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# Limited scope for reproductive senescence in wild populations of a short-lived fish

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## Abstract

Senescence in wild populations was long considered negligible but current evidence suggests that it is widespread in natural populations of mammals and birds, affecting the survival and reproductive output of older individuals. In contrast, little is known about reproductive senescence in species with asymptotic growth that can keep increasing their reproductive output as they grow older and larger. Using a cross-sectional study, we tested age-related decline in fecundity and relative allocation to reproduction in five wild populations of an annual killifish, *Nothobranchius furzeri* (Cyprinodontiformes). We did not detect any decline in absolute female egg production over their short lifespan in the wild. Relative fecundity (egg production controlled for female body mass) tended to decrease with age. This effect was driven primarily by a single population that survived 17 weeks, almost twice as long as the median persistence of the other four study populations. There was no decrease in relative ovary mass while in males, relative testes mass actually increased with age. Intra-population variation in relative ovary mass increased in older females suggesting heterogeneity in individual trajectories of female reproductive allocation. Overall, we demonstrate that annual killifish do not experience significant age-related decline in reproductive functions during their very short lifespan in the wild despite the marked deterioration of gonad tissue detected in captivity.

**Keywords** Reproductive ageing · Turquoise killifish · Gene by environment interaction · Plasticity · Population heterogeneity

## Introduction

Senescence is a decline in fitness (survival and reproductive success) with chronological age (Shefferson et al. 2017). While it was long assumed that senescence cannot be observed in wild populations since individuals succumb to predation, diseases or harsh environmental conditions before senescence are manifested (Kirkwood and Austad 2000; Nussey et al. 2013), and recent evidence demonstrated that senescence in the wild is common (Clutton-Brock and Sheldon 2010; Nussey et al. 2013; Lemaître and Gaillard 2017). Senescence is modulated both by abiotic (Kawasaki et al. 2008; Caruso et al. 2017) and biotic conditions (Reznick et al. 2004). Hence, studying senescence in wild populations provides valuable insight into how different components of natural selection interact and mediate senescence—a challenging question for the evolutionary ecology of senescence that cannot be fully understood in captivity.

Reproductive senescence is age-related decline in reproductive functions or structures, such as the ability to allocate energy to reproduction, acquire a mate or provide sufficient parental care (Shefferson et al. 2017; Lemaître and Gaillard 2017). While these complex traits strongly determine individual fitness, data on reproductive senescence from wild populations have only recently become available (Shefferson et al. 2017). The pace and shape of reproductive senescence varies among taxa (Jones et al. 2014), with wild mammals and birds often experiencing decreases in reproductive functions later in

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life (Bérubé et al. 1999; Reed et al. 2008; Bouwhuis et al. 2009; Martin and Festa-Bianchet 2011). In contrast, reproductive senescence in fish and other animals with asymptotic growth is considered negligible or even negative (Vaupel et al. 2004), as their reproductive potential increases as they grow larger (Barneche et al. 2018). For instance, female garter snakes increase their reproductive effort with age while the probability of reproductive failure remains constant throughout their life (Sparkman et al. 2007) and large old female fish are considered the key individuals supporting population-level recruitment (Barneche et al. 2018). However, at least some fish species do experience reproductive decline (Reznick et al. 2006), including wild populations (Benoît et al. 2018), suggesting that reproductive senescence is relevant even for animals with asymptotic growth.

Individual variation may obscure the recognition of senescence at population level (Bouwhuis et al. 2009; Hämäläinen et al. 2014). Both improvement (given continuous growth) and decline (senescence) in reproductive functions are plausible with increasing chronological age (Hamel et al. 2018). Temporal dynamics in intra-population variation of the trait may therefore indicate differential survival among individuals of different condition (manifested by, for example, body mass, immune response or functional performance), effectively masking the incidence of functional and reproductive decline at the individual level (Hämäläinen et al. 2014). In addition, facing an imminent risk that threatens long-term survival may result in a late-life increase in reproductive effort, so-called 'terminal investment' (Bonneaud et al. 2004), whose manifestation is contingent upon individual condition and thus varies among individuals.

