

Fluctuating temperatures extend median lifespan, improve reproduction and reduce growth in turquoise killifish

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ARTICLE INFO

Section editor: Sean Curran

Keywords:

Aging
Environmental stress
Life histories
Nothobranchius furzeri
Thermal regime
Vertebrate lifespan

ABSTRACT

In natural populations, individuals experience daily fluctuations in environmental conditions that synchronise endogenous biorhythms. Artificial alterations of environmental fluctuations can have negative consequences for life history traits, including lifespan. In laboratory studies of aging, the role of fluctuating temperature is usually overlooked and we know little of how thermal fluctuation modulates senescence in vertebrates. In this longitudinal study we followed individually-housed turquoise killifish, *Nothobranchius furzeri*, from two thermal regimes; ecologically relevant diel fluctuations (20 °C - 35 °C) and stable temperature (27.5 °C), and compared their survival, growth and reproduction. Fish experiencing fluctuating temperatures had a longer median lifespan but reached smaller asymptotic body size. Within-treatment variation indicated that extended lifespan in fluctuating temperatures was not causally linked to decreased growth rate or smaller body size, but occurred solely due to the effect of thermal fluctuations. Male body size was positively associated with lifespan in stable temperatures but this relationship was disrupted in fluctuating thermal regimes. Females exposed to fluctuating temperatures effectively compensated egg production for their smaller size. Thus, there was no difference in absolute fecundity between thermal regimes and body-size corrected fecundity was higher in females in fluctuating temperatures. Overall, despite a brief exposure to sub-optimal thermal conditions during fluctuations, fluctuating temperature had a positive effect on survival and reproduction. These results suggest that the expression of life history traits and their associations under stable temperatures are a poor representation of the relationships obtained from ecologically relevant thermal fluctuations.

1. Introduction

Environmental conditions modulate most organismal functions, from gene expression to behaviour (Fraser et al., 1993; Podrabsky and Somero, 2004). Temperature, light intensity and food availability fluctuate in predictable daily and seasonal cycles and organisms adapt their endogenous biorhythms to optimize performance (Claireaux and Lefrançois, 2007; Dillon et al., 2016). On the other hand, environmental fluctuation exposes organisms to periods of suboptimal conditions which may have negative consequences for survival (Zhang et al., 2018). Artificial desynchronization of environmental fluctuation may cause stress with long-term, senescence-related consequences (Almáida-Pagán et al., 2018). While high levels of stress may induce immediate mortality, mild stress typically has life-extending effects (Gems and Partridge, 2008). Organisms that have evolved under chronic environmental fluctuation are expected to possess efficient stress-coping

adaptations (Angilletta, 2009; Kern et al., 2015).

Temperature has a fundamental effect on lifespan and low body temperature is associated with longer lifespan in both ectotherms and endotherms (Flouris and Piantoni, 2015; Keil et al., 2015). In ectothermic vertebrates, chronic exposure to sub-optimal temperatures (i.e. temperatures outside the range of preferred body temperatures (Gvoždík, 2018)) has been studied particularly in fishes (Keil et al., 2015), confirming their role in lifespan extension. However, the effect on other life history traits was negative as a consequence of lower metabolism and slower development rate (Angilletta, 2009). The role of fluctuating temperatures on lifespan is less clear (Colinet et al., 2015). Fluctuating temperatures and their effect on lifespan have been studied primarily in invertebrates such as nematodes, crustaceans (*Daphnia* sp) or insects and variously demonstrated lifespan extension, reduction or no effect (Cedergreen et al., 2016; Colinet et al., 2015; Schwartz et al., 2016). The lifespan modulating effect of fluctuating temperature

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<https://doi.org/10.1016/j.exger.2020.111073>

Received 7 July 2020; Received in revised form 12 August 2020; Accepted 22 August 2020

Available online 26 August 2020

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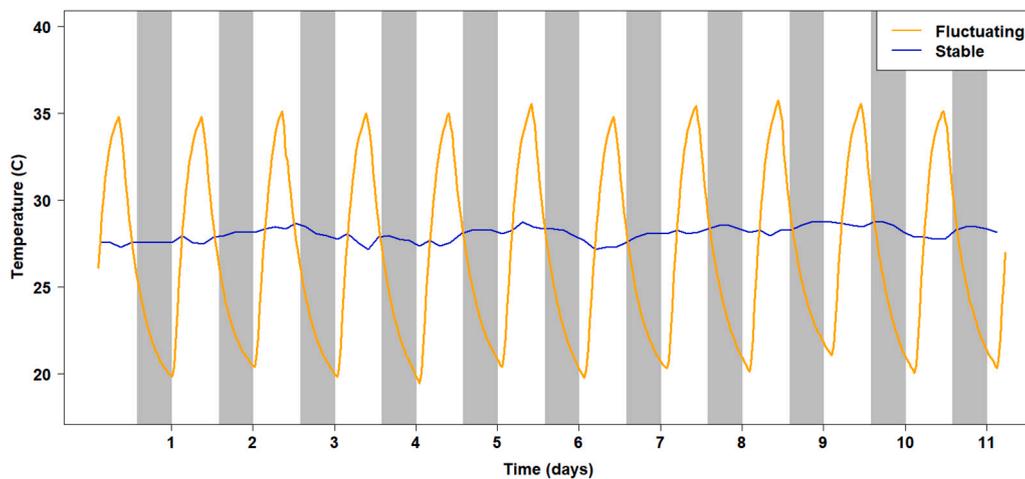


