The effects of thermal deterioration on the life history traits of *Daphnia magna*

Abstract

Adverse anthropogenic impacts on the Biosphere have produced environmental change at a rate unprecedented in the Cenozoic, shifting the environmental conditions outside of the physiological tolerance range of many species. Predicting the patterns of species' response to climate change requires tracking the variation in fitness-related traits (i.e. life histories) during exposure to thermally changing environments, because these traits may strongly correlate with the probability of survival.

A growing body of experimental studies demonstrated that an increase of mean temperatures significantly influences the response of several life history traits, namely reduction of development time and body size at maturation. However, there are still significant gaps in our understanding of how species respond to temperature change, as most research has focused only on abrupt changes to thermal conditions. To better reflect ecological reality, we need experimental designs that examine the effects of gradually and continuously changing thermal environments over longer time scales, because the population genetics of adaptation to stressful environments is strongly influenced by the rate of environmental change. Moreover, the extent to which these trait changes result from phenotypic plasticity versus genetic adaptation remains poorly understood.

To address these gaps, we conducted a controlled multigenerational evolutionary experiment with a plankton crustacean, *Daphnia magna.* We subjected the clonal populations to gradually increasing temperatures, a scenario more representative of ongoing climate change than abrupt thermal shifts. We monitored their lifespan, fecundity, somatic growth rate, age at maturation, body size, and extinction risk across generations, and used a reciprocal transplant experiment to disentangle genetic adaptation from phenotypic plasticity.

Our results revealed the two distinctive patterns of the response in life history traits under elevated temperatures. The adaptive response was manifested through an increase in fecundity of high-temperature populations relative to control populations. We interpret reduction in both their development time and mean adult body size as the maladaptive responses, given the positive correlations found between adult body size and fecundity, and between development time and adult body size.

However, we detected significant level of adaptive plasticity in our populations, as some clonal lines exhibited a markedly different response to elevated temperatures, opposite the population-level trend (increased development time under high temperature, leading to larger adult size and ultimately higher fecundity). This suggests a strong genotype-by-environment interaction (G X E), where genetic variation shapes the plastic response to environmental stress. The presence of such adaptive variants amidst a generally maladaptive mean response underscores the importance of intra-population variation in plasticity. This highlights the need to consider not only average plastic responses but also the distribution and evolutionary potential of genotype-specific trajectories in the face of climate change.

Key words: Temperature, deterioration, warming, Daphnia magna, adaptive plasticity

1. Introduction

Adverse anthropogenic impacts on the Biosphere have produced environmental change at a rate unprecedented in the Cenozoic (Barnosky et al., 2012). One of the most alarming consequences of the widely recognized issue of global change (Falkowski, 1994; Chivian and Bernstein, 2008; Barnosky et al., 2012) is the shifting of environmental conditions outside of the physiological tolerance range of many species (Parmesan, 2006). Recent studies predict the extinction of 14-35 % of extant species in the following decades (Thomas et al., 2004; Weins and Zelinka, 2024). Hence, understanding the factors which affect adaptation to changing environments has become one of the central problems of contemporary science, with the main goal to help mitigate the detrimental effects of global change. One of the most threatening aspects of global change is the continuous rise of temperature from the pre-industrial era (Hawkins et al., 2017) onwards, a trend predicted to accelerate within the next 50 years (Walther et al., 2002). Consequently, persistence of many extant species may be threatened due to the altered interspecies interactions (for example, food availability) and the limited physiological tolerances to high temperatures (Cahill et al., 2013). The latter factor is particularly pronounced in the lower latitudes, as many tropical species are already experiencing conditions close to their thermal optima (Deutsch et al., 2008).

Predicting the patterns of species' response to climate change requires tracking of the variation in fitness-related traits (i.e. life histories) during exposure to thermally changing environments, because these traits may strongly correlate with the probability of survival (Isaac, 2009). For example, slower life histories (long gestation and small litters) may enhance extinction risk due to the limited capacity of a population to recover from a decline caused by the natural or anthropogenically induced factors (Purvis et al., 2000). Moreover, these extinction-promoting traits are often negatively correlated with body size (McKinney

1997; Cardillo et al. 2005). Thus, in species with an intraspecific variation in body size, we might expect a shift to a smaller body size due to a lower extinction risk (Isaac, 2009). However, the opposite pattern can be predicted if an intraspecific variation in body size positively correlates with competitiveness (Isaac, 2009), survival (Yoccoz & Mesnager 1998) and fecundity (Du et al. 2005).