*Nothobranchius furzeri* is an iteroparous short-lived fish with very rapid life history adapted to ephemeral pools in the African savannah. The fish hatch synchronously when the pools are filled by seasonal rains (Reichard et al. 2017), grow fast, mature rapidly and then reproduce daily. All fish die when the pool desiccates (usually after 1–7 months), but populations may also disappear from the pool before its desiccation (Vrtílek et al. 2018a). During the dry phase, populations survive as resting embryos in dry pool sediment. In captivity, *N. furzeri* populations have a median lifespan of 4–7 months (Terzibasi Tozzini et al. 2013; Blažek et al. 2017) and exhibit senescence, including pathologies in reproductive organs (di Cicco et al. 2011) and decline in reproductive function (Kim et al. 2016). However, it is unclear whether reproductive senescence affects wild *N. furzeri*.

The abundance and density in wild *N. furzeri* populations decline with age (Vrtílek et al. 2018a) and mortality is stronger in males (Reichard et al. 2014; Vrtílek et al. 2018a). Importantly, the growth and maturation of *N. furzeri* are much faster in wild compared to captive conditions, with the onset of reproduction at the age of 3–5 weeks in captivity (Blažek et al. 2013, 2017; Cellerino et al. 2016) but as early as 2 weeks in wild populations (Vrtílek et al. 2018b). This is adaptive as the pools often desiccate rapidly (1–4 months after being filled) (Vrtílek et al. 2018a, b). Hence, natural selection should favour investment into early reproduction (Sibly and Calow 1986; Kirkwood and Rose 1991), despite its costs in terms of later reproduction or survival (Boonekamp et al. 2014; Lemaître et al. 2015; Zhang and Hood 2016), apparently manifested in rapid maturation and short intrinsic lifespan in *N. furzeri* (Valdesalici and Cellerino 2003; Blažek et al. 2013; Vrtílek et al. 2018b). Consequently, we predicted earlier onset and greater magnitude of reproductive senescence in wild populations of *N. furzeri* compared to captive conditions where no declines in gonad mass, clutch size and fertilisation rate were observed in fish aged 20–23 weeks (Blažek et al. 2017). Increased lipid retention in the liver has been suggested as a potential marker for senescence in captive *N. furzeri* (di Cicco et al. 2011), but lipids are also utilised in vitellogenin production in the liver (Selman et al. 1993) and we explored their role in mediating fecundity and reproductive allocation.

In the current study, we tested the existence of reproductive senescence in replicated wild populations of *N. furzeri*. We predicted that fecundity (reproductive output) and relative allocation to reproductive tissue (gonad mass) would decrease with age. We further predicted that intra-population variation in reproductive allocation would increase with age due to individual variation in response to challenging conditions in the wild. In addition, we tested how liver lipid retention level is associated with sex, age and reproductive allocation.

## Methods

### Study populations

We followed five populations of *Nothobranchius furzeri* (distributed across the entire species range in southern Mozambique) over their natural life cycle throughout the rainy season (17 January 2016–26 May 2016) (Table 1). Samples were collected at the age of 17–121 days (2.5–17 weeks) (collection licence issued by Mozambican Ministry of Sea, Inland Waters and Fisheries: ADNAP-170/7.10/16). In four populations, two seasonal samples were collected, with the first sample at 17–40 days and the second sample 47–74 days (with an interval of 19–34 days between samplings). The four populations had natural lifespan of 72–110 days. The fifth population had a lifespan of > 121 days and provided four seasonal samples (at 40, 70, 108 and 121 days).

Fish were always collected between 07:00 and 08:00 AM (i.e. shortly after sunrise), before the start of reproductive activity (Haas 1976; Polačik et al. 2016). A sample of fish was captured using a seine net (2.7-m long, 0.7-m deep, 4-mm mesh size) and a target subsample was randomly selected, sacrificed by an overdose of clove oil, stored in Baker solution