Fig. 1. Water temperature over a period of 11 days in stable (blue line) and fluctuating (orange line) temperature treatments. Grey areas represent the dark period of the day. A short time period was chosen for clear visualization. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

remains largely untested in vertebrates, while both negative and positive effects of fluctuating temperature have been demonstrated on developmental rate (Kern et al., 2015; Niehaus et al., 2012), growth rate (Diana, 1984; Vondracek et al., 1988) and reproduction (Boulé and Fitzgerald, 1989; Podrabsky et al., 2008).

Growth and reproduction are commonly linked to survival via trade-off in resource allocation (Kirkwood, 1977; Lee et al., 2013), antagonistic pleiotropic genetic effects (Williams, 1957) or positive pleiotropic effects (Maklakov et al., 2015). From an evolutionary perspective, it is important to study growth and reproduction together with survival to obtain more complete information on the functional senescence of an organism (Lemaître and Gaillard, 2017; Monaghan et al., 2008). At least in insects, there appears to be no consistent link between survival and other life history traits across studies that exposed animals to fluctuating temperatures (reviewed in Colinet et al. (2015)). This is likely due to wide variation in the distance between the range of preferred body temperatures and the fluctuations tested, unpredictability of extrapolation from studies in stable temperature, and species-specific thermal sensitivity of traits examined (Colinet et al., 2015; Kingsolver et al., 2008; Niehaus et al., 2012). To our knowledge, the extent to which fluctuating temperatures contribute to modification of lifespan and its association with fundamental life history traits in vertebrates is unknown.

In the present study, we used the short-lived turquoise killifish *Nothobranchius furzeri*, a promising vertebrate model for aging (Hu and Brunet, 2018), to examine the role of fluctuating temperature on vertebrate lifespan and its relationship to thermally sensitive life history traits – growth and reproduction.

2. Material and methods

2.1. Study species

The turquoise killifish *Nothobranchius furzeri* is adapted to thermally challenging ephemeral savanna pools in south-east Africa and has a naturally short lifespan of several months (Reichard and Polačik, 2019). During the peak season of killifish abundance, water temperature in ephemeral savanna pools fluctuates in an asymptote of 15–20 °C daily, usually from 22 °C in the early morning to 38 °C in late afternoon (Žák et al., 2018). Perhaps as an adaptation to a thermally unstable environment, the turquoise killifish has wide range of preferred body temperature, from 20 to 30 °C (Žák et al., 2018). The species is easily bred in the laboratory under a stable temperature regime, with a recommended breeding temperature of 25–28 °C (Genade et al., 2005; Polačik et al., 2016). In the laboratory, lowering the water temperature from 25 °C to 22 °C extended the median lifespan of *N. furzeri* by 11% (from 9 to 10 weeks) and delayed the onset of molecular and

behavioural markers of senescence (Milinkovitch et al., 2017; Valenzano et al., 2006). Nonetheless, laboratory studies used chronic exposure to lower temperature only, overlooking the fact that such conditions are unnatural for this species.

2.2. Fish maintenance

We used wild-derived population MZCS 222 (Cellerino et al., 2016) of turquoise killifish kept at the accredited breeding facility at the Institute of Vertebrate Biology of the Czech Academy of Sciences. Fish were hatched on 19 February 2018 following a standard breeding protocol (Polačik et al., 2016). During the first month after hatching, fish were kept under stable temperature of 27.5 °C ± 1 °C in two 60 L tanks. Fish were fed ad libitum 2–3 times per day, initially with live brine-shrimp nauplii and gradually (mixed feeding for 7 days) weaned onto frozen bloodworms (Chironomidae) as they grew larger.

At the age of 5 weeks (age at maturity of all individuals), 84 fish (40 females, 44 males) were individually housed in 2 L tanks within two independent recirculating systems (Aquamedic, Germany, www.aquamedic.de). Selection of experimental fish from initial stock was haphazard and fish from both original tanks were used across both treatments. Individually housed fish were fed ad libitum (amount consumed within 5 min) once per day between 9 and 10 am on thawed frozen bloodworms. Water conductivity was kept between 250 and 350 $\mu\text{S} \times \text{cm}^{-1}$ and the light regime was 14L:10D.

