 2017; Vrtílek, Žák, Blažek, et al., 2018) because individual-based longitudinal studies provide more accurate senescence estimates (Nussey et al., 2008; Warner et al., 2016). Fish and other animals with indeterminate and post-maturity growth have long been considered insusceptible to ageing, but recent studies demonstrate that ageing may be common in these species (Benoît et al., 2018; Depeux et al., 2020; Warner et al., 2016). Indeed, other fish species are reported to senesce, including anchovies (Parrish et al., 1986), guppies (Reznick et al., 2004) and herring (Benoît et al., 2018). Reproductive senescence among species with a long period of post-maturity growth does not appear to be restricted to those with rapid life history but rather to the availability of longitudinal data. For example, long-lived painted turtles, considered to lack senescence, were revealed to senesce in the wild, though the onset of senescence was postponed compared to animals that cease growing soon after sexual maturation (Warner et al., 2016). Limited

knowledge of reproductive senescence in some organisms, including model organisms, prevents a solid understanding of the mechanisms related to decline in this key fitness component (Lemaitre et al., 2020).

Low effective population size leads to the accumulation of mutation load (Cui et al., 2019). Inbreeding, an extreme version of limited effective population size, affects reproductive traits (DeRose & Roff, 1999). WT females matured at smaller size than GRZ females but maintained similar fecundity of their first batches as GRZ females. Maturing at small size and maintaining high fecundity may be beneficial to life in time-constrained environment such as ephemeral savanna pool inhabited by annual killifish. Given that captive environment is not time-constrained, delayed maturity in GRZ may arise from adaption to captivity.

Laboratory populations are selected for successful reproduction in captivity and, hence, are often more fecund (despite the cost of inbreeding) than WT strains under matching captivity conditions (Miller et al., 2002; Sgro & Partridge, 2000). A shorter life span is often linked to accelerated reproduction (Austad & Hoffman, 2018; Charnov et al., 2001; Miller et al., 2002). Short life span in GRZ may be associated with a high mutation load (Willemsen et al., 2020), which is supported by the fact that GRZ crosses with a WT population had an intermediate life span (Kirschner et al., 2012). Laboratory GRZ had a lower maximum clutch size, but their fertility and fecundity until the onset of senescence were similar to the WT population. It appears that population-specific fecundity may simply arise from an earlier onset of reproductive senescence in GRZ.

There are contrasting predictions for reproductive senescence between a fast life history and post-maturity growth. Evolutionary theories of ageing (Finch, 1990; Vaupel et al., 2004; Williams, 1957) predict that organisms with long post-maturity (or indeterminate) growth experience delayed senescence, or its absence, as a consequence of increased fitness of larger/older individuals. In contrast, short-lived organisms with high adult mortality are expected to evolve an early onset of senescence (Jones et al., 2008). We established that reproductive senescence in a short-lived species with post-maturity growth started much later than a deceleration in growth. Growth rate in organisms with a long post-maturity growth period is asymptotic rather than linear and decelerates at older age (Sebens, 1987; Warner et al., 2016). The prime age for fecundity coincided with 50% female survival and the consequent decline in fecundity suggests its association with somatic deterioration (Ricklefs et al., 2003). Comparing the onset of reproductive senescence with constraints imposed on annual killifish by the persistence of their natural habitats (Vrtílek, Žák, Polačik, et al., 2018) showed that senescence coincided with the time when only a few individuals would be expected to survive in rapidly desiccating pools. This correspondence of senescence with disappearance of the habitat provides the opportunity for late-acting detrimental mutations to be fixed in the population (Cui et al., 2019; Medawar, 1952). Reproductive senescence has, therefore, limited impact in natural populations (Vrtílek, Žák, Blažek, et al., 2018).

Functional reproductive senescence was greater in females. We detected a much lower reduction in fertilization capacity in old males than in old females. The observed decline in fertilization rate of eggs was mainly the result of a decreased capacity of females to produce viable eggs. This outcome is surprising as male killifish suffer higher mortality in the wild (Reichard et al., 2014) and twice as many histopathological lesions were reported in testicular in comparison with ovarian tissue under captive conditions (Di Cicco et al., 2011), leading to the prediction for earlier onset of reproductive senescence in males (Bonduriansky et al., 2008; Di Cicco et al., 2011). A weak association between gonad histopathology and reproductive function is puzzling, but has previously been observed in male southern platyfish *Xiphophorus maculatus* (Schreibman et al., 1983). A caveat is that we did not test spermatozoa traits or juvenile survival (Arslan et al., 2017), though our experimental data clearly demonstrate that older males were fully capable of fertilizing eggs.