**Table 1** Overview of populations studied. 'Clade' is a phylogeographic lineage of *N. furzeri* (Bartáková et al. 2013), 'hatching' is the date estimated based on otolith readings, 'final age' gives the number of days between estimated hatching and pool desiccation. Population Ch1 survived beyond our fieldwork period and fish from population LS5 died between days 75–86, before the pool desiccated. '*N* samples' gives number of sampling dates and, in parentheses, the number of females and males collected in total. Note that only a subsample was used for liver lipid retention analysis (see "Methods")

| Site | Coordinates | Clade | Hatching | Final age | *N* samples (F, M) |
|---|---|---|---|---|---|
| Ch1 | E 32° 53′ 54″, S 22° 16′ 33″ | Chefu | 26. 1. 2016 | > 121 | 4 (22, 16) |
| Ch2 | E 32° 43′ 38″, S 22° 33′ 17″ | Chefu | 26. 1. 2016 | 72 | 2 (15, 10) |
| LS1 | E 32° 22′ 45″, S 24° 11′ 40″ | Limpopo South | 18. 3. 2016 | 59 | 2 (17, 10) |
| LS2 | E 32° 36′ 55″, S 24° 18′ 15″ | Limpopo South | 10. 1. 2016 | 110 | 2 (26, 10) |
| LS5 | E 32° 46′ 30″, S 24° 24′ 59″ | Limpopo South | 11. 1. 2016 | 86 | 2 (24, 10) |

and returned to the laboratory for dissection. We aimed to collect 10 females and 5 males per sampling, though sample size varied due to fish availability (Table 1). Sampling frequency and size were kept to a minimum so as not to interfere with our long-term monitoring of the population dynamics of each study population. The study populations were monitored regularly, with 3–14 visits to record environmental parameters (temperature, conductivity, pool surface area, water depth, vegetation cover). The details of habitat conditions and population size dynamics are provided elsewhere (Vrtílek et al. 2018a). In general, pool area ranged from 780 $m^2$ (Ch2) to 3300 $m^2$ (Ch1) and declined seasonally. Population abundance and population density declined over the study period (Vrtílek et al. 2018a). Water temperature varied between 16.8 and 38.0 °C, with a pronounced diel periodicity that was much stronger than seasonal dynamics. All work was carried out in accordance with relevant guidelines and regulations.

## Fish dissection and histology

In the laboratory (IVB, Brno), fish were dissected and their total body mass, eviscerated body mass and gonad mass were weighed to the nearest 0.001 g. The number of mature (ovulated) oocytes was counted as a measure of current female fecundity. *Nothobranchius furzeri* ovulate overnight and lay their eggs singly during the following day. Hence, the number of mature eggs in the morning represents the investment in reproduction for a particular day. Livers were extracted in a subset of sampled fish (57 out of 103 females and 54 of 55 males), weighed, stored in Baker solution and sent to the laboratory at FLI in Jena for histological sectioning to estimate the level of cellular lipid retention (steatosis). At the FLI, livers were embedded in Paraplast, sectioned (5 μm) and stained in H&E. Lipid retention was scored blind to sample identity using the scale of Di Cicco et al. (2011) (score 0–3, 0: no lipid retention in liver cells, 1: minor diffused and localised lipid retention with some cellular swelling present, 2: moderate diffused or localised lipid retention, 3: substantial lipid retention) (Fig. S1).

## Fish age

For each population, the age of the fish was estimated from otolith samples. Three individuals (mixed sex) from each study population were collected prior to the first collection of samples for histology (age 8–26 days) and stored in 96% ethanol. Age estimates from younger fish are more precise (Reichard et al. 2017) and a single age cohort was present in each pool (Vrtílek et al. 2018a). Otoliths were dissected and polished and the number of daily increments was read by a commercial facility (Barcelona Otolith Reading Services, Spain). Date of hatching was calculated by subtracting the estimated age from the date of collection and subsequently used to calculate the age of fish in respective samples. This method was previously validated using captive individuals of known age (Polačik et al. 2011). The estimated age of fish from the same population was within 1 day, except for a single fish that was 3 days older than the rest of the sample (population LS2). The earliest estimate was used when different ages were recorded among individuals within a population to provide a population-level hatching date (Table 1). The full description of the age estimation method is provided in Reichard et al. 2017.