We used two treatments; stable temperature and fluctuating temperature, for a total period of 78 weeks. Stable temperature was maintained at 27.5 ± 1 °C (mean ± SD) throughout the experiment. Fluctuating temperature ranged from 20 °C ± 1 °C in early morning to 35 °C ± 1 °C in late afternoon (Fig. 1, Supplementary Fig. S1). The limits for fluctuating temperature were chosen to reflect the diurnal change in water temperature that killifish experience in the wild (Žák et al., 2018). The fluctuating temperature treatment was provided in one recirculating system by a combination of an aquarium chiller (TECO TR 10, Italy, www.tecoonline.com) and three aquarium heaters (2 × 200 W and 1 × 100 W, Eheim/Jäger, Wüstenrot, Germany). The second recirculating system was used for stable temperature (regulated by one 100 W heater, Eheim/Jäger, Wüstenrot, Germany). One temperature datalogger (HOBO Onset Computer, UA-002-64, Bourne, MA, USA) monitored temperature in the middle rack of each recirculating system. The consistency of thermal conditions and water parameters was checked every two weeks and the set-up of chiller or heaters was modified as necessary to maintain the same thermal regime irrespective of minor seasonal changes in room temperature over the 78 week duration of the experiment (room temperature ranged between 25 °C and 29 °C).

2.3. Data collection

Fish health status and survival were monitored daily. Fish body size was measured every week from the age of 29 days to the age of 300 days and every two weeks until the age of 466 days, using photographs taken in a plastic container with shallow water and a scale on the bottom (resolution of 1 mm). Photographs were imported to ImageJ v 1.46 (imagej.nih.gov) and body size was measured as Standard Length (from tip of the snout to the end of caudal peduncle). Individual specific growth rate (SGR, % SL per day) was estimated from the first 30 days of the experiment, when growth rate was highest and linear (Blažek et al., 2013, Fig. 4). The SGR was calculated as $(\ln SL_2 - \ln SL_1) \times (100/t)$ where SL_2 is terminal body length, SL_1 is initial body length and t is the length of time interval in days (Talbot, 1993).

Reproductive parameters were estimated monthly from spawnings in 2 L plastic containers with a 5 mm layer of fine sand as a spawning substrate. A pair of fish was placed in the container and allowed to spawn for 2 h, which is sufficient for release of all ovulated eggs from female ovaries (Polačik et al., 2016). The same pairings were used throughout the study, though when one individual from a pair died, it was replaced by another fish from the same treatment that lost its partner. At time of spawning termination, all pairs were moved back to recirculating system in a haphazard mode (alternating pairs from stable and fluctuating temperature) within 25 min. The periodicity of spawning was every week but eggs were counted for analysis only once per month. Weekly spawnings were performed to prevent egg retention in ovaries (Polačik et al., 2014), a common problem in female *N. furzeri* when housed in isolation (Polačik et al., 2016). In the present experiment, one female (4.5%) from stable temperature and three (13.6%) females from fluctuating temperature were suspected to have died due to inability to release their eggs (a significant reduction from 80% reported by (Polačik et al., 2014)). Those fish were omitted from the analysis of reproductive traits. The total number of eggs (fecundity; proxy of female reproductive output), number of fertilized eggs (fertility; proxy of reproductive fitness) and fertilization rate (proportion of fertilized eggs; proxy of oocyte quality; in *N. furzeri* fertilization rate is dependent on quality of eggs and almost independent of male traits, Polačik et al., 2016) were determined by sieving eggs from the sand 24 h after removal of parental fish from containers. Fertilized eggs were determined by the presence of the perivitelline space (Polačik et al., 2016).

2.4. Statistical analysis

Median lifespan was compared between treatments using a non-parametric Log-rank test, including sex-specific response. All survival curves were visualized by Kaplan-Meier plots.

The association between lifespan and other life history traits was analysed using a sex-specific Gamma Generalized linear model (GLM) with a log-link function. Sex-specific models were used because reproductive parameters - early fecundity (number of eggs after 30 days under each thermal regime) and fertilization rate (maximum fertilization rate achieved by a female) - were only recorded for females. Early fecundity, fertilization rate, thermal regime and body length at 59 days (period including both juvenile growth and the effect of thermal regime on growth) were included as predictors of female lifespan. SGR was not used for females, because it was collinear with body length (variance inflation factor > 4). For males, lifespan was modelled as a function of thermal regime, body length at 59 days and SGR during the first month of the experiment. In both models for males and females, all possible two-way interactions were included in the full model and removed when insignificant. Given a significant interaction between treatment and body size in males (Supplementary Table S2), thermal regime-specific analyses were conducted.

In addition, as an evaluation of disposable soma theory, we compared how the relationship between lifetime fecundity and female

lifespan varies between thermal regimes using Gamma GLM with lifespan as a response variable and lifetime fecundity in interaction with thermal regime as explanatory variables.

The growth trajectory was compared between thermal regimes with a sex-specific Gaussian Generalized Additive Model (GAM). Body size was the response variable, age was a smoothed predictor with the basis dimension 10 ($k = 10$) and thermal regime specified smoother (by = thermal regime). Thermal regime was added as a parametric predictor and Individual ID was used as a random factor. Specific growth rate from the first month in the experiment was compared using a GLM with SGR as a response variable and thermal regime in interaction with sex used as predictors. We also compared initial body size of fish between thermal regimes using a GLM with thermal regime and sex as explanatory variables.

