The University of South Bohemia in České Budějovice

Faculty of Science

Effects of stochastic thermal regime on life history of *Daphnia magna* (CRUSTACEA: CLADOCERA)

Master´s thesis

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Annotation:

Using two populations of *Daphnia magna*, the research elucidates the role of thermal average and fluctuations on different life history traits such as lifespan, reproductive output, body length and brood chamber volume.

Declaration:

I declare that I am the author of this qualification thesis and that in writing it I have used the resources and literature displayed in the list of used sources only.

České Budějovice, 12th April, 2024

Anand

ACKNOWLEDGEMENTS

Lastly, my eternal gratitude to my family for their love and support as I pursue my idiosyncratic passion for science.

"Last but not least, I wanna thank me. I wanna thank me for believing in me, for doing all this hard work, for never quitting. I wanna thank me for just being me at all times."

– Snoop Dogg

Towards next level!

Contents

Effects of stochastic thermal regimes on life history of *Daphnia magna* (CRUSTACEA: CLADOCERA)

1 INTRODUCTION

Aquatic ecosystems provide habitat to numerous organisms such as cellular microorganisms, plankton, fishes and also aquatic plants (McLusky *et al.,* 2004). Zooplankton such as cladocerans play an important role in shaping autotrophic and heterotrophic food web interactions. They act as primary consumers feeding on phytoplankton, bacteria and transfer energy to higher tropical consumers such as aquatic fish and insects (Ebert, 2022). But, these aquatic ecosystems face the biggest challenges of the century i.e. climate change, pollution and also their interaction resulting in changes in metabolic functioning, reproductive performance and species distributions (Dinh *et al.,* 2022). Global warming is rapidly changing Earth's climate through environmental variations at global, regional, and local scales and is widely recognised as one of the greatest threats to biodiversity and ecosystems (Bellard *et al.,* 2012; IPCC, 2023).

Continued warming will intensify climatic events (heatwaves, droughts) through strong fluctuating changes with climate change risks becoming increasingly complex (Meehl & Tebaldi, 2004; Tilman *et al.,* 2017). For example, lake heatwaves are projected to become more frequent with increase in lake surface water temperature (Woolway *et al.,* 2021a; Woolway *et al.,* 2021b). Woolway et al. (2021b) modelled a likely scenario of increase in average lake surface temperature from 3.7 ± 0.1 °C to 5.4 ± 0.8 °C under RCP 8.5 high-greenhouse gas emission event. Scientists are developing robust models to predict how climate change will exacerbate ecological catastrophic events. Till *et al.* (2019)'s climate models suggest a clear connection between the environmental conditions specific to a particular area during the hottest part of the year and the incidence of severe ecological events such as fish die-offs. Moreover, Tye *et al.* (2022) used combination of water and air temperature in their climatic models and showed their models were strong predictors of fish mortality events.

Temperature significantly influences individual performance in ectothermic organisms, as it determines their body temperature (Guderley, 2004; Seebacher & Murray, 2007) Variations in ambient temperature affect metabolic rate (Schulte, 2015), reproductive fitness (Angilletta *et al.,* 2004), and survival (Huey & Kingsolver, 1993). Changes at individual level processes leads to characteristic changes in species interactions that can propagate to higher levels of organisations, i.e. community and population level (Ohlberger *et al.,* 2013; Boukal *et al.,* 2019). In face of rapid environmental change, how does species respond in their survival and

reproductive strategy and to what larger extent does it affect at population level is a widely asked question (Hermisson & Pennings, 2005; Adamczuk, 2020).

Temperature-size rule (TSR), a phenotypic plastic response, is a biological pattern to explain temperature-dependent size variation where organisms tend to grow faster under warmer conditions but at the cost of small body size in comparison with conspecifics reared under colder conditions (Atkinson, 1994). When studying mechanism for TSR, Einum *et al.,* (2021) had inconsistent results in their theoretical model and empirical data failing to explain oxygen limitation as a driver for TS-rule in ectotherms (supporting previous studies: Lefevre *et al.,* 2017; Lefevre *et al.,* 2018) where *Daphnia* might not reach their absolute maximum size due to oxygen limitation required for metabolic activity and reproduction. And, *Daphnia* growing in colder environments are smaller compared to what their model predicts, while those in warmer environments might be closer to their predicted size. This is because the individuals adapt to fluctuating temperatures over time. Hence, Einum *et al.,* (2021) emphasized the use of fluctuating temperature regimes when studying the role of oxygen.

The upper and lower limits of thermal tolerance for normal functioning is also defined by the environment (i.e. temperature) in which the organsims live in (Pörtner *et al.,* 2000). The thermal tolerance window of *Daphnia magna* is directly influenced by temperature acclimation (i.e. organism adjusts to changes in temperature over time) and has lower anaerobic metabolism near to this acclimation temperature (Müller *et al.,* 2018). Müller *et al.,* 2018 also observed that abrupt temperature shifts had an immediate adverse effect on the filtration capacity. For painted turtle *Chrysemys picta* and red-ear slider *Trachemys scripta,* Les *et al.* (2009) reported that temperature fluctuations entering the optimal development range (ODR) are more favourable for successful embryonic development compared to constant conditions near the lower thermal limit.

However, these studies have tested the effects of increase in constant temperature, ignoring the fact that nearly all ectotherms live in fluctuating thermal environments (Payne & Smith, 2017) with diurnal (24h) and seasonal fluctuations. Thermal tolerance is also known to increase during fluctuating temperature regimes (Chen & Stillman, 2012; Manenti *et al.,* 2014). For example, tadpoles of the Limnodynastinae family experiencing daily thermal fluctuations had the capacity to increase their upper thermal tolerance limits (but showed reduced growth and development) (Kern *et al.,* 2015). Furthermore, most of the studies that examined the

effects of mean temperature with stochastic fluctuations are mostly with fishes and fewer especially for zooplankton (Pisano *et al.,* 2019; Steel *et al.,* 2012).

How a species responds to temperature fluctuations in temperature can be helpful for understanding species capacity to acclimate and adapt to changing environmental conditions. Thermal performance curves (TPCs) is a nonlinear unimodal describing species performance (i.e. metabolic rate, growth rate or heart rate) as a function of body temperature (T_b). The performance is zero below the minimum critical temperature (CT_{min}) and reaches a maximum value at intermediate optimum temperature (CT_{opt}). Above this, the performance drastically decreases towards the upper thermal limit (CT_{max}) (Angilletta, 2006). However, as the temperature fluctuates, so does the performance of an individual (Paajimans *et al.,* 2013; Kern *et al.,* 2015). According to the principle of Jensen's inequality, also known as the fallacy of the average, thermal performance at a constant environment would be unequal to the performance under fluctuating environmental condition with the same mean temperature (Ruel & Ayres, 1999). For instance, sepsis flies showed higher development rate by 4.3% and 12.9% under fluctuating regimes of 18 ± 3 °C and 18 ± 7 °C, respectively, than under constant 18 °C (Khelifa *et al.,* 2019). Predictions based on constant temperatures significantly differ from those incorporating fluctuations (Bozinovic *et al.,* 2011). For striped marsh frogs (*Limnodynastes peronii*), frogs developing at fluctuating temperatures were smaller and shorter than frogs at constant temperatures (Bozinovic *et al.,* 2011). Furthermore, models either over- or underpredicted embryonic growth rate and development depending on daily temperature fluctuations when compared to constant temperatures (Niehaus *et al.,* 2012).

Recent climate change models also forecast a rise in temperature variability and stochasticity, leading to a higher likelihood of extreme warm temperatures and heatwave occurrences (Angélil et al., 2017; Guo et al., 2018; Stillman, 2019; IPCC, 2023). For example, Nancollas & Todgham (2022) integrated submerged conditions or low tide cycle with different levels of thermal complexity (magnitude, stochasticity) using experimental tanks to see how it shapes the biochemical and physiological responses for mussel *Mytilus californianus*. Mussels from the stochastic thermal regime exhibited the highest glycogen content, yet there was no variation in the expression of heat shock proteins across different thermal regimes. This implies that mussels allocate energy reserves to cope with stochastic low tide conditions. The mussels subjected to fluctuating thermal regimes exhibited reduced gill anaerobic metabolism, indicating enhanced metabolic capacity (Nancollas & Todgham, 2022). Burton *et al.,* (2018)

incorporated the effect of mean, variability and predictability of temperature and found that *Daphnia magna* adjusted their physiological response, i.e. heat tolerance, based on changes in constant temperature only. This is supported by their subsequent work (Burton *et al.,* 2020) on upper thermal limits where T_{imm} (the temperature at which individual becomes immobilised) had a positive correlation with increasing acclimation temperature. Researchers have thus focused on incorporating both mean as well as levels of temporal fluctuations in temperature regimes.

Propagation of effects (continuation of influences or consequences from one generation to the next generation) can also be seen where selection acting on individual's parents, grandparents can influence the fitness of subsequent generations. Numerous studies have been done to understand the impacts of parental conditioning of individuals using multi-generational experiments: do the advantages obtained through parental conditioning endure across successive generations? And if they do, what are the mechanisms accountable for sustaining these alterations? (Rodríguez-Romero *et al.,* 2016; Shama *et al.,* 2016; Munday *et al.,* 2017). For example, decreased reproductive body size and fecundity was found when marine worms *Ophryotrocha labronica* were subjected to multi-generational exposure to warming (30 °C) (Gibbin *et al.,* 2017). They also found increased juvenile development rates across the generations. Their findings do not align with those of Chakravarti et al. (2016), where no changes in development rate were observed for *Ophryotrocha labronica* within-generational (exposure of offspring from control-reared parents to treatment conditions; e.g. control to warming) or transgenerational exposure (exposure of offspring to same treatment conditions; e.g.warming to warming) to warming (30 °C). However, Gibbin *et al.,* (2017) point out that multi-generational exposure for six generations was too short to observe the genetic adaptation in marine worms *Ophryotrocha labronica*.

While multi-generational experiments shed light on the enduring impacts of parental conditioning, theoretical models offer additional frameworks for understanding how temperature fluctuations impacts fitness traits. A theoretical model proposed by Marshall *et al.,* (2008) describes optimal offspring size relationship with environments: for stochastic environment, having narrow range of viable offspring sizes and varying offspring sizes within brood is favored while in stable environment, offspring with wide range of viable sizes and consistent offspring size within broods is selected. Smith & Fretwell's model (1974) states that the maternal fitness decreases with higher energy investment to produce larger and higher

performing individual offspring. Variation in neonate body length according to the environmental cues is a favourable and viable strategy via diversified bet-hedging strategy than having a constant body length. This ensures that mothers produce different offspring phenotypes thereby increasing the likelihood chance of survival of few, if not all, although maternal effects are suggested to have nonadaptive significance with no correlation between the maternal-offspring fitness (Fox & Czesak, 2000; Kielland *et al.,* 2017).

To date, based on the existing literature, there have been no studies explicitly investigating the multigenerational impacts of stable vs. stochastic thermal regimes on *Daphnia magna*. The objective of this study was to see how multi-generational impacts of the thermal regimes would influence the life-history traits of *Daphnia magna.* In this study, several life history traits of *Daphnia magna* (lifespan, total reproductive output, body length at death and of neonates, brood chamber size) were measured, in response to contrasting environmental regimes (stable vs. stochastic thermal regimes). The experiment comprised *D. magna*, originating from different clones, and studied for three generations. The study aimed to answer the following questions: (1) How do stable and stochastic thermal regimes affect *Daphnia* life histories? (2) Do lineages respond differently to these thermal regimes? I hypothesised that: 1) there would be differences in performance in life-history traits between the two thermal regimes. Specifically, organisms under the stochastic regime would be more sensitive to random fluctuations, thereby having a greater impact on their fitness performance; 2) the lineages used in this experiment would exhibit differential performance responses to the thermal regimes in terms of their life-history strategies.