## Statistical analysis

Change in absolute fecundity (number of mature oocytes) was tested using Generalized Linear Mixed Models (GLMM) with negative binomial error distribution to account for a major overdispersion in residuals in GLMM with a log-link, with age as a continuous fixed factor. The population ID was modelled as a random intercept, with age as a random slope. The same approach was used to analyse relative fecundity, with body mass added as a covariate. To test the change in allocation to reproductive tissue (gonad mass corrected for body mass), we fitted a General Least Squares model (GLS) with compound symmetry correlation structure defined by population ID. The GLS modelling approach is equivalent to Linear Mixed Models (LMM) with population treated as a

random intercept (Pekár and Brabec 2016), but additionally enabled correction for heteroscedasticity that was present in our dataset. The sexes were analysed separately, because ovary mass and testes mass are known to differ markedly. Age was a continuous fixed effect, with body mass used as a covariate in the analysis of relative allocation to reproductive tissue.

We calculated the coefficient of variation (CV, the ratio of the standard deviation in the sample to the mean value) in the male and female Gonadosomatic Index (relative allocation to reproductive tissue calculated as a proportion of gonad mass per eviscerated body mass) for each age by population combination (the first and second samples) and compared the level of intra-population variation between younger and older fish using a non-parametric Wilcoxon signed rank test. Given our specific directional prediction (individual heterogeneity in senescence can increase but not decrease intra-population variation), we used one-tailed version of the test.

We tested the association between the level of cellular lipid retention in the liver and the number of mature oocytes using GLMM with negative binomial error distribution with a log-link and the random intercept and slope. We tested the association between lipid retention in liver and ovary mass using GLS. Cellular lipid retention score (ordinal scale with four levels, 0 to 3) was added as a fixed variable. We also performed exploratory analysis of liver cellular lipid retention between the sexes with respect to age including their interaction using Poisson GLMM with log-link with population ID as a random intercept. All explanatory variables were zero-centred (mean = 0) and scaled to one unit of variance (1 standard deviation). Residuals were tested for overdispersion and negative binomial error distribution was employed when overdispersion was detected. We examined heteroscedasticity in all models and corrected for it where necessary (ovary mass GLS model). We removed a single female outlier (population Ch1, age 40 days) from all analyses because she had atrophied ovaries (ovary mass 0.006 g; interquartile range of ovary mass was 0.082–0.164 g and the second smallest ovary was 0.034 g). We used the packages 'nlme' v3.1-137 (Pinheiro et al. 2018), 'lme4' v1.1-18-1 (Bates et al. 2015) and 'MuMIn' v1.42.1 (Barton 2018) in R v3.5.0 (R Core Team 2018) for all statistical analyses. The data and R code used for the analysis are publicly available at Figshare repository (doi: https://doi.org/10.6084/m9.figshare.7321751).

## Results

### Fecundity

The number of mature oocytes did not decrease with female age (GLMM, age $-0.054 \pm 0.057$ (estimate ± standard error), $z = -0.96$, $P = 0.338$; $N = 103$) (Fig. 1a). Relative fecundity (i.e. number of mature oocytes with body mass as a covariate) tended to decline with age, though the effect was not statistically significant (GLMM, age $-0.145 \pm 0.076$, $z = -1.91$, $P = 0.056$; body mass $0.398 \pm 0.070$, $z = 5.72$, $P < 0.001$, $N = 103$).

### Allocation to reproductive tissue

Relative ovary mass did not significantly decrease with female age (GLS, age $-0.008 \pm 0.005$, $t = -1.56$, $P = 0.121$; body mass covariate $0.053 \pm 0.006$, $t = 8.33$, $P < 0.001$; $N = 103$) (Fig. 1b). Relative testes mass increased, rather than decreased, with male age (GLS, age $0.002 \pm 0.001$, $t = 2.90$, $P = 0.005$; body mass covariate $0.006 \pm 0.001$, $t = 8.12$, $P < 0.001$; $N = 55$) (Fig. 1c).

The variation in relative reproductive allocation among females within a population (CV of Gonadosomatic Index per sample) was low in young females, but increased with age (left-tailed Wilcoxon signed rank test: $z = 2.02$, $P = 0.031$; $N = 10$) (Fig. 2a). In contrast, the variation in Gonadosomatic Index among males did not increase in older males (left-tailed Wilcoxon signed rank test: $z = 1.48$, $P = 0.938$; $N = 10$) (Fig. 2b).