Fecundity was analysed using a negative-binomial GAM with the number of eggs as a response variable and age (continuous, in days) as a smoothed parameter ($k = 7$). The addition of thermal regime-specific smoothers was not needed (see Results). Parametric terms were thermal regime and body size. Female ID was specified as a random factor. The same model structure was used for fertility analysis, with number of fertilized eggs used as a response variable instead of total number of eggs.

Fertilization rate was analysed with a binomial GAM, with raw binomial data as a response variable (number of fertilized eggs, number of unfertilized eggs), age as a smoothed predictor ($k = 5$, with k chosen on the basis of superior model fit, as estimated by AIC) and thermal regime as a parametric predictor. Female ID was specified as a random factor. The model was accounted for data overdispersion.

All procedures were performed in R 3.6.1. environment using *survival* 2.44.1.1, *lme4* 1.1.21, *mgcv* 1.8.28, *splines* 3.6.1, *car* 3.0.4 and *effects* 4.1.3 packages (Bates et al., 2015; Fox and Weisberg, 2019; R Core Team, 2019; Therneau and Grambsch, 2000; Wood, 2017).

3. Results

3.1. Effect of thermal regime on lifespan

Fish in fluctuating temperatures had a 93% longer median lifespan than fish kept in stable temperature (log-rank, $\chi_1^2 = 6.3$, $p = 0.010$, $N = 84$, Fig. 2a). A longer median lifespan in fluctuating temperatures was recorded in males ($\chi_1^2 = 5.4$, $p = 0.020$, $N = 44$) but this difference, although numerically larger, was non-significant in females ($\chi_1^2 = 1.5$, $p = 0.200$, $N = 40$, Fig. 2b). Overall, median lifespan did not differ significantly between males and females within thermal regimes (fluctuating: $\chi_1^2 = 3.5$, $p = 0.06$, $N = 42$; stable: $\chi_1^2 = 0$, $p = 0.90$, $N = 42$).

3.2. Effect of thermal regime on lifespan correlates

In females, thermal regime did not affect the relationship between lifespan and fundamental life history traits. A positive association between lifespan and fertilization rate ($\chi_1^2 = 9.23$, $p = 0.002$, Supplementary Table S1, Fig. 3a) was congruent across both thermal regimes ($\chi_1^2 = 1.41$, $p = 0.234$, $N = 35$). There was no effect of body size, early fecundity or their interaction with thermal regime on female lifespan (Fig. 3a, Supplementary Table S1).

There was positive relationship between lifespan and lifetime fecundity in females, with steeper increase detected in stable than in fluctuating temperature (Gamma GLM, lifetime fecundity:thermal regime interaction, $\chi_1^2 = 10.84$, $p = 0.001$, $N = 35$, Supplementary Fig. S2, Table S2).

In males, a positive relationship between lifespan and body size was detected in stable temperature ($\chi_1^2 = 5.01$, $p = 0.025$, $N = 21$) but not in fluctuating temperature ($\chi_1^2 = 0.022$, $p = 0.873$, $N = 21$, Fig. 3b, Supplementary Tables S3, S4). There was no effect of SGR on male lifespan (Supplementary Tables S3, S4).

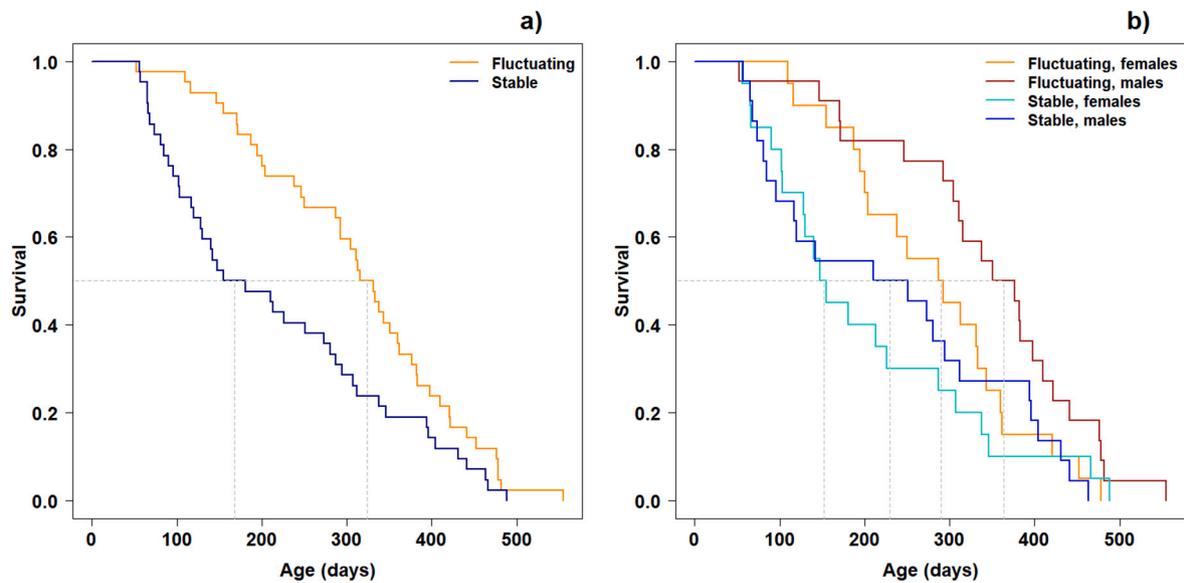


Fig. 2. Different survival of *Nothobranchius furzeri* in stable (blue, $N = 42$) temperature and in fluctuating (orange, $N = 42$) temperature a). Survival was longer in fluctuating temperature ($p = 0.010$). Sex and thermal regime specific survival of *Nothobranchius furzeri* b). Both survival plots are Kaplan-Meier plots. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.3. Effect of thermal regime on growth