Given the complex interactions among life history traits, clarifying their relative contributions to fitness and survival under rising temperatures requires experimental studies that manipulate temperature and directly monitor trait changes. To date, most of these studies have been carried out using insects as model organisms or various plankton species cultivated in the outdoor mesocosms (Van Doorslaer et al., 2007; Van Doorslaer et al., 2010; Musolin et al., 2010; Ciota et al., 2014; Geerts et al., 2015; Laughton et al., 2017; Cambronero et al., 2018; Iltis et. al., 2019; de Leon et al., 2023; Albini et al., 2025).

Most experimental studies with insects revealed negative correlation of temperature with lifespan, development time, and body size (e.g. Ciota et al., 2014; Laughton et al., 2017; Iltis et. al., 2019). Importantly, these effects of an elevated temperature on the life history traits may depend on the different phenological parameters such as season. For example, an increase in temperature during the warmest part of the season was found to reduce development and increase mortality in the heteropteran species *Nezara viridula*, while adults showed a decline in body size as well as reduced life span and fecundity (Musolin et al., 2010). The negative effects of elevated temperature on life history traits may also vary with geographical distribution of species. In a study of four *Protaphorura* species (Collembola) sampled across a latitudinal gradient, heat stress reduced fecundity in all species, but the most severe effects were observed in the boreal species, which was the most heat-sensitive (de Leon et al., 2023).

Another common experimental approach includes monitoring of freshwater populations under semi-natural conditions (outdoor mesocosms). Mean body size of the plankton species showed variation in response to the elevated temperatures, depending on their cultivation environment. A consistent decline in mean body size has been observed under temperature increases of up to 6°C compared to ambient conditions, including in both single-species setups and more complex multispecies zooplankton communities (Cambronero et al., 2018; Albini et al., 2025). However, increases in body size at maturity have also been reported under moderate warming (+4°C), likely due to interactions between abiotic factors (e.g., temperature) and biotic factors such as interspecific competition (Van Doorslaer et al., 2010).

In addition, numerous studies indicate that the effects of elevated temperature on fecundity vary with latitude. While boreal species suffered a reduction of fecundity at higher temperatures (Giovannini et al., 2023), this correlation was positive for temperate-origin species (Van Doorslaer et al., 2007; Wang et al., 2023; but see Cambronero et al., 2018). In contrast, the response of age at maturation to elevated temperatures appears to be relatively uniform regardless of the environmental conditions, with experimental studies repeatedly reporting earlier maturation at higher temperatures (Van Doorslaer et al., 2007; Cambronero et al., 2018; Giovannini et al., 2023; Wang et al., 2023).

Despite substantial experimental research, significant gaps remain in our understanding of how life history traits respond to temperature change, particularly because most studies have focused on abrupt shifts in temperature, typically involving a single-step increase. To better reflect ecological reality, we need experimental designs that examine the effects of gradually and continuously changing thermal environments over longer time scales. This is important for two reasons. First, the rate and therefore the impact of global warming varies across regions, showing positive correlation with both latitude (Root et al., 2003; Deutsch et al.,

2008) and altitude (Pepin et al., 2022). Second, experimental studies have shown that the adaptive dynamics of populations to stressful environments are strongly influenced by the rate of environmental change (Collins and de Meaux, 2009; Lindsey et al., 2013). Because eco-evolutionary dynamics are shaped by the rate of environmental change, life history traits may respond differently under gradual shifts than they do under the abrupt changes typically modeled in experimental studies.