2 MATERIALS and METHODS

2.1 Study organisms and culture

Daphnia magna (hereinafter referred to as *Daphnia*) individuals were collected in a fishpond in Stropnice, Czech Republic (48.7419925N, 14.7588725E). Sixty gravid females were placed individually in a 250 mL glass beaker containing 100 mL of aerated Aachener Daphnien Medium (ADaM; Klüttgen *et al.* 1994). From the 60 individuals, only 15 cultures could be fully established before the beginning of the experiment, as the rest either died early or did not reproduce well. The cultures were maintained in thermostatic-controlled cabinets (Lovibond TC 445S, Tintometer Inc.) under standardised laboratory conditions according to the EPA and OECD guidelines (US-EPA, 2002; OECD, 2012). The third-clutch neonates, produced by parthenogenesis, were used for establishing subsequent cultures.

The photoperiod in the chambers followed 14h light: 10h dark cycle and the cultures were kept at constant 22 °C. Medium was changed thrice a week and the stock cultures was fed daily with 1 mL/individual of *Chlorella* food powder medium. *Chlorella* powdered medium was prepared by diluting 1 g of dry *Chlorella* powder (Alnatura food-grade Bio *Chlorella*, Czech Republic) in 1 L of ADaM culture medium according to Magester *et al.* (2021) and mixed at 350 rpm for 10 min. Sedimentation of the particles was allowed for one day. A second sedimentation was done by keeping the supernatant in refrigerator for three hour and the final supernatant was used as the food.

2.2 Experimental setup

The effects of thermal regimes on three generations of *Daphnia* (named hereafter F1, F2 and F3) were studied. Two lineages (hereinafter named M11 and M16) were selected among the 15 cultures based on their overall optimal performance in terms of reproductive output (data not shown). The third clutch neonates of the fourth generation from the establishment of the cultures was used for the experiment. Thirty offspring (<24 hours old) were randomly selected from each of the two lineages. Each founder (F1) was transferred into a 250 mL glass beaker filled with 100 mL of ADaM culture medium and place in a climatic test chamber (Cooled incubator ST 500, Pol-Eko Aparatura, Wodzislaw Poland). Each individual followed either (1) a stable thermal regime (daily mean (21 °C), with daily temperature range between 19 – 23 °C) or (2) a stochastic thermal regime (the daily mean temperature differed between the consecutive days but the overall mean temperature per cycle was 21 °C, the daily temperature

amplitude was same for each day (4 °C), the temperature change from mean temperature 21 °C was not larger than 4.2 °C and not smaller than -4 °C; see Fig. 1 for the experimental design and Fig. 2 for the temperature regimes). A single full cycle of the stochastic thermal regime lasted for two weeks and was repeated until the end of the experiment. The programmed temperature profiles for the two temperature regimes are provided in supplementary Table S1. During the experiment, the medium was changed thrice a week and individuals were fed daily with *Chlorella* food (1 mL/individual).

For each generation, the experimental design was 2 thermal regimes × 2 lineage × 15 neonates = 60 individuals (for three generation = 180 individuals in total). Third-clutch neonates of the surviving individuals of F1 and F2 generations were pooled for each lineage for each thermal regime. Neonates were then picked randomly to start the following generation.

Mortality, reproduction and number of offspring per clutches were recorded during the experiment. The experiment ended when all the individuals had died or were older than 74 days. Dead individuals and neonates (when not used in the experiment) were preserved in Ethanol 75% conc. + 4% Glycerol for body length measurement. To measure body length, each individual was photographed with a Lumenera ® INFINITY camera mounted on Olympus SZX10 stereomicroscope with constant 1.25× zoom magnification and 1.25× objective. Body length (in μm) was measured from the top of the eye to the base of the tail spine (QuickPHOTO software; Fig. 3). Only neonate body lengths from 1st, 3rd and last clutches were measured and analysed in this study.

The temperature was recorded in four media filled plastic containers, placed on the upper and on the bottom shelves in each chamber, throughout the experiment (HOBO 4-channel analog logger UX120-006M, HOBOware software, version 3.7.26; supplementary Fig. S3). It was observed that the daily temperature range for stable thermal regime fell outside the programmed temperature profile. Therefore, temperature correction was done on day 40 by +0.5 °C and on day 41 by +0.3 °C for the stable thermal regime.
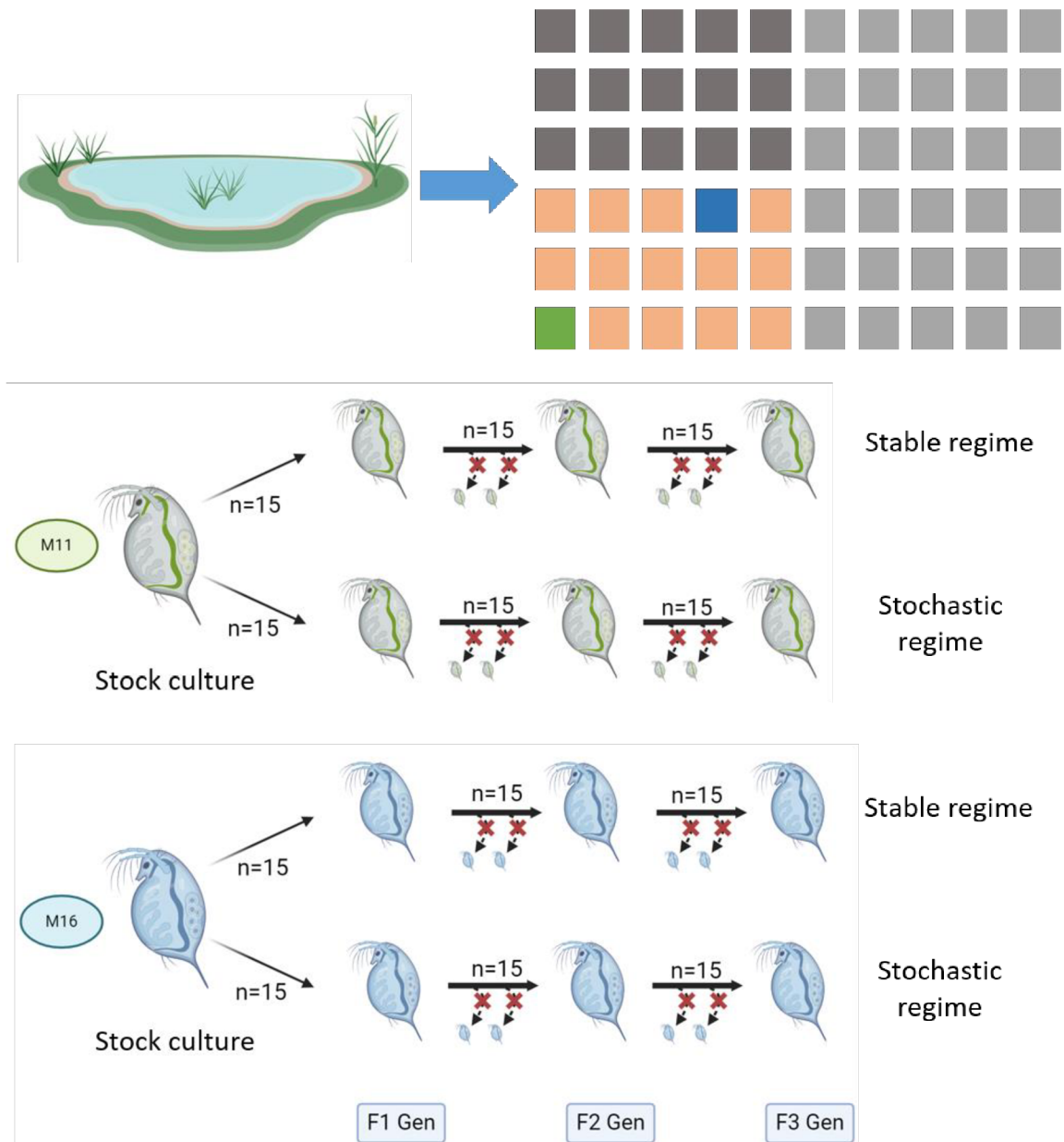
Fig. 1 Schematic representation of the experimental design. (A) Collection of *Daphnia magna* from the fish pond; (B) 60 gravid females were collected to establish the cultures in the lab and represent the initial pool of cultures (60 boxes). Out of these, 15 cultures were established successfully (orange boxes). Two lineages (M11 and M16) were selected to start the experiment (green and blue boxes); (C) Experimental design where *Daphnia* was reared in two different thermal regimes across three generations. Fifteen neonates from the third clutch were used for each treatment combination. F1 = founders, F2 =daughters, F3 = granddaughters. Red crosses represent 1st and 2nd clutch neonates which were preserved and 3rd clutch neonates was used for the next generation. Created with BioRender.
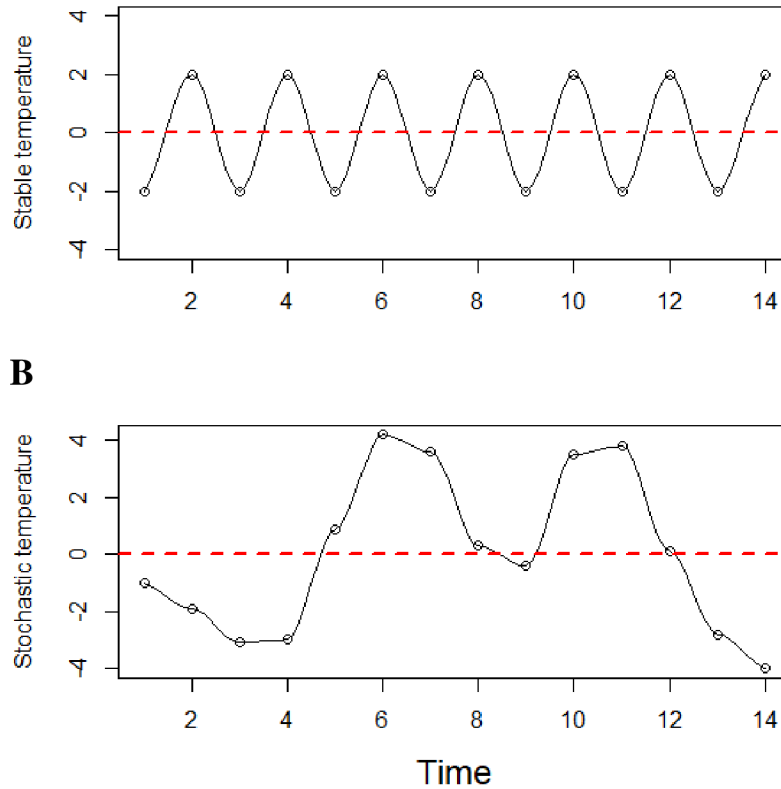
Fig. 2 Stable thermal regime (A) and stochastic thermal regime (B). Over a complete single cycle, both environments are at overall same mean temperature (= 21 °C) represented by red dashed line. Adapted from Burton *et al.* (2020).

Fig. 3 Measurement of *Daphnia magna* body specifications from top of eye to end of the body (excluding apical spines). Reference photo: Dieter Ebert, Wikipedia.

2.3 Statistical Analyses

Generalised linear models (GLMs) and generalised linear mixed models (GLMMs) were used to assess the effects of the two thermal regimes on *Daphnia* life history traits. The model predictors included a categorical variable representing the two thermal regimes (*stable* or *stochastic*), a categorical variable representing the two lineages (*M11* or *M16*), a categorical variable representing the three generations (*F1, F2* and *F3*) and their interaction terms (either pairwise or three-way interaction). Lifespan (in days), body length at death (μm), total reproductive output (expressed as the total number of neonates produced by one *Daphnia*), neonate body length (μm) at 1st, 3rd and last clutch represented the response variables. Fixed effects included neonate clutch number (1st clutch and 3rd clutch) and last clutch reproductive size (number of neonates produced by one *Daphnia* in the final clutch). Preliminary inspection of the data showed that body length at death (μm) increased with lifespan (ln-transformed) as additional fixed effect and thus this predictor was included in the model. Preliminary inspection of the data showed that total reproductive output increased and decreased, for body length at death (μm) and neonate body length (μm), respectively. And, these fixed effects were only included as main effects in the model.