Biological age can often describe senescent patterns more reliably than chronological age, as it controls for selective disappearance of less fit individuals from the population with time (Froy et al., 2019; Hämäläinen et al., 2014; Hayward et al., 2013). On the scale of biological age (weeks to death), we detected only a slight terminal decrease in fecundity in WT population while fecundity actually increased with biological age in GRZ. The fecundity pattern observed in GRZ may be interpreted as a terminal investment (e.g. Froy et al., 2013). However, we believe rather that it is related to the contribution of short-lived individuals to terminal population-specific biological age, having died at a chronological age when their fecundity was still steeply increasing. In WT fish, reproductive senescence in fertilization rate was detected since it declined throughout biological age. In contrast, in the GRZ population, reproductive senescence in fertilization rate was asymptotic. Despite this insight into reproductive senescence from the perspective of biological age, the observed age-related patterns of reproductive function in *N. furzeri* were better explained by models of chronological age. Terminal reproductive senescence was a feature of long-lived females because no detectable decline was observed in short-lived females (Figure S4) which corroborates the conclusion that chronological age was more important.

We speculate that the difference between GRZ and WT in reproductive senescence at biological age is related to the relatively premature death of GRZ fish, corresponding with a lower expression of phenotypic markers of ageing in the GRZ strain compared to wild-derived populations (Terzibasi et al., 2008; Valdesalici & Cellerino, 2003). Indeed, only 16% of GRZ fish survived to an advanced age when a decline in fertilization rate (from the perspective of chronological age) emerged, contrasting with 50% survival of WT females at the age when their fertilization rate started to decline.

A positive association between early reproduction and life span, and a positive association between early and late reproduction are incongruent with reproduction–life span trade-offs (Kirkwood, 1977; Stearns, 1992). High allocation into early reproduction is likely under strong selection in annual killifish, since

natural pools can disappear in <3 weeks after initial inundation (Vrtílek, Žák, Polačik, et al., 2018). While we acknowledge that captive conditions are not ideal for testing life-history trade-offs given the absence of resource limitation in captivity (Ricklefs & Cadena, 2007), we can discount the confounding effects of a novel laboratory environment (Service & Rose, 1985) and inbreeding (Rose, 1984). Our study populations differed in both these aspects, but retained the same pattern of positive association between early reproduction and life span. Silver spoon effect may play the role in observed pattern because it predicts that favourable conditions (such as captivity conditions) early in life have a long-term positive effect on fitness (Grafen, 1988). Additionally, the shorter life span and lower fecundity of GRZ compared to WT fish is congruent with results reported for *Drosophila melanogaster*, where mutations decrease early adult fitness through reduced life span (Kimber & Chippindale, 2013). This observation would support positive pleiotropy (Maklakov et al., 2015). We acknowledge that our data cannot distinguish among those concepts and further experimental research is needed to disentangle mechanisms behind positive correlation of phenotypic traits in annual killifish.

We estimated senescence in a limited number of reproductive traits and focused mainly on female traits. The focus on female reproductive senescence in our study was driven by the impossibility of extracting *N. furzeri* sperm non-invasively (Dolfi et al., 2020). Reproductive senescence in males was negligible but we acknowledge that other male reproductive traits such as sperm traits, ornaments (Cornwallis et al., 2014) or offspring performance (Preston et al., 2015), unmeasured in our study, can be subject to reproductive senescence. Following offspring survival and performance beyond 24 hr post-fertilization was not feasible during our study, especially given the number of eggs we analysed (N = 12,480 fertilized eggs) and developmental variability of diapausing killifish eggs (Polačik et al., 2014). A dedicated longitudinal study may provide insights into the effect of parental reproductive senescence on offspring performance.

In conclusion, we demonstrate strong reproductive senescence in a short-lived annual fish, a vertebrate with a long post-maturation growth period. It remains to be tested how common reproductive senescence is among fish, as well as other species with post-maturity and indeterminate growth and diverse life histories, including long-lived species. These data would illustrate the evolutionary constraints on reproductive function and fitness.

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AUTHORS' CONTRIBUTIONS

J.Ž. and M.R. designed the study and wrote the paper; J.Ž. performed the research and analysed the data.

DATA AVAILABILITY STATEMENT

Data supporting the results and the R codes of all our analyses are available from Figshare Repository https://doi.org/10.6084/m9.figshare.12095961 (Žák & Reichard, 2020).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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