Experimental studies with microbial systems highlight the importance of warming rate for adaptive dynamics. For example, populations exposed to gradual temperature increases are more likely to persist and evolve higher thermal tolerance, consistent with predictions of evolutionary rescue (Killeen and Kaltz, 2017). Similarly, slower environmental change can promote growth at high temperatures but may come at a cost to long-term survival due to correlated selection (Liukkonen et al., 2021). These findings underscore how the pace of environmental change can shape evolutionary outcomes. However, such studies have primarily focused on growth rate in unicellular organisms. We still lack a clear understanding of how more complex life history traits, such as body size, fecundity, and age at maturation, respond to gradual thermal change, particularly in multicellular species. Most experimental work on these traits has been based on abrupt or one-step temperature increases, limiting ecological relevance.

In addition, the extent to which these trait changes result from phenotypic plasticity versus genetic adaptation remains poorly understood. Organisms may respond to thermal shifts through adaptive plasticity, adjusting trait expression without genetic change (Lalejini et al., 2021). For example, *Daphnia magna* populations can modify hemoglobin expression in response to temperature (Yampolsky et al., 2014). Yet, genetic responses to warming have also been observed in *D. magna*, including short-term adaptation in laboratory populations (Seefeldt and Ebert, 2019) and long-term responses in the wild (Geerts et al., 2015).

Importantly, plasticity itself can evolve (Schaum et al., 2022), making it difficult to disentangle the roles of plasticity and genetic change in trait evolution. Properly distinguishing between these mechanisms requires experiments that track life history traits under common garden conditions. To date, studies that have attempted such distinctions have almost exclusively focused on abrupt temperature shifts (for example, Van Doorslaer et al., 2010), leaving the effects of gradual temperature deterioration largely unexplored.

To address these gaps, we conducted a controlled multigenerational evolutionary experiment with three clonal lines of *D. magna* to test how multiple life-history traits respond to gradually increasing temperatures, a scenario more representative of ongoing climate change than abrupt thermal shifts. Populations were propagated through non-overlapping generations under either constant conditions (control) or a warming regime that increased by 1 °C every 4–6 generations. We monitored fecundity, somatic growth rate, age at maturation, body size, and extinction risk across generations, and used a reciprocal transplant experiment to disentangle genetic adaptation from phenotypic plasticity. This design allowed us to evaluate both the pace and nature of trait change under sustained warming. Our results provide insight into how gradual thermal deterioration can alter life-history strategies in a key model species, informing predictions of population responses to ongoing climate change.

2. Materials and Methods

2.1 Experimental procedures

Model Organism and Culture Conditions

Daphnia magna Straus (1820) is a planktonic freshwater crustacean (class Phyllopoda) that reproduces via cyclic parthenogenesis, producing clutches of up to 100 eggs after each adult molt (Ebert, 2005). Eggs develop directly into juveniles (neonates), bypassing a resting stage. The generation time is temperature-dependent, with the first clutch typically produced

171 5–10 days after birth at the optimal temperature of 20°C (Ebert, 2005). Due to its clonal
172 reproduction and tractable life cycle, *D. magna* is a widely used model for studying
173 phenotypic plasticity and local adaptation (Yampolsky et al., 2014).

174 Three clonal lines were used: HU-HO-2 and TR-EG-1 (University of Basel), and An (Middle
175 East Technical University, Ankara). Cultures were maintained in 250 ml glass beakers with
176 150 ml of ADaM medium (Klüttgen et al., 1994), replenished weekly. All populations were
177 kept under a 16:8 h light:dark cycle and fed with *Chlamydomonas reinhardtii* at 50,000
178 cells/ml three times per week. Temperature treatments varied according to experimental
179 design.

180 **Preliminary Thermal Performance Assay**

181 Before the main experiment, we conducted a baseline thermal tolerance assay following
182 Seefeldt and Ebert (2019) to evaluate the performance limits of the three *D. magna* clones
183 and to inform temperature treatment design.

184 Five replicate populations per clone (HU-HO-2, TR-EG-1, An) containing five adults were
185 maintained at 22°C in 100 ml beakers containing ADaM medium, replenished weekly.
186 Populations were acclimated to increasing thermal conditions by raising the temperature to
187 25°C in week one and to 28°C in week two. Juvenile females (1–3 days old) were then
188 sampled from each replicate and individually exposed to one of four test temperatures (30°C,
189 31°C, 32°C, or 33°C) in 60 ml jars. Each temperature was replicated at least three times per
190 clone. Over two weeks, individuals were monitored daily for evidence of reproduction
191 (presence of eggs in brood chambers or neonates in the medium). This assay established
192 the upper thermal limits for each clone and confirmed elevated extinction risk beyond 30°C
193 (see Results section).