I used a Gaussian GLM with log link function for the lifespan of *Daphnia*, a Gaussian GLM model with log link function for the body length at death, a GLM with Genpois error distribution and log link function for total reproductive output, and a GLM with Gaussian error distribution and log link function for the first-clutch and third-clutch neonate body length. Finally, to test the offspring size-number trade-off, i.e. whether the number of neonates produced in a brood chamber was restricted by neonate body length and whether this relationship differed between the temperature regimes, lineages or generations, a GLM model with Gaussian error distribution and log link function was used. The model included last clutch-neonate body length as the response variable and inverse of last clutch reproductive size (i.e. the number of neonates produced in the last clutch) as fixed effects. For this model, data were subset using only individuals with mortality within 0-4 days after their last reproduction as criteria. Since *Daphnia* grow continuously during their lifetime, it would also mean that the relationship between the number of neonates vs. size of the neonates would continuously change. By focusing on the last clutch, it is more accurate to assess how resources are allocated towards reproduction towards the end of the reproductive cycle. This can provide insights into trade-offs between reproduction and offspring size.

GLM's were ran using the 'glmmTMB' function from the *glmmTMB* package (Brooks *et al.,* 2017). Model residuals were assessed with the *DHARMa* package (Hartig, 2020) to detect overdispersion and residuals. The models were ranked using Akaike information criterion (Akaike, 1974, 1998; AIC), and the most parsimonious model was identified for each response variable. In instances when the parsimonious model exhibits overall deviations in the qq-plot and residual patterns in the residuals plot, models with ΔAICc < 2 (considered to be strongly supported by the data (Burnham & Anderson, 2002)) were checked for overdispersion/residuals and those models without any statistical deviations was then chosen as the best-fit model. The parameter values for the model were predicted with *ggeffects* package (Lüdecke, 2018) along with mean and 95% confidence interval. Model results were visualised using the *ggplot2* package (Wickham, 2016).

All statistical analyses were performed in R 4.3.2 (R Development Core Team, 2023).

3 RESULTS

3.1 Water temperature

For the entire experimental duration, the daily mean temperature of the stable regime was 20.25 ± 0.91 °C and for the stochastic regime, the daily mean temperature was 19.91 °C with a minimum of 16.66 °C and a maximum of 24.07 °C. Thus, the actual temperature profiles slightly varied from our target (21.0 °C). The measured temperature profiles for the regimes for the entire experimental duration is provided in supplementary Fig. S2, S3 and Table S1.

3.2 Life-history traits summary

Total reproductive output (total number of neonates produced by one *Daphnia*) was highest for the F1 *Daphnia* from the M11 lineage in the stable regime (80.53 ± 4.86; Table S2) and lowest for the F3 *Daphnia* from the M11 lineage in the stochastic regime (10.50 ± 1.83; Table S2). The lifespan was higher in the M16 lineage in the stable regime for the *Daphnia* from F1 (40 ± 5.53; Table S2) and lowest in the M11 lineage in the stochastic regime for the *Daphnia* from F3 (16.93 ± 3.02; Table S2). Neonate body length (μm) from the first clutch differed, with the M16 lineage exhibiting the highest value at F2 in the stable regime (801.08 ± 33.95; Table S2) and the M11 lineage the lowest body size at F3 in the stochastic regime (698.25 ± 19.28; Table S2). Similarly, neonate body length (μm) in the third clutch varied, with the M16 lineage showing higher value at F2 in the stable regime (849.46 ± 42.02; Table S2) and the M11 lineage the lowest body size at F3 in the stable regime (747.58 ± 19.26; Table S2). Lastly, body length at death (μm) exhibited notable differences, with the M16 lineage displaying the highest value at F1 in the stable regime (3103.33 ± 95.23; Table S2) and the M16 lineage the lowest body size at F3 in stochastic regime (1814.57 ± 286.70; Table S2).

3.3 Lifespan

The thermal regime and the generations significantly decreased the lifespan of *Daphnia* individuals (Table S3). There was an overall decrease in the lifespan of the *Daphnia* individuals across the three generations in both thermal regimes. In the stochastic regime, the lifespan of the individuals decreased significantly across the three generations, while in stable thermal regime the lifespan of the individuals decreased for F1 and F2 but showed a slight increase for F3 (Fig 4; Table 1). Furthermore, the interactive effect between stochastic regime and generations is seen on the lifespan (Fig 4; Table 1).

Table 1 Parameters of the most parsimonious Gaussian GLM model of the *Daphnia*. Trt = thermal regime; Lin = lineage; Gen = generation. Intercept includes thermal regime (stable), lineage (M11) and generation (F1) as baseline levels.

Model parameter	Estimate (and 95 % confidence intervals)	t-value	p
Intercept	3.623 (3.502 to 3.734)	61.370	<0.001
Trt$_{(stochastic)}$	-0.067 (-0.214 to 0.077)	-0.914	0.361
Gen$_{(F2)}$	-0.653 (-0.885 to -0.449)	-5.940	<0.001
Gen$_{(F3)}$	-0.500 (-0.703 to -0.3151)	-5.113	<0.001
Lin$_{(M16)}$	0.065 (0.085 to 0.177)	1.153	0.250
Gen$_{(F2)} \times$ Trt$_{(stochastic)}$	0.360 (0.085 to 0.648)	2.529	<0.05
Gen$_{(F3)} \times$ Trt$_{(stochastic)}$	-0.193 (-0.510 to 0.108)	-1.240	0.217
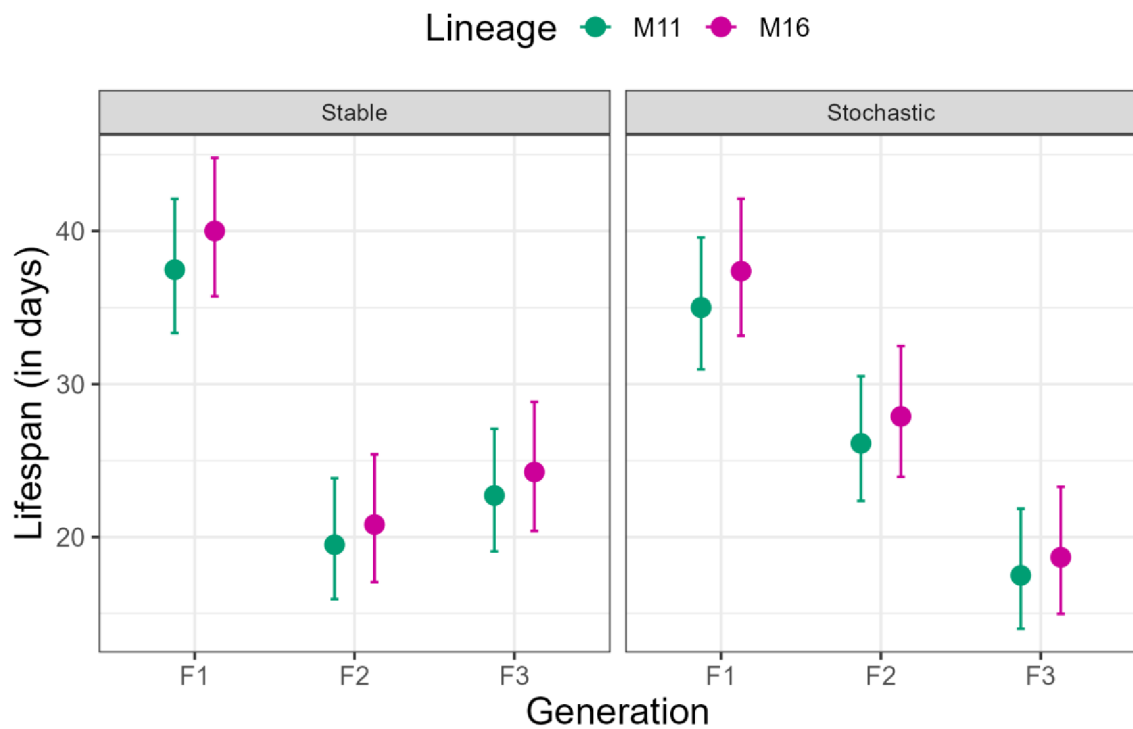
Fig. 4 Estimated mean lifespan of *Daphnia* individuals across the generations, lineages and thermal regimes. Model estimates are based on the most parsimonious model and include 95 % confidence intervals. *ggeffects* package predicts the quantity and values of data points (in this case, y-axis values).

3.4 Body length at death

The thermal regime and generations significantly decreased the body length at death with an interactive effect (Fig. 5, Table 2, Table S4). Additionally, the lifespan had a significant and additive effect on the body size at death (Fig. 5, Table 2, Table S4). Indeed, across the three generations, the body size at death decreased and was the lowest at F3. Furthermore, the stochastic regime had a significant and negative effect on the body size (Fig. 5).

Table 2 Summary of Gaussian GLM to model body at death as the response. Trt = thermal regime; Gen = generation; log (age) = log-transformed lifespan. Intercept includes thermal regime (stable) and generation (F1) as baseline levels.

Model parameter	Estimate (and 95 % confidence intervals)	t-value	p
Intercept	7.363 (7.166 to 7.560)	73.22	<0.001
Trt$_{(stochastic)}$	-0.008 (-0.064 to 0.048)	-0.28	0.776
Gen$_{(F2)}$	-0.034 (-0.104 to 0.035)	-0.96	0.337
Gen$_{(F3)}$	-0.159 (-0.238 to -0.081)	-3.99	<0.001
Log (age)	0.190 (0.136 to 0.244)	6.91	<0.001
Gen$_{(F2)}$ × Trt$_{(stochastic)}$	-0.061 (-0.151 to 0.028)	-1.35	0.178
Gen$_{(F3)}$ × Trt$_{(stochastic)}$	-0.078 (-0.199 to 0.041)	-1.29	0.198
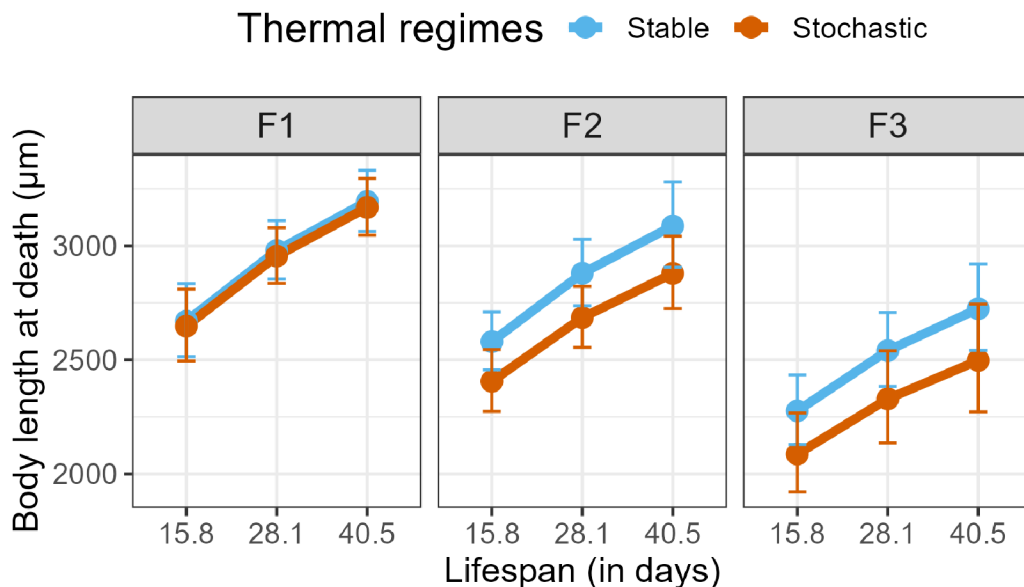
Fig. 5 Estimated body length at death across the generations, lineages and thermal regimes. Model estimates are based on the most parsimonious model and include 95 % confidence intervals. *ggeffects* package predicts the quantity and values of data points (in this case, x- and y-axis values).