Overall, fish in fluctuating temperature were smaller than fish in stable temperature (Gaussian GAM, males: $F_{1,1008} = 7.08$, $p = 0.009$, $N_1 = 44$ fish, $N_2 = 1066$ measurements; females: $F_{1,973} = 41.33$, $p < 0.001$, $N_1 = 40$, $N_2 = 1036$; Fig. 4, Supplementary Table S5). After the first month, female SGR was 53% lower in fluctuating temperature than in stable temperature. In males, the reduction in SGR between fluctuating and stable temperatures was 20% (thermal regime:sex: ANOVA, $F_{1,77} = 5.31$, $p = 0.024$, $N = 84$, Supplementary Table S6). At the beginning of the experiment, fish in both thermal regimes had similar body size (treatment: $F_{1,82} = 0.196$, $p = 0.660$, $N = 84$, Supplementary Table S7).

3.4. Effect of thermal regime on reproductive traits

Relative fecundity was 80% higher in females in fluctuating temperature (Neg.Bin. GAM, $\chi^2 = 7.93$, $p = 0.005$, $N_1 = 35$, $N_2 = 223$; corrected for female body size: $\chi^2 = 8.68$, $p = 0.003$). Females in fluctuating temperature effectively compensated their egg production despite their smaller size and, overall, there was no significant difference in absolute fecundity between thermal regimes ($\chi^2 = 0.74$, $p = 0.391$). The age-related pattern of relative fecundity (age: edf = 3.736, $\chi^2 = 127.19$, $p < 0.001$; Fig. 5a) was similar in both thermal regimes (model with treatment-specified smoothers AIC = 1836, without treatment-specified smoother AIC = 1832). Likewise, fertility did not differ between the treatments (Supplementary Table S8). There was no significant difference in fertilization rate between thermal regimes (Binomial GAM: $F_{1,177} = 0.188$, $p = 0.665$;

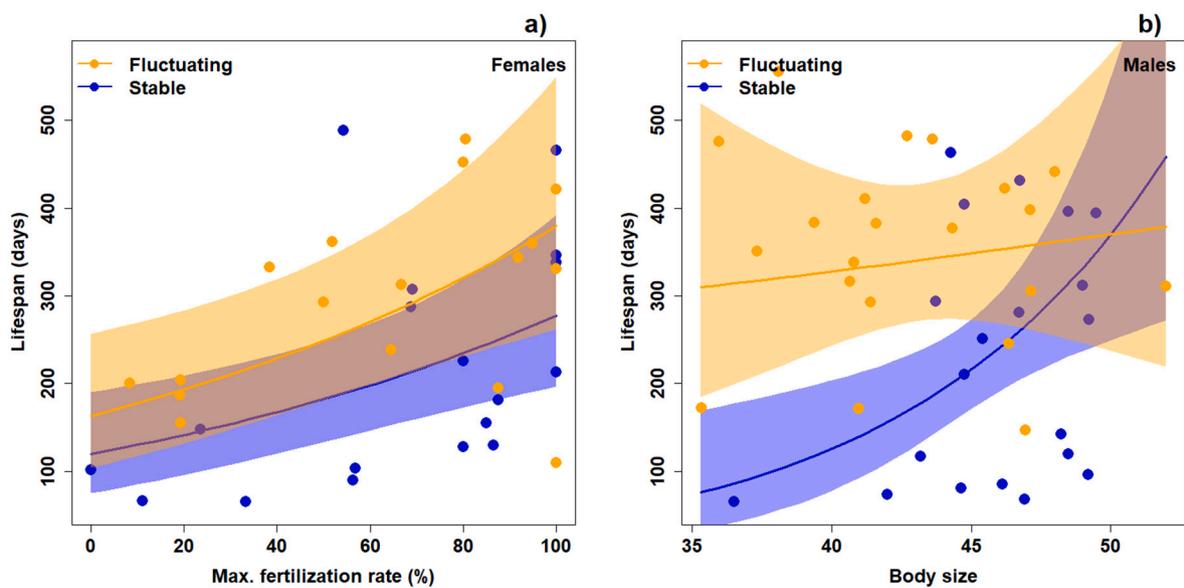


Fig. 3. Life history traits with significant sex-specific association with lifespan. (a) Positive association between fertilization rate and lifespan in females ($p = 0.002$, $N = 35$). (b) Treatment-specific association between body size and lifespan in males (stable: $p = 0.025$, $N = 21$; fluctuating: $p = 0.873$, $N = 21$). The curves were fitted by Gamma Generalized linear models. Shaded areas represent 95% confidence intervals. Filled circles denote raw data.