### Lipid retention in livers

The number of mature oocytes tended to be negatively associated with the level of liver lipid retention (GLMM, lipid retention score $-0.100 \pm 0.056$, $z = -1.79$, $P = 0.073$; $N = 57$). Relative allocation to reproductive tissue (with age and body mass as covariates) was not associated with lipid retention in females (GLS, lipid retention score $-0.005 \pm 0.004$, $t = 1.34$, $P = 0.187$; $N = 57$) or males ($0.001 \pm 0.001$, $t = 1.53$, $P = 0.132$; $N = 54$). In general, lipid retention in livers was higher in males than in females (GLMM $1.135 \pm 0.186$, $z = 6.09$, $P < 0.001$; $N = 111$), but did not change with age ($0.104 \pm 0.102$, $z = 0.98$, $P = 0.329$) and there was no interaction between sex and age ($z = -1.13$, $P = 0.259$ in the full model).

## Discussion

Senescence was long assumed to be manifested only in captive conditions where the elimination of external sources of mortality enables animals to live long enough to experience senescent decline in functional traits (Kirkwood and Austad 2000). Recent research on natural, free-ranging populations of birds and mammals has confirmed that particular traits, including reproductive traits, are commonly subject to senescent declines in the wild (Nussey et al. 2013; Mourocq et al. 2016). For example, long-term demographic studies have demonstrated decreased lamb production in older wild bighorn ewes (Bérubé et al. 1999) and lower numbers of fledglings in older

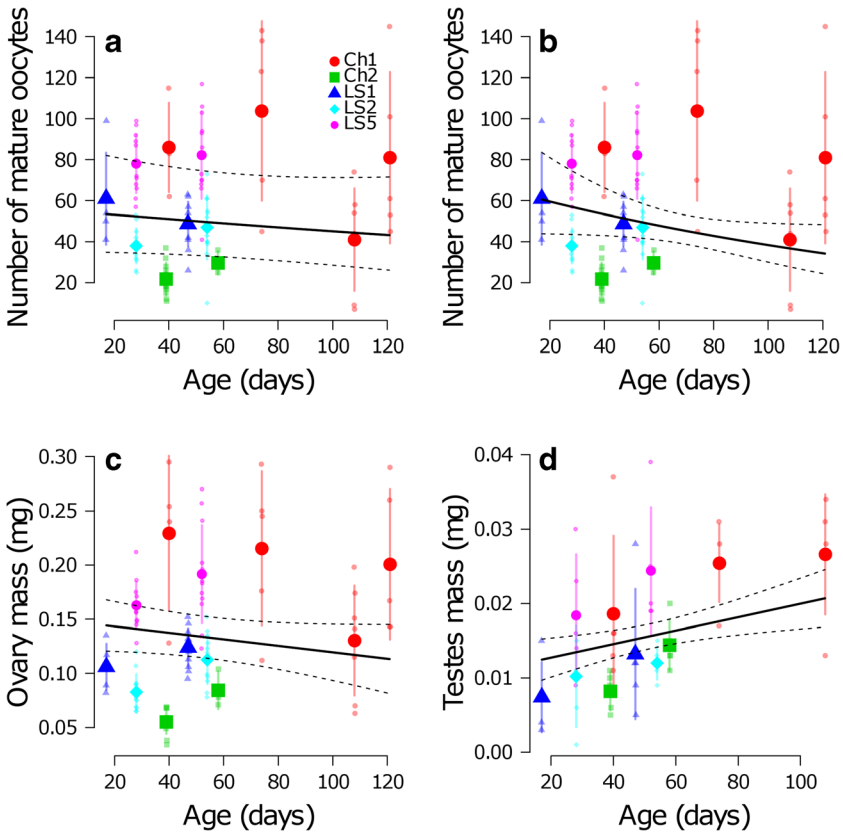


**Fig. 1** Age-related reproductive output in 5 wild populations of *N. furzeri*—**a** absolute female fecundity (number of mature oocytes uncorrected for body size), **b** relative female fecundity (predicted effect of age on number of mature oocytes when accounting for body mass as a covariate), **c** female and **d** male relative gonad mass (with body mass as a covariate). The horizontal solid line shows the predicted relationship between each response variable and age, with dashed lines indicating 95% confidence intervals. The small points in the background are raw values, the solid points represent sample-specific mean values and the vertical error bars denote one standard deviation

great tits (Bouwhuis et al. 2009). A few recent studies on fish and other animals with asymptotic growth have also reported marked senescence in reproductive functions in semi-natural (Massot et al. 2011) and natural populations (Benoît et al. 2018), suggesting that reproductive senescence may be more widespread in nature than is generally recognised. Here, we focus on reproductive senescence in wild populations of *N. furzeri*, a species with an extremely condensed life history. Our cross-sectional study demonstrated no significant age-related decline in fecundity or relative gonad mass among *N. furzeri* females. Intra-population variation in relative gonad mass was much higher in older females but this variation was not associated with liver lipid retention. In males, relative gonad mass even increased with age.