3.5 Reproductive output

There was no effect of the thermal regimes on the total reproductive output (Table S5). However, there was an interactive effect of the generations and the lineages (Fig. 6, Table 3). Indeed, the total reproductive output decreased across the three generations and was lower for M11 in comparison to M16, especially at F3 (Fig. 6, Table 3).

Table 3 Summary of Genpois GLM to model reproductive output as the response with neonate body length as fixed effect. Lin = lineage; Gen = generation; NL = neonate body length (both 1st and 3rd clutch). Intercept includes lineage (M11) and generation (F1) as baseline levels.

Model parameter	Estimate (and 95 % confidence intervals)	z-value	p
Intercept	4.530 (4.044 to 5.016)	18.274	< 0.001
Lin$_{(M16)}$	-0.0004 (-0.122 to 0.122)	-0.07	0.994
Gen$_{(F2)}$	-0.878 (-1.037 to -0.719)	-10.857	< 0.001
Gen$_{(F3)}$	-1.651 (-1.907 to -1.395)	-12.618	< 0.001
NL$_{(neonate\ body\ length)}$	-0.0002 (-0.0008 to 0.0003)	-0.723	0.470
Gen$_{(F2)}$ × Lin$_{(M16)}$	0.052 (-0.188 to 0.292)	0.426	0.670
Gen$_{(F3)}$ × Lin$_{(M16)}$	0.751 (0.408 to 1.096)	4.286	< 0.001
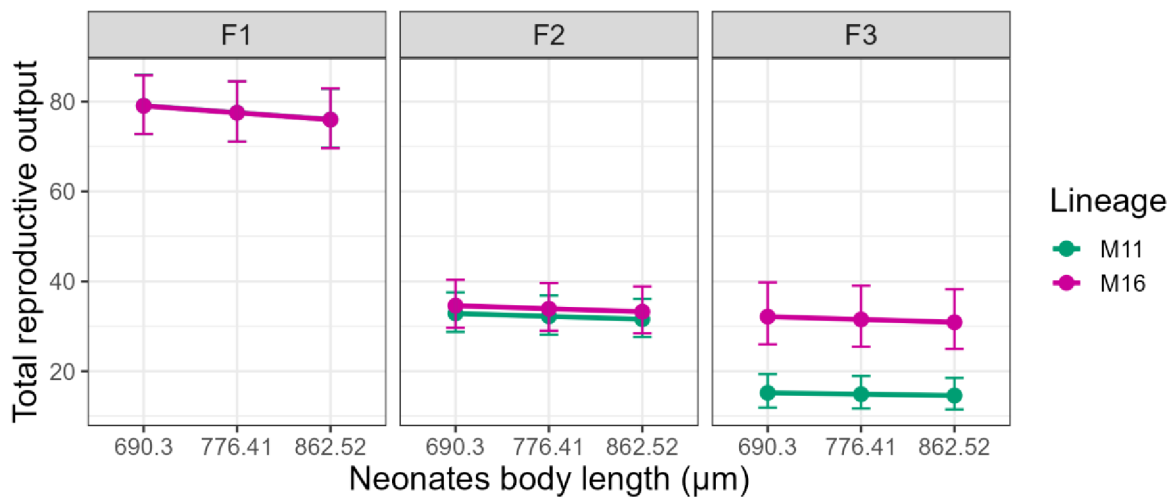
Fig. 6 Estimated total reproductive output across generations for the two lineages. Model estimates are based on the most parsimonious model and include 95 % confidence intervals. In F1 generation, lineage M16 overlaps lineage M11. *ggeffects* package predicts the quantity and values of data points (in this case, x- and y-axis values).

However, when tested with the body length at death as a fixed effect, there was a strong interactive effect of the thermal regimes, the generations and the lineages on the reproductive output (Table S5). Indeed, the total reproduction significantly decreased across the generations (Fig. 7, Table 4). While it was lower in M16 in comparison to M11 in the stable regime, the opposite was observed in the stochastic regime (Fig. 7). Finally, while the total reproduction was higher in the stable regime than in the stochastic regime at F1, results were contrasted at F2 and F3 (Fig. 7).

Table 4 Summary of Genpois GLM to model reproductive output as the response with body length at death as fixed effect. Lin = lineage; Gen = generation; ML = body length at death. Intercept includes thermal regime (stable), lineage (M11) and generation (F1) as baseline levels.

Model parameter	Estimate (and 95 % confidence intervals)	z-value	p
Intercept	-0.562 (-1.560 to 0.436)	-1.104	0.270
$Trt_{(stochastic)}$	-0.170 (-0.446 to 0.105)	-1.210	0.226
$Lin_{(M16)}$	-0.194 (-0.476 to 0.088)	-1.348	0.178
$Gen_{(F2)}$	-0.618 (-1.560 to 0.436)	-3.073	< 0.01
$Gen_{(F3)}$	-1.249 (-1.012 to -0.224)	-2.509	< 0.05
$ML_{(body\ length\ at\ death)}$	0.0016 (0.0012 to 0.0018)	10.327	< 0.001
$Gen_{(F2)} \times Lin_{(M16)}$	-0.872 (-1.627 to -0.118)	-2.268	< 0.05
$Gen_{(F3)} \times Lin_{(M16)}$	-1.091 (-2.325 to 0.143)	-1.733	0.083
$Gen_{(F2)} \times Trt_{(stochastic)}$	0.269 (-0.272 to 0.811)	0.976	0.329
$Gen_{(F3)} \times Trt_{(stochastic)}$	-0.024 (-1.283 to 1.233)	-0.038	0.970
$Lin_{(M16)} \times Trt_{(stochastic)}$	0.317 (-0.073 to 0.707)	1.593	0.111
$Gen_{(F2)} \times Lin_{(M16)} \times Trt_{(stochastic)}$	0.955 (0.037 to 1.873)	2.040	< 0.05
$Gen_{(F3)} \times Lin_{(M16)} \times Trt_{(stochastic)}$	1.718 (-0.013 to 3.450)	1.945	0.052
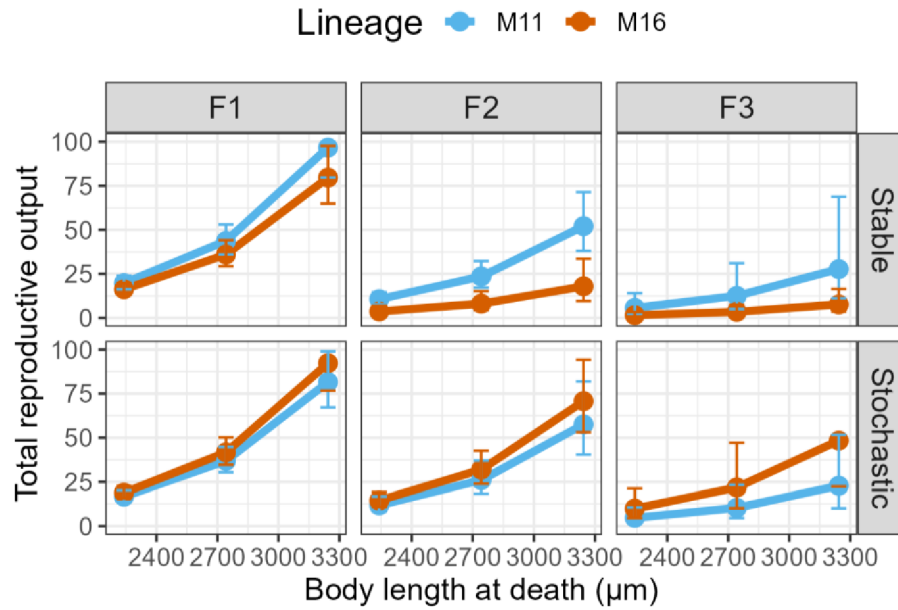
Fig. 7 Total reproductive output in relation with body length at death across the three generations, for the two lineages. Model estimates are based on the most parsimonious model and include 95 % confidence intervals. *ggeffects* package predicts the quantity and values of data points (in this case, x- and y-axis values).

3.6 Neonate body length

The first clutch neonate body length differed significantly between the thermal regimes (Table S6) while only the body length of the mother at death had a significant effect on the third-clutch neonate body length (Table S6). Indeed, the stochastic regime had a negative effect on the first-clutch neonate body length (Table 5).

Table 5 Summary of Gaussian GLM to model neonate body length at 1st and 3rd clutch as the response. Trt = thermal regime; ML = body length at death. Intercept includes thermal regime (stable) as baseline level for 1st clutch response variable.

Model parameter	Estimate (and 95 % confidence intervals)	t-value	*p*
1st clutch:			
Intercept	6.59e+00 (6.45e+00 to 6.73e+00)	95.43	< 0.001
Trt$_{(stochastic)}$	-4.61e-02 (-8.19e-02 to -1.04e-02)	-2.53	< 0.05
ML$_{(body\ length\ at\ death)}$	1.02e-05 (-3.66e-05 to 5.70e-05)	0.43	0.670
3rd clutch:			
Intercept	6.80 (6.60 to 7.00e+00)	66.40	< 0.001

ML(body length at death)	-0.00003(-0.0001 to-3.35e-05)	-0.98	0.326

The neonate body length differed significantly between the first and third clutch reproduction (Table S7) and the lineages and generations significantly increased the neonate body length in the third clutch when compared to first clutch (Fig. 8, Table 6).

Table 6 Summary of Gamma GLM to model neonate body length of both 1st and 3rd clutch as the response. Lin = lineage; Gen = generation. Intercept includes thermal regime (stable), lineage (M11), generation (F1) and clutch number (1st clutch) as baseline levels.

Model parameter	Estimate (and 95 % confidence intervals)	z-value	*p*
Intercept	6.573 (6.546 to 6.600)	474.4	< 0.001
Lin(M16)	0.023 (-0.003 to 0.050)	1.8	0.079
Gen(F2)	0.045 (0.016 to 0.075)	3.0	< 0.01
Gen(F3)	0.010 (-0.025 to 0.045)	0.5	0.586
Clutch number(3rd clutch)	0.102 (0.076 to 0.128)	7.7	< 0.001
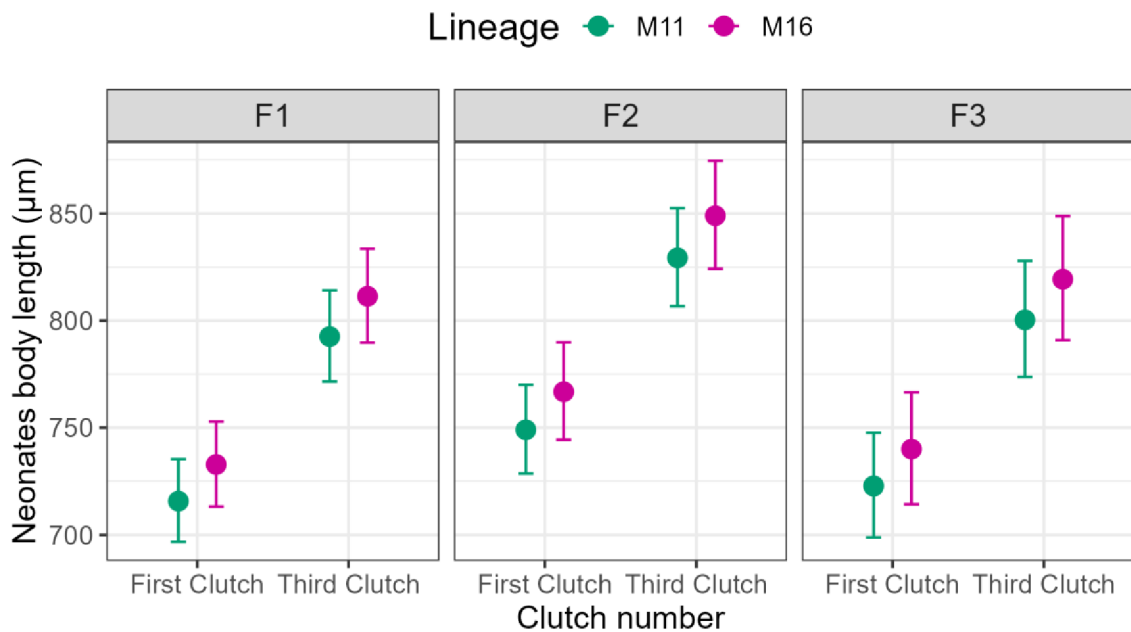
Fig. 8 Neonate body length in relation to neonates clutch number across the three generations, for the two lineages. Model estimates are based on the most parsimonious model and include 95 % confidence intervals. *ggeffects* package predicts the quantity and values of data points (in this case, x- and y-axis values).