194

Experimental Design and Temperature Treatments

A total of 24 experimental populations were established, comprising 12 treatment and 12 control lines (3 clones × 4 replicates each). Each population consisted of 15 individuals per generation. Non-overlapping generations were maintained by transferring the first 15 neonates to a fresh beaker of medium at each generation (see Figure 1 for more details regarding the experimental design and transfer procedures).

In the treatment group, temperature increased gradually over time, beginning at 26°C. Temperature was raised by 1°C every 4 generations until it reached 29°C, and then by 1°C every 6 generations thereafter. This slowing of the rate beyond 29°C was intended to minimize heat-induced extinction risk, based on prior findings (Mitchell and Lampert, 2000) and the results of our baseline assay.

Control populations were maintained at a constant 26°C for the duration of the experiment.

Life History Trait Assays

To quantify evolutionary responses to gradually deteriorating (increasing) temperature, life history traits were measured at five timepoints: 26°C, 27°C, 28°C, 29°C, and 30°C, corresponding to generations 4, 8, 12, 18, and 24 in the treatment populations.

At each timepoint, the first three mature individuals (i.e., individuals with eggs visible in the brood chamber) were isolated from each population and placed individually in 60 ml jars containing ADaM at the population's current temperature. Each female’s first and second clutches were counted to estimate fecundity. The first clutch was discarded after hatching to avoid size bias, as first-clutch offspring tend to be smaller (Ebert, 1991). After release of the second clutch, the mother was removed.

Six neonates from the second clutch were randomly selected and reared individually in the same jars. Pilot tests had shown that higher densities could trigger unwanted male

219 production or diapause (ephippia) formation. Neonates were photographed at birth and again
220 at maturation using brightfield microscopy (BRESSER Advance ICD Stereo Microscope with
221 Full HD eyepiece camera at 10x magnification). Body size was measured via gut length (GL)
222 of the relaxed animals in ImageJ and converted to dry mass (DM) using the equation: DM =
223 $0.00679 \times GL^{2.75}$ (Fossen et al., 2018).

224 Neonates were monitored daily for development and survival. Age at maturation was defined
225 as time when the first eggs were visible in their brood chambers. Lifespan was defined as
226 the number of days from birth until death. Somatic growth rate was calculated as the
227 difference in dry mass between birth and maturation, divided by the number of days between
228 the two measurements. The clutch interval (time between first and second clutches) was also
229 recorded.

230 **Extinction Monitoring**

231 Each population was visually inspected daily. Populations were recorded as extinct when no
232 live individuals remained in the beaker.

233 **Reciprocal Transplant Assay**

234 To disentangle the effects of phenotypic plasticity and genetic adaptation, a reciprocal
235 transplant was conducted at generation 25. Three mature individuals were sampled from
236 each control and treatment population. From each adult, six second-clutch neonates were
237 reared in the environment opposite to their lineage's ancestral condition.

238 Control-derived neonates were raised at 30°C, representing novel heat exposure. Treatment-
239 derived neonates were raised at 26°C, representing a return to ancestral temperature. These
240 individuals were reared until maturation, and their adult body size and somatic growth rate
241 were quantified using the same methods as described above.