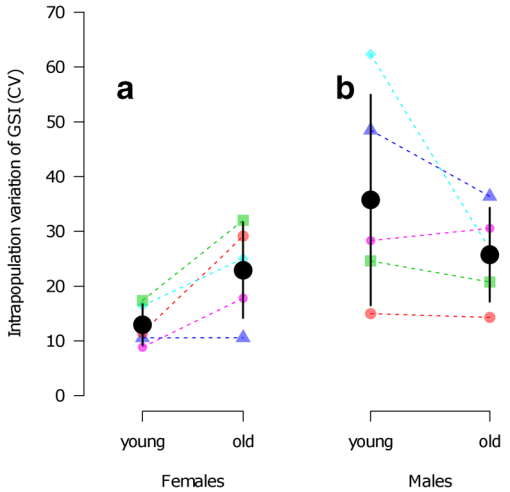


**Fig. 2** Intra-population variation in Gonadosomatic Index (GSI) in young and old females (**a**) and males (**b**). The coefficient of variation (CV) was calculated as a measure of variability within each sample and is shown by population-specific colour and symbol, corresponding to the specifications used in Fig. 1. The large black points illustrate mean values across the populations. The error bars represent one standard deviation

At the physiological level, there is evidence of reproductive senescence from captive populations of female *N. furzeri* and closely related species, but at an age far beyond 10 weeks that is the average population-level survival in the wild (Vrtílek et al. 2018a). In two laboratory strains of *N. furzeri*, follicular degeneration and fibrosis was detected in approximately 30% of old females (aged 16 and 28–32 weeks for the respective strains), while the incidence of neoplasia in ovaries was relatively low (< 10%) compared to other organs (di Cicco et al. 2011). Old (12 months) *Nothobranchius guentheri* females from a laboratory population suffered higher incidence of atretic oocytes and lower production of vitellogenins (Liu et al. 2017), glycolipoproteins that supply ovulating eggs with yolk (Selman et al. 1993; Reading and Sullivan 2011). This indicates a decline in the ability to efficiently transform the available resources into mature eggs, potentially explaining our observation that older females did not decrease their

relative reproductive allocation (relative gonad mass) while their relative fecundity (number of mature eggs corrected for body size) tended to decline. This tendency in reproductive fecundity decline is unlikely due to decreased resource availability for ovulating older females, as condition factor (Fulton Index) in females did not decrease with age (Fig. S2). In addition, a previous laboratory study demonstrated that food-deprived female *N. furzeri* reduced reproductive allocation along with fecundity (Vrtílek and Reichard 2015). It is possible that a particular (yet unidentified) pathway of female reproductive physiology becomes altered at older age, resulting in reduced production of mature eggs.

There was no evidence of a decline in reproductive allocation and fecundity with age in captive *N. furzeri* populations (Blažek et al. 2017) estimated at the age of 10 and 20 weeks. In the longer-lived *N. orthonotus* and *N. pienaari*, estimates over a period of 50–60 weeks demonstrated clear age-related declines in fecundity (Blažek et al. 2017). The two species are sympatric and closely related to *N. furzeri* but their lifespan in captivity is approximately 50–75% longer (Terzibasi Tozzini et al. 2013; Blažek et al. 2017). Reproductive allocation did not decline in any of the eight populations of four *Nothobranchius* species tested by Blažek et al. (2017), in agreement with the outcome of the present study.