3.7 Brood chamber volume

There was no effect the thermal regimes on the brood chamber size at death (Table S8). Only the generation had a strong significant and negative effect on the brood chamber size, and the lineage a smaller effect (Fig. 9, Table 7, Table S7).

Table 7 Summary of Gaussian GLM to model relationship between clutch size and neonate body length. Lin = lineage; Gen = generation; inverse of last clutch reproductive size. Intercept includes lineage (M11) and generation (F1) as baseline levels.

Model parameter	Estimate (and 95 % confidence intervals)	z-value	p
Intercept	6.816 (6.763 to 6.870)	249.71	< 0.001
Lin$_{(M16)}$	-0.047 (-0.107 to 0.013)	-1.54	0.123
Gen$_{(F2)}$	-0.042 (-0.105 to 0.022)	-1.28	0.199
Gen$_{(F3)}$	-0.130 (-0.215 to -0.045)	-3.00	< 0.01
Inverse(1 / last clutch reproduction size)	-0.071 (-0.203 to 0.060)	-1.06	0.288

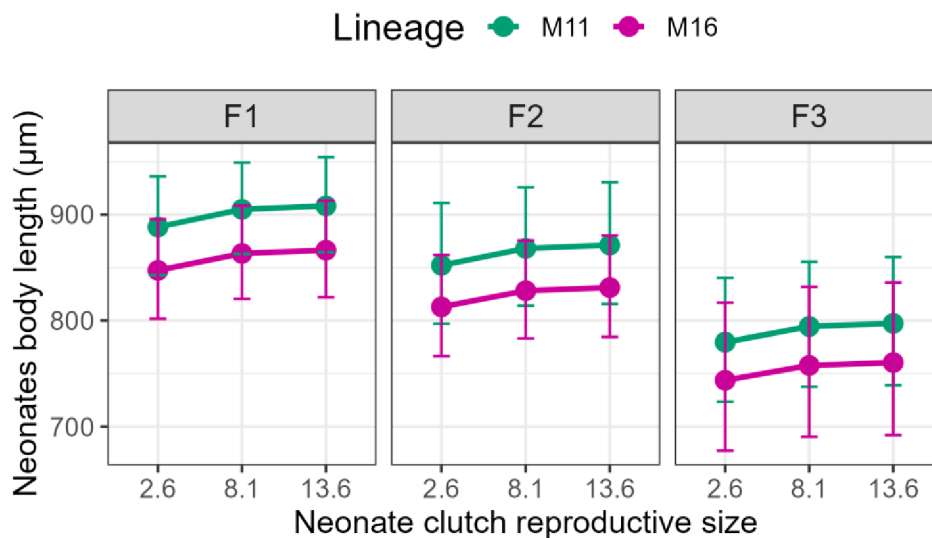**Fig. 9** Relationship between last clutch reproductive size and last clutch neonate body length across the three generations, for the two lineages. Model estimates are based on the most parsimonious model and include 95 % confidence intervals. *ggeffects* package predicts the quantity and values of data points (in this case, x- and y-axis values).

4 DISCUSSION

The study had the following objectives: (1) How do stable and stochastic thermal regimes affect *Daphnia* life histories? (2) Do lineages respond differently to these thermal regimes? Our results showed that the thermal environments (stable vs stochastic regime) had an effect on the life-history traits of *Dphnia magna* and also generations had a strong impact for most of the life-history traits signifying propagation of the fitness effects from founders to granddaughters. Meanwhile, the performance in life-history traits did not differ between the two lineages.

4.1 Fluctuations at same thermal average had an impact on *Daphnia* performance

Climate change is happening at an unprecedented rate with reports on increase in mean temperature and random thermal fluctuations showing that small shifts in the amplitude of environmental fluctuations can cause abrupt and irreversible changes in the ecosystems (Bathiany *et al.,* 2018). In our experiment, there was no indication that *Daphnia* performance was directly dependent on the mean and variance of the thermal regimes but our results showed significant overall reduction in life-history traits performance across the generations. Burton *et al.,* (2018) found a positive correlation with increasing acclimation temperature for *Daphnia magna* adjusted to heat tolerance, based on changes in constant temperature only while Burton *et al.,* (2020) report that the T_{imm} (the temperature at which individual becomes immobilised) had a positive correlation with increasing acclimation temperature. In our experimental study, the performance and life-history traits did depend upon thermal regimes and individuals performed differently in the thermal regimes.

Jensen's inequality states that the organismal performance at average body conditions would be dissimilar to the average performance under fluctuating temperature (Denny, 2017). By extending Jensen's inequality, there should have been dissimilarity between the thermal regimes in lifespan in our study. Also, thermal stress contributes to increased lifespan in fluctuating temperatures via heat-shock protein expression (HSP's) (Sørensen *et al.,* 2003; Žák & Reichard, *et al.,* 2020). In a laboratory experiment on brook stout (*Salvelinus fontinalis*), survivorship was higher in a combination of stable and low stochasticity regimes than in a combination of constant and high-stochasticity regimes (Pisano *et al.,* 2019) and survival

probabilities was lower for constant regime than for fluctuating regimes. Hokanson *et al.,* (1977) demonstrated that temperature variability influenced the survival rates of the rainbow trout (*Oncorhynchus mykiss*) positively or negatively depending on whether the mean temperature was higher or lower than 16 °C. Interestingly, within fluctuating regimes, the trout under a mean of 22 °C exhibited substantially higher mortality rate of up to 46% when compared to those under a mean of 21 °C within a 5-day period. However, in my study, no differences were observed in lifespan for individuals subjected to stable or stochastic thermal regime despite the fact that *Daphnia* individuals in the stochastic regime experienced different temperature amplitudes throughout their lifecycle. Constant reduction in lifespan in the stochastic regime is seen while disruption in pattern was observed between F2 and F3 generation in the stable regime. For the giant gourami (*Osphronemus goramy*) fish larvae, Prakoso *et al.* (2021) reported that constant regime had higher survival rate than those under fluctuating regimes, suggesting that constant regime temperature was optimal for the survival of the fish larvae. Our study finds no difference in lifespan between the thermal regimes which goes in contrast with Žák & Reichard (2020) who report that, for turquoise killifish *Nothobranchius furzeri*, fluctuating thermal regime (20-35 °C) had higher median lifespan than stable thermal regime (27.5 °C) and this increase in lifespan in fluctuating thermal regime may have been influenced by the stress-protective response.

The concept of Jensen's inequality can also be applied to body length variable (which is a function of growth rate). Using the non-linear and time-dependent relationship of fluctuating temperature, Kingsolver *et al.* (2015) observed maximal growth rate under fluctuating temperatures and thereby promoting large body size in ectotherms. Furthermore, marine stickleback (*Gasterosteus aculeatus*) were larger when reared at stochastic fluctuating temperature ranging from 14 to 23 °C when compared to constant 21 °C and those under 17 °C and stable thermal environment (ranging from 17 °C to 21 °C) were similarly sized (Shama, 2017). Vajedsamiei *et al.,* (2021) conducted a similar experiment on marine mussel *Mytilus* at four thermal means (18.5 °C, 21 °C, 23.5 °C, 26 °C) with three different degrees of daily fluctuating amplitude (± 0 °C, ± 2 °C, ± 4 °C). The authors observed improved mussel growth rates under high thermal mean (26 °C) with large thermal amplitude (± 4 °C) and for 23.5 °C intermediate-amplitude (± 2 °C) had no minor impact on the mussel growth rate when compared to large-amplitude fluctuations (± 4 °C) which showed decreased growth rates. Their findings suggest that fluctuations mitigate heat stress impacts only at critically high

mean temperatures and that both thermal means and temperature fluctuations were influential for mussel growth and development. In my study, the body length at death did differ between the two thermal regimes (dependent on the temperature fluctuations) and the individuals reared under stable regime showed higher body length than those under stochastic regime in F2 and F3 generation. While our results for lifespan goes in contrasts with Žák & Reichard (2020), the body length at death for *Daphnia* in our study is higher in stable thermal regime than for stochastic thermal regime which is similar to Žák & Reichard (2020) in which they found higher growth rate for stable thermal regime (27.5 °C) than under fluctuating thermal regime (20-35 °C). Žák & Reichard (2020) suggested that the smaller body size observed under fluctuating conditions could be attributed to periodic exposure to cold temperatures, leading to increased energy expenditure during warm periods. This higher energy demand might exceed the maximum metabolic rate, resulting in reduced energy available for growth.

According to Darwin's (1874) fecundity advantage hypothesis, i.e. large females producing more offspring, has received considerable support, especially for invertebrates and ectothermic vertebrates (Bonnet *et al.*, 2000; Wootton, 2012). During periods of energy or resource limitation, a trade-off between growth and reproduction is expected. Allocation of resources to growth reduces allocation to reproduction resulting in decreased reproductive output for individuals with larger body (Shine, 1988). The findings in our study support this theory. Total reproductive output of *Daphnia* individuals decreased significantly with increasing body length in F2 and F3 generation, indicating that *Daphnia* favoured allocation of resources to growth rather than reproduction (Kozłowski *et al.*, 2004).

Bartosiewicz *et al.*, (2015) tested if the reproductive investment was dependent on the brood chamber volume of *Daphnia* species with varying food resource conditions. The authors found that reproductive investment would be constrained by brood space at least during early reproductive clutches and not by available resources. For *Daphnia longispina,* despite high food levels and high lipid levels in body, the reproduction at third clutch was limited by physiological constraints (capacity of oocytes to absorb resource material) and not by brood space or resources (Bartosiewicz *et al.,* 2015). Meanwhile, Pettersen *et al.,* (2023) report that the embryonic energy expenditure did not depend on temperature fluctuations and the embryonic energy expenditure was similar in two fluctuating thermal regimes with mean 24 ± 2 °C and 24 ± 6 °C in common wall lizards. Our results align with these findings, indicating

that the number of neonates produced in the brood chamber was independent of the neonate body length (as an extension of available brood space). However, we were able to estimate the brood chamber size only for the last clutch. For future experimental studies, by measuring neonate body length for multiple clutches, understanding how *Daphnia* mothers allocate resources for reproductive investment over its lifetime can be better understood.