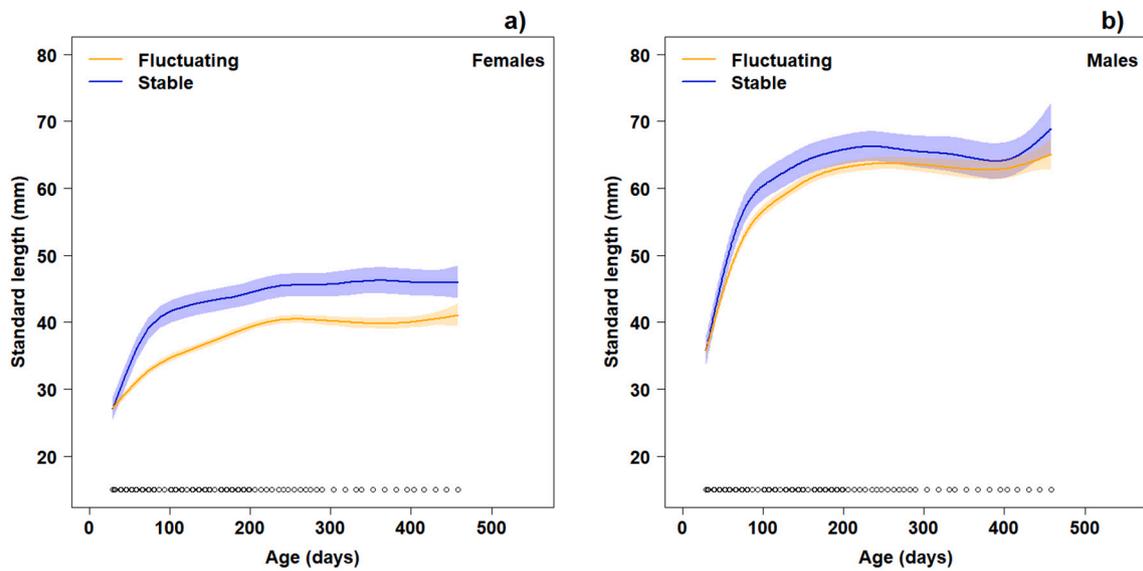


Fig. 4. Growth of (a) females ($p < 0.001$, $N = 40$) and (b) males ($p = 0.009$, $N = 44$) was higher in stable than in fluctuating temperature. The curves were fitted by Gaussian Generalized Additive Models with specified treatment-specific smoothing factor. The lines represent means and shaded areas are 95% confidence intervals. Empty circles at the bottom of plots represent time-points when body-size measurements were taken. Due to age-related dying (Fig. 2) sample size was decreasing with age.

$N_1 = 35$, $N_2 = 209$); the data demonstrate a decrease in fertilization rates in both treatments, followed by a short terminal increase caused by the longest surviving females (edf = 3.852, $F = 8.291$, $p < 0.001$, Fig. 5b). A treatment-specific smoother for age was not needed ($\Delta AIC = 9$).

4. Discussion

Previous studies have detected a detrimental effect of stable temperature on early development of ectothermic vertebrates (Ashmore and Janzen, 2003; Niehaus et al., 2012). Using the short-lived fish *Nothobranchius furzeri*, we demonstrated that stable temperature shortened lifespan and decreased relative fecundity, but increased growth

rate compared to individuals experiencing fluctuating temperatures.

Chronic exposure to stable temperature shortened the median lifespan of turquoise killifish despite the fact that fish in fluctuating temperatures spend approximately a quarter of the day in temperatures 5–6 °C above the upper range of their preferred body temperatures (i.e. 30 °C (Žák et al., 2018)). Jensen's inequality, validated on stable temperatures, postulates that organismal performance is disproportionately more sensitive to warm temperatures than to low temperatures (Martin and Huey, 2008). Thus, it is predicted that the upper limit would have a disproportionately greater (i.e. lifespan-shortening) effect. Nonetheless the effect of fluctuating temperature on life history traits appears to be contingent upon the range of experimental temperatures and their distance from optimal temperatures (Colinet et al., 2015; Kingsolver