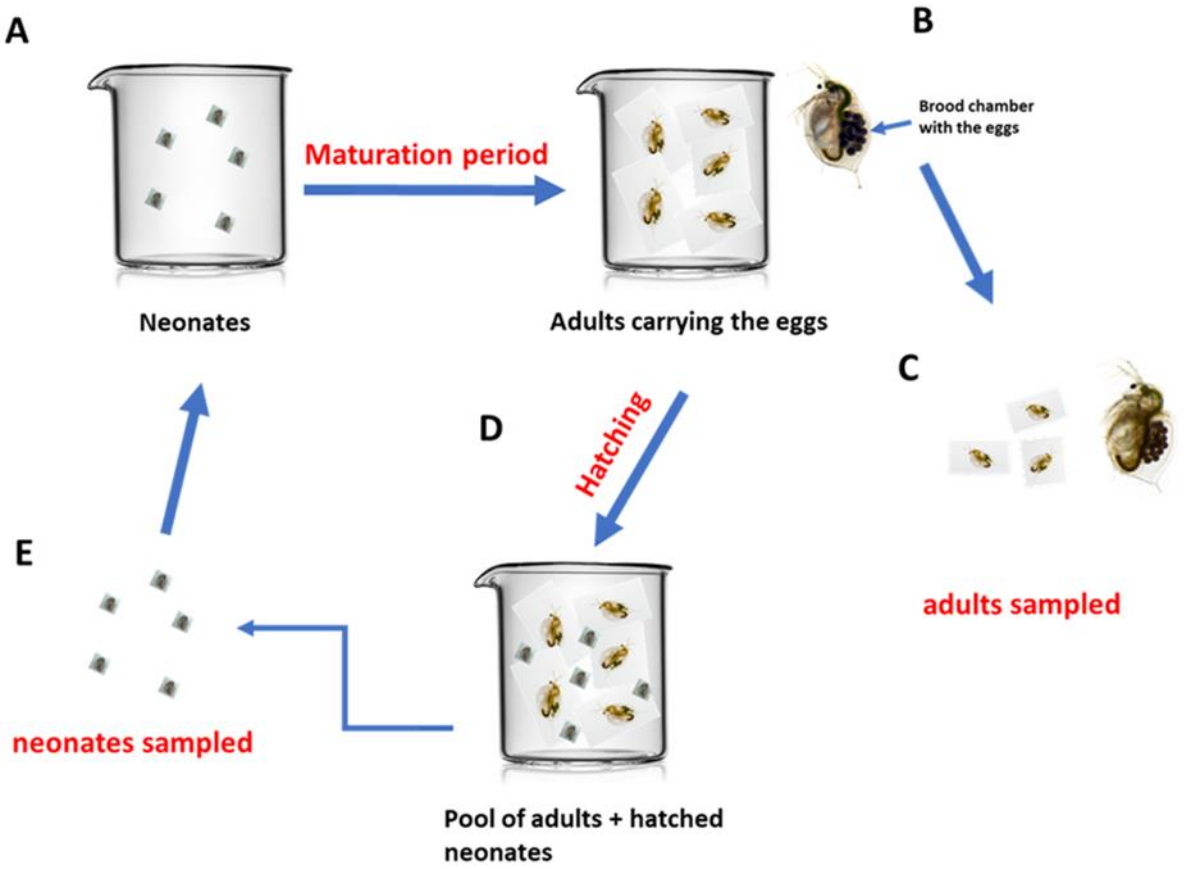

Figure 1 – Transfer procedure and data collection regime of experimental populations; (A) 15 neonates per beaker were cultivated until they reached maturation (B), defined as the time when the first eggs appeared in their brood chambers (note that both neonates and adults are downscaled relative to the beakers for an easier representation); C) after each round of temperature increase, 3 adults were sampled for the main assays for quantification of life history traits; D) hatching (the release of the newborn neonates from the brood chambers) resulted in formation of pool of both adults and neonates (note that here, for simplicity, the ratio of newborns vs adults is drawn as 1:1; typically, a larger number of neonates is present); E) after each new generation of neonates appeared, 15 individuals were sampled and transferred to a new beaker (i.e. the next generation of an experimental population has been initiated.

2.2 Data Analysis

2.2.1 Baseline Heat Tolerance (Preliminary Thermal Performance Assay)

To assess the baseline thermal tolerance of ancestral clones, we fit a generalized linear mixed model (GLMM) with a binomial error distribution. The presence or absence of offspring

(eggs or neonates) was treated as a binary response variable. Clone identity was included as a fixed categorical predictor, and temperature was included as a continuous covariate.