Taking into account of extreme climatic events, sudden random fluctuations between the hottest and the cooler part of the day would incur large physiological damage where the stress accumulation would be greater than the rate of recovery (Kovacevic *et al.,* 2019). The fluctuations employed in our experiment reflects the natural conditions commonly experienced by cladocerans in the wild (Mitchell & Lampert, 2000; Bruijning *et al.,* 2018). Indeed, maximum growth rate was found to be between 23-29 °C (Mitchell & Lampert, 2000) while thermal optimum for *Daphnia magna* ranged between $16 - 22$ °C (Bruijning *et al.,* 2018). Geerts *et al.,* (2015) report a 3.6 °C higher CT_{max} (upper thermal limits) than those *Daphnia* exposed to +4 °C ambient temperature.

Within generations, organisms undergoing diel thermal variability can also develop plastic responses such as acclimation (physiological/behavioural adaptation), heat shock response (through synthesis of heat shock proteins) and hardening (rapid response to extreme temperature for short period) (Bowler, 2005; Loeschcke & Sørensen, 2005). It is also possible that the fluctuations do not affect the individuals, but in fact have beneficial effects through its refuge effect (Kefford *et al.,* 2022). Vajedsamiei *et al.,* (2021) provide a framework suggesting an interplay between thermal acclimation-stress accumulation dynamics. When individuals were at high temperatures, heat tolerance build-up (by HSP expression or metabolic supply) would ensure their survival. As the stochastic thermal regimes had cooler days after the hot days, this "refugee effect" from chronic high temperature would provide relief to the *Daphnia* individuals. This would explain why fluctuations in both thermal regimes behaved similarly in terms of *Daphnia* performance. However, as the *Daphnia* individuals in our study undergoes temperature fluctuations between highest and lowest temperature over the cycle, repeated exposures would lead to physiological stress build-up which may/may not be reversible (Pörtner *et al.,* 2007) and when the maximum stress capacity is reached, the individual dies. When individuals (i.e. *Daphnia*) are exposed to temperature above the thermal optimum in consecutive diel cycles, sufficient stress is accumulated leading to mortality

(Pörtner, 2010). In our case, if adult individuals had accumulated stress, then the environmental stimulus may have affected the neonates inside the brood pouch of the F1 mother before its release (see Arrighi *et al.,* 2013; Bateson *et al.,* 2014; Sikkink *et al.,* 2014; Perez & Lehner, 2019; Zawlodzki *et al.,* 2022). As observed in our study, F2 generation individuals had decreased lifespan, reproduction and body length at death and one possible reason for this could be that the third clutch neonates of F1 probably carried the stress from the F1 mothers (similar for F3 generation).

Since all the individuals were fed *ad libitum* with same the food and the water changed every two days, the possibility of the effect of food and oxygen limitation on the fitness traits can be ruled out. There are very few studies that include the effect of food while studying daily thermal fluctuations (Van Baelen *et al.,* 2023) and are mostly related to constant temperatures (Masclaux *et al.,* 2009; Martin-Creuzburg *et al.,* 2019). Periodicity of thermal fluctuations determines the growth rate of *Daphnia magna* and food resource influenced the size and direction of the growth rate. Van Baelen *et al.,* (2023) conclude that understanding the food resource is crucial as they are shown to influence temporal dynamics of phenotypic plasticity under temperature fluctuations.

As remarked by Engqvist and Reinhold (2016), "Possibly, the lack of an effect was due to the fact that the study was performed under benign laboratory conditions (or, in case of field studies, during a particularly good year)". It would be valuable to assess whether *Daphnia* individuals collected from different seasons exhibit similar performance and whether experimenting with different stochastic regimes will give us different performance response is something that can be implemented in future studies.

4.2 Conspecific populations exhibited similar performance

Studies on intra-population variation to the thermal regimes have been reported previously in conspecific populations of *Daphnia magna* (Hoegnagel *et al.,* 2018; Bruijning *et al.,* 2018) and for inter-population (Altermatt *et al.,* 2008; Dziuba, *et al.,* 2020; Dziuba *et al.,* 2021; Vanvelk *et al.,* 2021) Observations from our study revealed no profound differences between the two *Daphnia* populations in their overall responses to various life history traits while most of the models include lineage as an important predictor term signifying that it has to some extent an influence in its response to the environmental cues.

During extreme conditions (low or high temperature, hypoxia, low food resource), *Daphnia* species produce dormant eggs by sexual reproduction to ensure its survival (Kleiven 1992; Slusarczyk & Rybicka, 2011). These dormant eggs or ephippia can introduce novel clones into population (Carvalho & Wolf, 1989). Using clones developed from ephippia, Fossen et al. (2021) studied if genetic differences in how *Daphnia magna* reproduce at different temperatures can lead to variations in their ability to enter a dormant stage. Their results showed that ephippia production was higher at 12 °C than at 22 °C and large differences in ephippia production per generation was seen among clones at 12 °C. Kielland *et al.,* (2017) also studied the effect of daily thermal fluctuations using *Daphnia magna* clones reared from ephippia eggs and found no-clonal specific response to fluctuations in body mass or metabolic rate. The lack of clonal-specific responses would indicate that the *Daphnia magna* have either adapted to cope with a range of thermal conditions or individuals can adjust their physiological traits in response to environmental changes, regardless of their genetic makeup.

Thus, it is important to consider that the experiment included only two lineages from the established cultures and whether the M11 lineage is different to that of M16 is unknown. Also, the *Daphnia* individuals in our study were collected from a pond and was not reared directly from the ephippia eggs. Furthermore, the individuals collected from the same pond would mean less spatial variation and perhaps the individuals come from the same population. *Daphnia pulex* individuals collected from a pond had identical genotypes at five different loci (Lyberger & Schoener, 2023) and based on this study, there is a high chance that the two populations in our study are in fact sister lineages or very closely related. Future studies should explore sampling *Daphnia* ephippia (dormant egg banks in benthic layer has larger reservoir of genetic lineages; Brendonck & De Meester, 2003) or individuals from spatially-separated ponds which would give us genetically diverse populations of *Daphnia magna* populations. Those lineages that perform better within a population shifts the average phenotype towards a new, optimal fitness peak in response to the prevailing environmental conditions (Sunday *et al.,* 2014).

5 CONCLUSIONS & FUTURE DIRECTIONS

The prominent findings in this study are that environmental stochasticity by itself had a smaller role in explaining life-history trait variation of *Daphnia magnna* individuals but the environmental fluctuations did influence the mothers and their offspring across the generations in various life-history traits (lifespan, body length at death, reproductive output, neonate body length at first clutch). It was also found that lineages did not differ in their response to fluctuations. This would either mean that the lineages used in this study are closely related or two separate non-identical lineages exhibiting similar responses to thermal regimes.

The following insights from this study can be used for future experimental studies:

1. Factoring more/diverse lineages and zooplankton species

In the initial experimental phase of F1 generation, three lineages were included as part of the study. The third (M12, discarded later) lineage produced no offspring although no mortality was observed during the first three weeks. M12 lineage in the lab culture (at constant 22 °C) displayed optimal performance similar to the rest of the cultures. A possible limitation of this study is studying two lineages that were reared from individuals collected from the same pond. Future research could investigate the sampling of individuals from ponds that are geographically separated. This approach would allow for the study of genetically diverse populations of *Daphnia magna* across different locations. Also, evolutionary responses to climate change have been extensively focused on single species. It would be interesting to see how different cladocerans species behave to these thermal fluctuations (Verheyen & Stoks, 2019; Vanvelk *et al.,* 2021).

2. Match/mismatch transgenerational plasticity experiment

Another well explored arena is transgenerational plasticity and match/mismatch or reciprocal experiments. In transgenerational plasticit, offspring are translocated among alternative environments differing from their parental environment leading to 'match/mismatch' (Donelson *et al.,* 2012) which helps determining whether any changes observed are driven by adaptation or phenotypic plasticity. This would help us understand the genotype x environment interaction. For transgenerational experiment, the future experimental design would be exposing *Daphnia magna* to control, stable regime and stochastic regime for few generations and later performing reciprocal transplants among these regimes (as in

Chakravarti *et al.,* 2016, Gibbin *et al.,* 2017). Taking consideration of the assumptions held by this experimental design as explained by Engqvist & Reinhold (2016) would likely call for more empirical and theoretical work to disentangle the effects of within-generational and transgenerational plasticity.

3. Variation in stochastic regime

Due to the fluctuating nature of the environment, *Daphnia* can undergo different stochastic fluctuations within/across the generations and conversely. It is also plausible for *Daphnia* to experience same stable/stochastic fluctuations in consecutive generations (stable-stable-stable or stochastic-stochastic-stochastic like in this study). As epigenetic changes are easily reversible where phenotype can either switch back immediately or within few generations (Klironomos *et al.,* 2013; Burggren, 2014) it would be informative to see if such interactions is seen for different cladoceran species or populations and for different stochastic regimes (either experimenting with different amplitude fluctuations or by employing several different regimes with varying predictability across generations).

Over the past years, theoretical as well as empirical experiments have focused on studying fluctuating temperatures for better understanding species response to climate change (Niehaus *et al.,* 2012; Vasseur *et al.,* 2014; Saarinen *et al.,* 2018). Mean temperature and thermal fluctuations have also gained a significant threshold in thermal biology (Steel *et al.,* 2012; Dowd *et al.,* 2015; Drake *et al.,* 2017; Shama, 2017; Morash *et al.,* 2018; Pisano *et al.,* 2019; Vajedsamiei *et al.,* 2021; Hammill & Dart, 2022). However, to date, as per literature review, no such studies have explicitly tested for multigenerational effects of thermal average and fluctuations on zooplankton species, thus this study presents an important extension of this growing body of work.

To understand how future thermal environment conditions will influence species- and population-level responses, it is critical to incorporate various aspects of thermal complexity (periodicity, amplitude, and duration). Recognizing these crucial elements of thermal variability will enable better designed experiments, enhancing our ability to predict how organisms will react to climate change (Helmuth *et al.,* 2014).

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Supplementary information

Fig S1 Climatic test chamber with the experimental samples. Each tray contains 15 *Daphnia* individuals for one of the lineage placed in individual 250 mL beakers. Shown here are F1 (top row) and F2 generation (middle row) individuals for one of the thermal regimes.
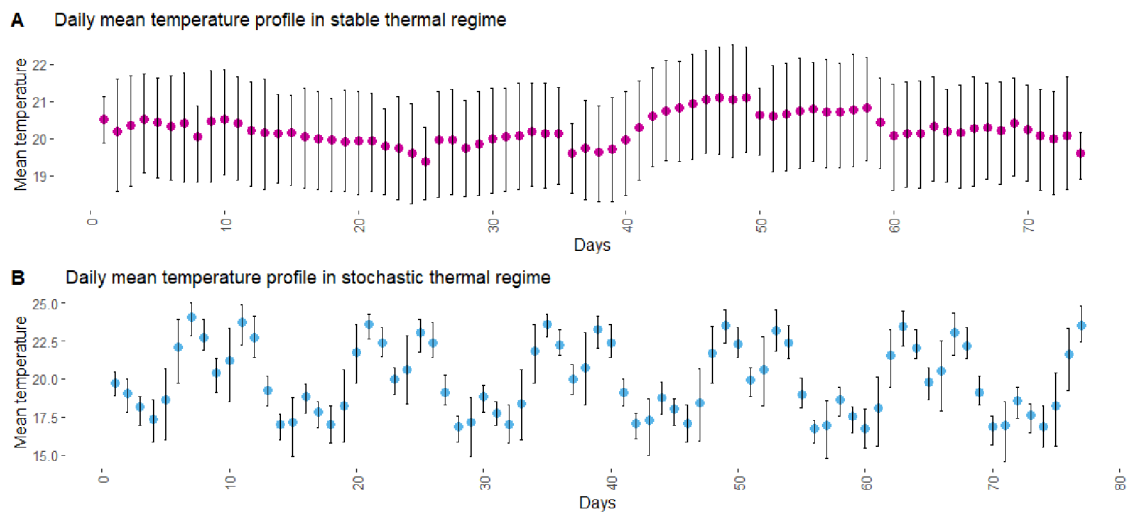
Fig S2 Daily mean temperature ± standard deviation (SD) of the two thermal regimes, stable (A) and stochastic (B) regimes, recorded for the entire experimental duration. The points represent daily mean temperatures and the whiskers indicate CI's. Daily mean temperature calculated from all data loggers.