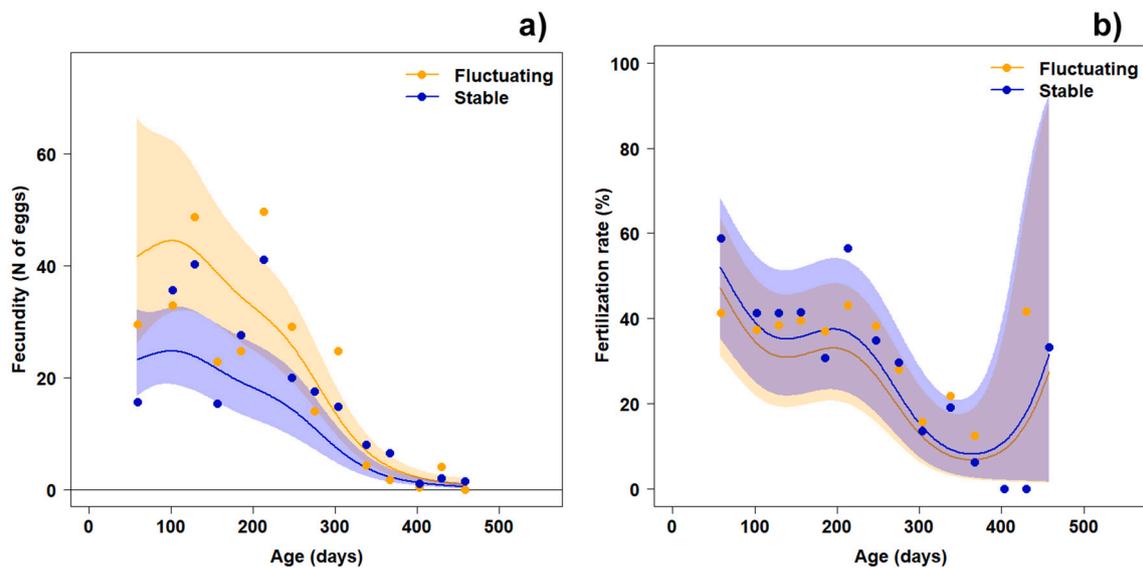


Fig. 5. Age-related patterns of (a) fecundity and (b) fertilization rate of females ($N = 35$) kept in stable (blue) and fluctuating (orange) temperature. Fecundity (corrected for body size) was analysed using negative-binomial Generalized additive model (GAM) and was higher in fluctuating temperature treatment ($p = 0.005$). The fertilization rate was fitted by binomial GAM and similar in both regimens ($p = 0.665$). Circles represent mean values computed from raw data. Note that empty circles do not necessarily follow the curve due to random factor effects and, for fecundity estimates, correction for body size. Due to age-related mortality (Fig. 2), sample size was decreasing with age. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

et al., 2008). We fluctuated the temperature between 20 °C and 34 °C, which reflects conditions commonly experienced by turquoise killifish in the wild (Reichard et al., 2009; Žák et al., 2018). The length of time the killifish experienced these temperatures was probably too short and/or temperatures too close to the optimal range to have a detrimental effect on lifespan. Close proximity to optimal temperatures is supported by the fact that the upper range of fluctuating temperatures was 7 °C below the lethal critical thermal maximum for this species (Philippe et al., 2018). Therefore, it is reasonable to suggest that turquoise killifish possess efficient physiological mechanisms to cope with temperatures 5–6 °C above the upper limit of their preferred body temperatures.

The observed increase in lifespan in fluctuating temperature may have been induced by a stress-protective response (Gems and Partridge, 2008; Judy et al., 2013; Sorensen et al., 2003). Thermal stress increases production of reactive oxygen species and induces up-regulation of heat-shock proteins (Hsps) (Banh et al., 2016; Podrabsky et al., 2008). It is assumed that metabolism and oxygen consumption during the warm phase of fluctuation is higher than at a similarly warm stable temperature (Beauregard et al., 2013; Lyttikainen and Jobling, 1998; Martin and Huey, 2008). This may induce mild stress in the form of dietary restriction which may contribute to lifespan extension (Schwartz et al., 2016). However, it is unlikely the case in our study, as dietary restriction commonly reduces the fecundity (Vrtelek and Reichard, 2015) while we observed relative increase in fecundity. Higher metabolic rate in fluctuating temperature contributes to detrimental oxidative stress and hence accelerates functional senescence (Banh et al., 2016; Kregel and Zhang, 2007). Hsps are a large group of proteins with various effects on lifespan and life-history traits when up-regulated (Lithgow, 1996; Sorensen et al., 2003). They are able to repair protein damage induced by both thermal and oxidative stresses and they are a candidate mechanism for why we observed longer lifespan in fluctuating temperature (Lithgow, 1996; Sorensen et al., 2003). The association of specific Hsp and lifespan in turquoise killifish is unknown, but previous work on the Neotropical annual killifish *Austrofundulus limnaeus* showed that fluctuating temperature up-regulated small Hsps (Hsp22, Hsp27) while stable temperature conditions led to the up-regulation of large Hsps (Hsp70, Hsp90) (Podrabsky and Somero, 2004). We believe that it is reasonable to assume that up-regulation of different Hsps may contribute to the observed difference in lifespan between thermal regimes. Further, the activation of other stress protective mechanisms such as progranulin pathway may also play a role (Judy et al., 2013).

Fish in fluctuating temperature experienced slower growth and reached smaller body size. The temperature-size rule postulates that, in fish, oxygen supply is more important for anabolism than food supply (Forster et al., 2012; Pauly, 1981) and oxygen availability for tissues is lower at higher temperatures. Hence, fish experiencing warmer temperatures reach smaller sizes, especially among tropical fish species (Audzijonyte et al., 2020). We believe that the main mechanism behind the smaller body size of fish in fluctuating temperature is the combination of periodic cold exposure and an increase in mandatory energy consumption higher than the maximum metabolic rate during warm periods, reducing the amount of available energy to growth (Gvoždík and Kristín, 2017; Kingsolver and Gomulkiewicz, 2003; Lyttikainen and Jobling, 1998). Alternatively, smaller asymptotic body size in fluctuating temperatures could be explained by the cost of repairing mechanisms (Sorensen et al., 2003) or by smaller cell size (Adrian et al., 2016). Irrespective of the mechanism, fluctuating temperature constrained the maximum body size of turquoise killifish.