In wild populations, we detected a tendency for relative fecundity to decline with age. This pattern was primarily driven by a single population that was sampled over a period of 17 weeks, while the trend was inconclusive in the remaining four populations monitored for a considerably shorter time (6–11 weeks). Twenty-week-old females in captivity did not exhibit senescence in egg production (Blažek et al. 2017). We hypothesised that rapid reproductive senescence in wild populations of *N. furzeri* might arise from a greater trade-off between early development and late-life fitness. Under natural conditions, juvenile development is extremely rapid, with fish commonly reaching maturity in 2 weeks after hatching (Vrtílek et al. 2018b) while in captivity, the juvenile period can be twice as long (3–5 weeks) (Blažek et al. 2013, 2017; Cellerino et al. 2016). The costs of rapid growth and high investment into early reproduction are paid later in life, either in terms of decreased survival (Descamps et al. 2006; Boonekamp et al. 2014) or reduced late-life reproduction (Nussey et al. 2006; Reed et al. 2008). Our analysis does not support the hypothesis that a decline in egg production is manifested in the wild, at least over ecologically relevant longevity.

Intra-population variation in female relative reproductive allocation increased with age in the study populations, suggesting considerable individual heterogeneity. Given that wild *N. furzeri* females maintained the same reproductive allocation on average, increased late-life intra-population variation in relative reproductive allocation suggests that reproductive fitness improved with age in some females but declined in others. The level of liver lipid retention could be an appropriate marker of condition and positively correlate with female fecundity. While a high level of lipid retention in the livers is pathological in mammals (including humans: Vernon et al. 2011), the liver serves as a lipid storage organ in many fishes (Sheridan 1988). We observed sex differences in lipid retention in livers in wild fish, a feature that has been repeatedly observed in captive *N. furzeri* (di Cicco et al. 2011; Cellerino et al. 2016). We speculate that this represents a sex-specific pattern of lipid metabolism. Female fish utilise lipids stored in livers by allocating them to vitellogenins, representing the major resource for developing embryos (Selman et al. 1993; Reading and Sullivan 2011). We found that the most fecund females tended to have relatively less lipid in their liver, but there was no relationship between lipid retention in livers and female relative gonad mass. This might suggest that females may utilise stored lipids for egg production (increased resource use). We fully acknowledge that we made no particular a priori prediction of the direction of the relationship and this correlation should be taken with caution. The increased intra-population variation in female reproductive allocation could also have been a consequence of low male density, with some females accumulating eggs that were not spawned on the previous day (Fig. S3). Overall, the elevated inter-individual variation in reproductive traits at older age demonstrates the importance of studying intra-population variation rather than just the population mean (Hayward et al. 2015; Hamel et al. 2018) and points towards individual-specific patterns of reproductive senescence in female *N. furzeri* that shall be further explored in longitudinal studies.

The sexes differ in the magnitude and trajectories of senescent declines, including reproductive traits (Cornwallis et al. 2014). In some species, males are able to maintain high reproductive success over their lifetime (Hayward et al. 2015; Lemaître and Gaillard 2017), but males of other species exhibit steep reproductive senescence (Dean et al. 2010). We focused on a single measure of male reproductive ability—the relative mass of testes. While large testes result in high male reproductive success in species with promiscuous mating systems (Preston et al. 2003), the cost of sperm production is higher than usually assumed (Wedell et al. 2002; Smith et al. 2009), making senescent decline in testes size plausible (Hayward et al. 2015; Johnson et al. 2018). In our study, wild male *N. furzeri* showed a marked increase in relative testes mass with age. Captive-bred male *N. furzeri* maintained relatively high fertilisation success at the age of 20 weeks (Blažek et al. 2017), suggesting they did not suffer from reproductive senescence. Also, at the histological level, only rare and limited testes pathology was observed at the age of 11 weeks (di Cicco et al. 2011). Testis fibrosis, degeneration and atrophy in various combinations and degrees affected most male *N. furzeri* only at the age of 28–32 weeks (di Cicco et al. 2011), which is beyond the maximum lifespan of males in wild populations.

Overall, we demonstrated that wild females of extremely short-lived fish species do not suffer significant age-related decline in fecundity during their short natural lifespan of 4 months, despite a tendency for reduced relative fecundity over that period. Individual variation in reproductive allocation among females within each population increased with age, but this heterogeneity was not associated with the extent of lipid retention in livers. Survival in natural populations of *N. furzeri* appears too short for reproductive senescence to be manifested despite its expression in captivity.

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