Table S1 Temperature program for a) stable regime; b) stochastic regime spanning across two weeks. S1 – S6 refers to breakdown of temperature time-scale for a day. For stochastic regime, the differences is calculated from the baseline temperature of 21 ℃ and temperature is changed regularly each day and the cycle is repeated every two weeks until the end of the experiment. The overall mean temperature for both thermal regimes is 21 ℃.

	S1	S2	S3	S4	S5	S6	differences	amplitude	mean
a) Stable regime									
day	22	21	20	19	21	23	0	4	21
b) Stochastic regime									
day1	21	20	19	18	20	22	-1	4	20
day2	20.1	19.1	18.1	17.1	19.1	21.1	-1.9	4	19.1
day3	18.9	17.9	16.9	15.9	17.9	19.9	-3.1	4	17.9
day4	19	18	17	16	18	20	-3	4	18
day5	22.9	21.9	20.9	19.9	21.9	23.9	0.9	4	21.9
day6	26.2	25.2	24.2	23.2	25.2	27.2	4.2	4	25.2
day7	25.6	24.6	23.6	22.6	24.6	26.6	3.6	4	24.6
day8	22.3	21.3	20.3	19.3	21.3	23.3	0.3	4	21.3
day9	21.6	20.6	19.6	18.6	20.6	22.6	-0.4	4	20.6
day10	25.5	24.5	23.5	22.5	24.5	26.5	3.5	4	24.5
day11	25.8	24.8	23.8	22.8	24.8	26.8	3.8	4	24.8
day12	22.1	21.1	20.1	19.1	21.1	23.1	0.1	4	21.1
day13	19.2	18.2	17.2	16.2	18.2	20.2	-2.8	4	18.2
day14	18	17	16	15	17	19	-4	4	17

Table S2 Means and standard errors for life-history traits of *Daphnia magna* lineages for each thermal regimes across the generations. a) F1 generation

Generation	F1			
Thermal regime	Stable		Stochastic	
Lineage	**M11**	**M16**	**M11**	**M16**
Total reproduction output	80.53 ± 4.86	77.80 ± 10.29	67.73 ± 7.32	78.33 ± 8.63
Lifespan	37.46 ± 1.49	40.00 ± 5.53	35.33 ± 2.1	37.06 ± 2.46
Neonates body length at 1st reproduction (µm)	721.23 ± 5.10	733.91 ± 12.82	720.91 ± 9.46	722.22 ± 24.30
Neonates body length at 3rd reproduction (µm)	833.31 ± 17.67	796.75 ± 20.54	804.70 ± 14.88	827.54 ± 21.46
Body length at death (µm)	3072.83 ± 77.76	3103.33 ± 95.23	3100.72 ± 63.20	3080.15 ± 102.90

Table S2 continued. b) F2 generation

Generation	F2			
Thermal regime	Stable		Stochastic	
Lineage	**M11**	**M16**	**M11**	**M16**
Total reproduction output	23.06 ± 3.38	22.44 ± 5.72	26.86 ± 4.77	28.92 ± 4.10
Lifespan	22.93 ± 2.13	17.60 ± 1.76	25.87 ± 2.57	28.13 ± 2.92
Neonates body length at 1st reproduction (µm)	782.54 ± 15.75	801.08 ± 33.95	707.63 ± 11.98	732.68 ± 28.94
Neonates body length at 3rd reproduction (µm)	811.31 ± 20.47	849.46 ± 42.02	838.05 ± 24.67	873.69 ± 34.84
Body length at death (µm)	2733 ± 46.86	2626.64 ± 87.71	2571.20 ± 132.43	2645.92 ± 93.85

Table S2 continued. c) F3 generation

Generation	F3			
Thermal regime	Stable		Stochastic	
Lineage	**M11**	**M16**	**M11**	**M16**
Total reproduction output	11.00 ± 1.39	13.14 ± 5.41	10.50 ± 1.83	26.28 ± 5.63
Lifespan	20.00 ± 2.03	26.80 ± 1.84	16.93 ± 3.02	19.20 ± 3.18
Neonates body length at 1st reproduction (μm)	768.58 ± 17.79	781.39 ± 35.33	698.25 ± 19.28	757.76 ± 15.31
Neonates body length at 3rd reproduction (μm)	747.58 ± 19.26	830.32 ± 8.09	804.81 ± 44.27	823.79 ± 68.18
Body length at death (μm)	2085.80 ± 198.33	2612.91± 146.49	2376.83 ± 157.73	1814.57 ± 286.70

Table S3 Effect of the temperature regime on the lifespan. Table represents comparison of all candidate models for lifespan. Most parsimonious selected model for the response variable is in bold. Corresponding information is shown – degree of freedom (df), predictor terms, LogLik difference (ΔLogLik), AICc difference (ΔAICc – selection criterion) and Akaike weight. "trt" = thermal regime; "lin" = lineage; "gen" = generation.

Response	Model name	Model structure	df	ΔLogLik	ΔAICc	weight
Lifespan	**age.9**	**trt × gen**	**7**	**38.519**	**0**	**0.49**
	age.10	(trt × gen) + lin	8	39.207	0.815	0.326
	age.3	gen	4	33.316	3.982	0.067
	age.7	lin + gen	5	33.818	5.095	0.038
	age.5	trt + gen	5	33.374	5.984	0.025
	age.13	gen × lin	0	35.064	6.91	0.015
	age.8	trt + lin + gen	6	33.875	7.122	0.014
	age.15	trt × gen × lin	13	41.727	7.126	0.014
	age.14	(gen × lin) + trt	8	35.145	8.939	0.006
	age.12	(trt × lin) + gen	7	33.897	9.244	0.005
	age.1	~ 1	2	0	66.454	0
	age.4	lin	3	0.383	67.757	0
	age.2	trt	3	0.019	68.486	0
	age.6	trt + lin	4	0.401	69.814	0
	age.11	(trt × lin)	5	0.421	71.89	0

Table S4 Effect of the temperature regime on body length at death. Table represents comparison of all candidate models for body length at death of *Daphnia* individuals with lifespan [log (age)] as fixed effect. Most parsimonious selected model for the response variable is in bold. Corresponding information is shown – degree of freedom (df), predictor terms, LogLik difference (ΔLogLik), AICc difference (ΔAICc – selection criterion) and Akaike weight. "trt" = thermal regime; "lin" = lineage; "gen" = generation.

Response	Model name	Model structure	df	ΔLogLik	ΔAICc	weight
Body length at death	bl.5	trt + gen + log (age)	6	17.363	0	0.406
	bl.3	gen + log (age)	5	15.406	1.708	0.173
	bl.9	**(trt × gen) + log (age)**	**8**	**18.714**	**1.817**	**0.164**
	bl.8	trt + lin + gen + log (age)	7	17.398	2.172	0.137
	bl.7	lin + gen + log (age)	6	15.424	3.878	0.058
	bl.15	(trt × gen × lin) + log (age)	14	24.409	4.957	0.034
	bl.14	(gen × lin) + trt + log (age)	9	17.656	6.252	0.018
	bl.13	(gen × lin) + log (age)	8	15.809	7.627	0.009
	bl.1	log (age)	3	0	28.22	0
	bl.2	trt + log (age)	4	1.062	28.229	0
	bl.4	lin + log (age)	4	0.082	30.189	0
	bl.6	trt + lin + log (age)	5	1.134	30.254	0
	bl.11	(trt × lin) + log (age)	6	2.141	30.444	0
	bl.10	(trt × gen) + lin + log (age)	9	NA	NA	NA
	bl.12	(trt × lin) + gen + log (age)	8	NA	NA	NA

Table S5 Effect of the temperature regime on for total reproductive output. Table represents comparison of all candidate models for total reproductive output of *Daphnia* individuals with neonate body length (NL; 1st and 3rd clutch) or body length at death (ML) or lifespan as fixed effect. Most parsimonious selected model for the response variable is in bold. Corresponding information is shown – degree of freedom (df), predictor terms, LogLik difference (ΔLogLik), AICc difference (ΔAICc – selection criterion) and Akaike weight. "trt" = thermal regime; "lin" = lineage; "gen" = generation.

Response	Model name	Model structure	df	ΔLogLik	ΔAICc	weight
Total reproductive output + neonate body length (fixed)	**nbcl.13**	**(gen × lin) + NL**	**8**	**113.311**	**0**	**0.689**
	nbcl.14	(gen × lin) + trt + NL	9	113.596	1.634	0.304
	nbcl.15	(trt × gen × lin) + NL	14	114.487	11.251	0.002
	nbcl.3	gen + NL	5	103.713	12.727	0.001
	nbcl.7	lin + gen + NL	6	104.721	12.843	0.001
	nbcl.5	trt + gen + NL	6	104.256	13.772	0.001
	nbcl.8	trt + lin + gen + NL	7	105.329	13.783	0.001
	nbcl.12	(trt × lin) + gen + NL	8	105.546	15.53	0.0003
	nbcl.9	(trt × gen) + NL	8	104.385	17.852	0.0001
	nbcl.10	(trt × gen) + lin + NL	9	105.397	18.031	0.0001
	nbcl.4	lin + NL	4	4.87	208.303	0
	nbcl.6	trt + lin + NL	5	4.875	210.403	0
	nbcl.11	(trt × lin) + NL	6	5.346	211.592	0
	nbcl.1	NL	3	0	215.955	0
	nbcl.2	trt + NL	4	0.022	217.998	0
Total reproductive output + body length at death or lifespan (fixed)	**nb.15**	**(trt × gen × lin) + ML**	**14**	**61.65**	**0**	**1**
	na.15	(trt × gen × lin) + log (age)	14	52.905	17.491	0.0002
	na.16	(trt × gen × lin) × log (age)	25	68.181	18.06	0.0001
	nb.16	(trt × gen × lin) × ML	25	50.977	52.469	0
	nb.1	~ ML	3	5.606	86.534	0
	n.1	trt × gen × lin	13	13.888	92.995	0
	na.1	~ log (age)	3	0	97.746	0

Table S6 Effect of the temperature regime on (a) neonate body length at first clutch and (b) neonate body length at third clutch with body length at death (ML) as fixed effect for both set of models. Table represents comparison of all candidate models. Most parsimonious selected model for the response variable is in bold. Corresponding information is shown – degree of freedom (df), predictor terms, LogLik difference (ΔLogLik), AICc difference (ΔAICc – selection criterion) and Akaike weight. "trt" = thermal regime; "lin" = lineage; "gen" = generation.