Slower growth rate is often associated with longer lifespan (Lee et al., 2013). This relationship held for the gross contrast between treatments, with fish in fluctuating temperature reaching smaller body size and living longer. At the individual level, the same association was demonstrated for males in the stable thermal regime, as reported in previous studies (Baumgart et al., 2016; Kirschner et al., 2012).

However, the relationship did not hold for males in fluctuating temperature, indicating that extended male lifespan in fluctuating temperature is not causally linked to decreased growth rate or smaller body size. This illustrates that the assumptions derived under stable temperatures are of limited utility for predicting organismal performance under ecologically relevant temperature fluctuations (Colinet et al., 2015; Niehaus et al., 2012).

Females experiencing fluctuating temperature were more fecund, relative to their body size. Even when fecundity was not corrected for body size, their fecundity was similar to females at stable temperature despite their smaller body size. Increased thermal fluctuation may be a signal of water level decrease and thus induce higher investment to current reproduction, as reported for *N. wattersi* under experimental conditions (Grégoir et al., 2017). Surprisingly, Podrabsky et al. (2008) found that fluctuating temperature had a negative (rather than positive) effect on fecundity and fertilization rate in the Neotropical annual killifish *Austrofundulus limnaeus*, despite the fact that *A. limnaeus* in the wild experience fluctuating temperatures closely resembling the thermal dynamics in ephemeral pools inhabited by the turquoise killifish (Podrabsky et al., 1998; Žák et al., 2018). Inconsistent findings between our study and that of Podrabsky et al. (2008) may be due to the different range of temperatures used, the period for which fish were subjected to fluctuating temperature (which was only 2 months in Podrabsky et al., 2008), the effect of different food rations (fish fed twice per day in Podrabsky et al., 2008) or the different thermal requirements of the two species.

The thermal regime did not affect patterns of reproductive senescence in fecundity, fertilization rate or fertility despite the fact that females in fluctuating temperature tended to have longer lifespans. Across both treatments, females with higher fertilization rates lived longer and early fecundity was not negatively associated with individual lifespan. Overall, there was positive relationship between female lifespan and lifetime fecundity in both thermal regimes (despite slope of the relationship was thermal-regime specific) which indicates that short-lived females did not compensate their fecundity to shorter lifespan. This is in contrast to the trade-off assumptions of the disposable soma theory of senescence (Kirkwood, 1977) which assume that high investment in early reproduction has costly consequences expressed later in life. In that respect, our data support the predictions of positive pleiotropy where “good quality” individuals possess superior fitness traits (at least in a given environment), including reproductive outcome and survival (Maklakov et al., 2015).

Our findings demonstrate that the expression of life history traits and their associations observed under stable temperature are a poor representation of relationships arising in the fluctuating thermal conditions of natural habitats. The lifespan of *N. furzeri* males was significantly extended under fluctuating temperature and the same trend was present in females. Females in fluctuating temperature had higher fecundity, with no detrimental effects on reproductive senescence or survival, suggesting a lack of allocation trade-offs or negative pleiotropy. Overall, our results have important consequences for future laboratory studies on functional and actuarial senescence, as most laboratory studies are conducted in stable experimental temperature. Future studies should study the physiological and molecular mechanisms underlying lifespan and life history differences in *N. furzeri* exposed to different thermal regimes.

CRediT authorship contribution statement

Conceptualization: JZ, MR.
 Data curation: JZ, MR.
 Formal analysis: JZ.
 Funding acquisition: MR, JZ.
 Investigation: JZ.
 Methodology: JZ, MR.
 Project administration: MR, JZ.

Resources: MR.
 Supervision: MR.
 Validation: JZ, MR.
 Visualization: JZ.
 Writing - original draft: JZ, MR.
 Writing - review & editing: JZ, MR.

Acknowledgements

We thank Vašek Homolka, Jiří Farkač and all other members of Reichard-Lab who helped with maintenance of the killifish colony. We thank Rowena Spence for English correction and Lumír Gvoždík, Radim Blažek, Lisandrina Mari, Matej Poláčik and Milan Vrtílek for helpful comments on early version of the manuscript. All experimental procedures were in accordance with institutional and national laws.

Funding

This study was supported by Czech Science Foundation grant (19-01781S). The work of Jakub Žák from Department of Zoology, Faculty of Science at Charles University, Prague, was partially supported by SVV 260571/2020.

Data availability

Data and R code for the analyses are available at Figshare (doi: <https://doi.org/10.6084/m9.figshare.12546632>).

Declaration of competing interest

The authors declare no conflict of interests.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.exger.2020.111073>.

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