Response	Model name	Model structure	df	ΔLogLik	ΔAICc	weight
(a) Neonate body length at first clutch	**fm.2**	**trt + ML**	**4**	**3.098**	**0**	**0.336**
	fm.5	trt + gen + ML	6	4.637	1.416	0.165
	fm.6	trt + lin + ML	5	3.285	1.848	0.133
	fm.9	(trt × gen) + ML	8	6.122	3.14	0.07
	fm.11	(trt × lin) + ML	6	3.697	3.295	0.065
	fm.8	trt + lin + gen + ML	7	4.768	3.475	0.059
	fm.1	~ ML	3	0	4.021	0.045
	fm.3	gen + ML	5	1.886	4.646	0.033
	fm.10	(trt × gen) + lin + ML	9	6.486	4.838	0.03
	fm.12	(trt × lin) + gen + ML	8	5.08	5.224	0.025
	fm.4	lin + ML	4	0.25	5.696	0.019
	fm.7	lin + gen + ML	6	2.04	6.609	0.012
	fm.14	(gen × lin) + trt + ML	9	4.912	7.988	0.006
	fm.13	(gen × lin) + ML	8	2.283	10.817	0.002
	fm.15	(trt × gen × lin) + ML	14	7.295	16.236	0.0001
(b) Neonate body length at third clutch	**tm.1**	**~ ML**	**3**	**0**	**0**	**0.38**
	tm.4	lin + ML	4	0.289	1.634	0.168
	tm.2	trt + ML	4	0.266	1.681	0.164
	tm.6	trt + lin + ML	5	0.533	3.415	0.069
	tm.3	gen + ML	5	0.528	3.425	0.069
	tm.11	(trt × lin) + ML	6	1.472	3.868	0.055
	tm.7	lin + gen + ML	6	0.863	5.087	0.03
	tm.5	trt + gen + ML	6	0.846	5.121	0.029
	tm.8	trt + lin + gen + ML	7	1.158	6.89	0.012
	tm.12	(trt × lin) + gen + ML	8	1.897	7.871	0.007
	tm.9	(trt × gen) + ML	8	1.739	8.187	0.006
	tm.13	(gen × lin) + ML	8	1.628	8.41	0.006
	tm.10	(trt × gen) + lin + ML	9	1.899	10.395	0.002
	tm.14	(gen × lin) + trt + ML	9	1.825	10.541	0.002
	tm.15	(trt × gen × lin) + ML	13	3.993	17.06	0.0001

Table S7 Effect of the temperature regime on neonate body length (of both first clutch and third clutch) with clutch number (clno) as fixed effect. Table represents comparison of all candidate models. Most parsimonious selected model for the response variable is in bold. Corresponding information is shown – degree of freedom (df), predictor terms, LogLik difference (ΔLogLik), AICc difference (ΔAICc – selection criterion) and Akaike weight. "trt" = thermal regime; "lin" = lineage; "gen" = generation.

Response	Model name	Model structure	df	ΔLogLik	ΔAICc	weight
Neonate body length for both clutches	**ftl.7**	**lin + gen + clno**	**6**	**5.838**	**0**	**0.284**
	ftl.3	gen + clno	5	4.316	0.913	0.18
	ftl.8	trt + lin + gen + clno	7	6.096	1.642	0.125
	ftl.13	(gen × lin) + clno	8	7.063	1.886	0.111
	ftl.5	trt + gen + clno	6	4.527	2.623	0.077
	ftl.12	(trt × lin) + gen + clno	8	6.377	3.258	0.056
	ftl.14	(gen × lin) + trt + clno	9	7.477	3.264	0.056
	ftl.10	(trt × gen) + lin + clno	9	6.886	4.445	0.031
	ftl.4	lin + clno	4	1.155	5.125	0.022
	ftl.1	~ clno	3	0	5.347	0.02
	ftl.9	(trt × gen) + clno	8	5.075	5.863	0.015
	ftl.6	trt + lin + clno	5	1.396	6.751	0.01
	ftl.2	trt + clno	4	0.204	7.026	0.008
	ftl.11	(trt × lin) + clno	6	1.822	8.033	0.005
	ftl.15	(trt × gen × lin) + clno	14	8.745	12.129	0.001

Table S7 Effect of the temperature regime on brood chamber. Relationship between neonate body length at last clutch with reproductive size at last clutch (Inverse *(1 /lrep)) as fixed effect is used. Table represents comparison. Most parsimonious selected model for the response variable is in bold. Corresponding information is shown – degree of freedom (df), predictor terms, LogLik difference (ΔLogLik), AICc difference (ΔAICc – selection criterion) and Akaike weight. "trt" = thermal regime; "lin" = lineage; "gen" = generation; "I *(1 /lrep)" = inverse of last clutch reproductive size.

| Response	Model name	Model structure	df	ΔLogLik	ΔAICc	weight
Neonate body length at last clutch	**lc.3**	**gen + I *(1 /lrep)**	**5**	**3.75**	**0**	**0.243**
	lc.7	lin + gen + I *(1 /lrep)	6	4.915	0.036	0.238
	lc.13	(gen × lin) + I *(1 /lrep)	8	6.492	1.828	0.097
	lc.5	trt + gen + I *(1 /lrep)	6	3.896	2.072	0.086
	lc.8	trt + lin + gen + I *(1 /lrep)	7	5.013	2.275	0.078
	lc.12	(trt × lin) + gen + I *(1 /lrep)	8	6.12	2.572	0.067
	lc.1	~ I *(1 /lrep)	3	0	2.968	0.055
	lc.14	(gen × lin) + trt + I *(1 /lrep)	9	6.881	3.637	0.039
	lc.4	lin + I *(1 /lrep)	4	0.287	4.626	0.024
	lc.2	trt + I *(1 /lrep)	4	0.098	5.005	0.02
	lc.10	(trt × gen) + lin + I *(1 /lrep)	9	6.148	5.104	0.019
	lc.9	(trt × gen) + I *(1 /lrep)	8	4.811	5.19	0.018
	lc.6	trt + lin + I *(1 /lrep)	5	0.349	6.801	0.008
	lc.15	(trt × gen × lin) + I *(1 /lrep)	13	10.22	8.157	0.004
	lc.11	(trt × lin) + I *(1 /lrep)	6	0.639	8.587	0.003
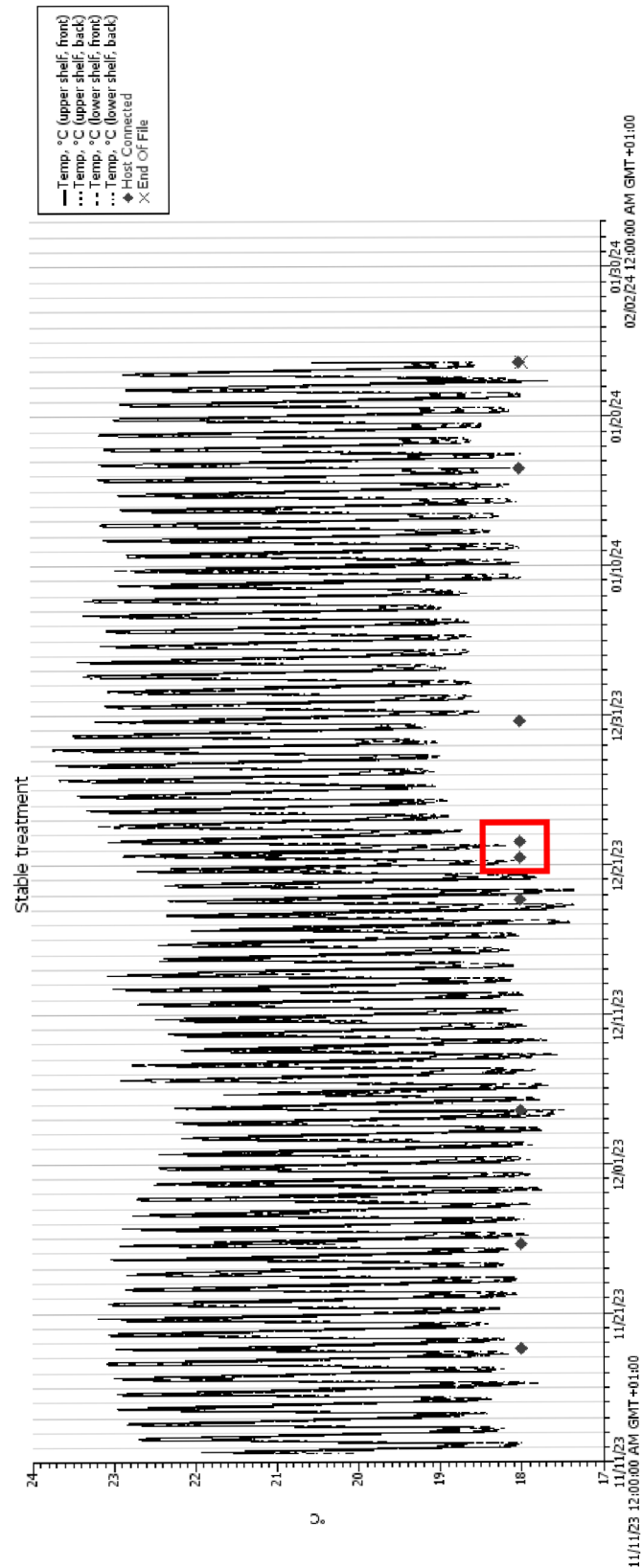
Fig S3 Temperature profile for the experiment with water flea *Daphnia magna* subjected to (a) stable fluctuating temperature. Temperature correction was done on day 40 and day 41 by +0.5 °C and by +0.3 °C for stable regime (denoted by red box in figure)
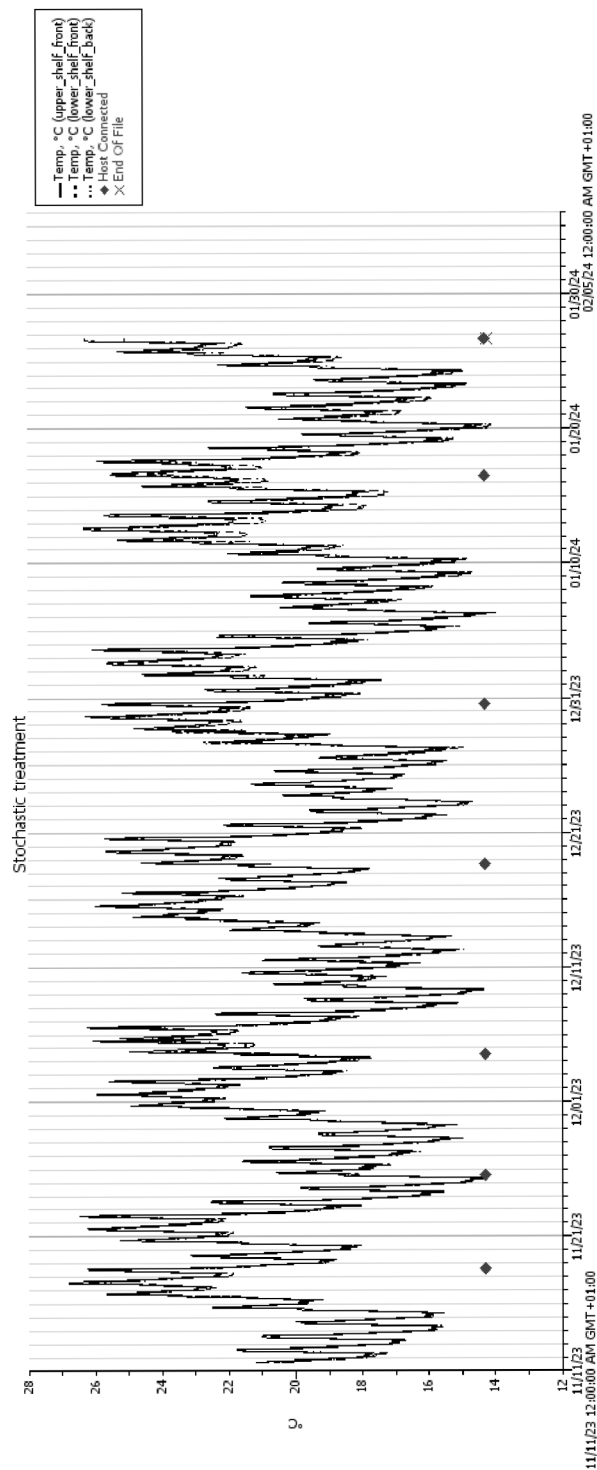
Fig. S3 (continued) (b) for stochastic fluctuating temperature. Daily time scale for each environments was: 2 ½ h at temperature 1; 2 ½ h of dowload ramping at temperature 2; 2 ½ h of download ramping at temperature 3; 8 ¾ h of download ramping at temperature 4; 2 ¾ h of upload ramping at temperature 5; 5 h of uploading ramping at temperature 6. Temperature measured by HOBO data loggers and visualised in HOBO software.

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