2.2.2 Life History Trait Assays

The datasets containing life history trait values from the main assays were analyzed using linear mixed models (LMMs). The models included “treatment” and “clone” as categorical fixed effects, “generation” as a continuous fixed effect, and “population” and “individual” as categorical random effects to account for hierarchical structure. Each of the seven life history traits was modeled separately as a continuous response variable. Given the limited sample sizes within each dataset, model parameters were estimated using restricted maximum likelihood (REML). The potential covariation among life history traits in the experimental populations was examined using Pearson’s correlation test, based on pairwise comparisons between traits.

Span between the clutches

Two extreme values were identified as potential outliers using Grubbs’ test ($p < 0.05$) and were excluded from model comparison.

Neonate body size

Four extreme values were identified as potential outliers using Grubbs’ test ($p < 0.05$) and were excluded from model. Model selection was based on a comparison of AIC values and a likelihood ratio test. A simplified model excluding interaction terms (AIC = -1274.3) was favored over a more complex model that included the interactions (AIC = -1263.1), as the later did not significantly improve model fit according to a likelihood ratio test (χ^2, P = 0.9).

Adult body size

A single extreme value was identified as potential outliers using Grubbs' test ($p < 0.05$) and was excluded from model.

The development time

Two extreme values were identified as potential outliers using Grubbs' test ($p < 0.05$) and were excluded from model comparison. A simplified model excluding interaction terms (AIC = 1198.7) was favored over the full interaction model (AIC = 1208.5; LRT P = 0.77).

Somatic growth rate

Model selection was based on a comparison of AIC values and a likelihood ratio test. A simplified model excluding interaction terms (AIC = - 107.35) was favored over a more complex model that included the interactions (AIC = -97.86), as the later did not significantly improve model fit according to a likelihood ratio test (χ^2, P = 0.72).

2.2.3 Extinction dynamics

The extinction dynamics of each treatment group were analysed by fitting Weibull regression model with two fixed factors: 'treatment' and 'clone'. The interaction between the factors was included in the model. The best fit was obtained using extreme hazard function.

2.2.4 Reciprocal Transplant Assay

The dataset from the reciprocal transplant assay was analyzed using linear mixed models (LMMs). Fixed effects included "environment" (ancestral or novel), "treatment" (control or thermal deterioration), and "clone" (all categorical). Random effects accounted for hierarchical structure by including "population" and "individual" as categorical variables. Each of the two monitored life history traits—age at maturation and growth rate—was modeled separately as a continuous response variable. Given the limited number of observations in

305 each dataset, model parameters were estimated using restricted maximum likelihood
306 (REML).

307 All the analyses were carried out using RStudio (version 2024.12.1; R Core Team, 2017).
308 The packages: 'gplots' (v3.1.3; Warnes et al., 2022), 'ggplot2' (v3.4.2; Wickham, 2016),
309 'plothrix' (v3.8.2; Lemon, 2006), 'RColorBrewer' (v1.1-3; Neuwirth, 2022) were used for
310 visualization of the datasets: the high-temperature tolerance of the ancestors; the main
311 assays; the reciprocal transplant experiment. The package 'plyr' (v1.8.8; Wickham, 2011)
312 was used for summarizing the data prior to visualization. 'Survival' (v3.2-13; Therneau, 2021)
313 and 'survminer' (v0.4.9; Kassambara et al., 2021) were used for the analysis and
314 visualization, respectively, of extinction dynamics. For fitting of the mixed models, the
315 packages lme4 (Bates et al., 2014) and lmerTest (Kuznetzova et al., 2017) were used.

316 **4. Results**

317 **4.1 Preliminary thermal performance assay**

318 The reproductive ability of the ancestral clones depended on the test temperature
319 (Generalised Linear Mixed Model; df = 1; $F_{1,247}$ = 11.76; P < 0.001). While the average
320 successful reproduction rate at the temperature range between 30°C and 32°C was 28%,
321 only a single individual (0.01%) successfully reproduced at 33°C (Figure 2A). We detected a
322 significant effect of the genetic background of tested individuals (Generalised Linear Mixed
323 Model; df = 2; $F_{2,246}$ = 6.7; P = 0.001) (Figure 2B), with the Tr clone achieving 2.66 times
324 higher percentage of successfully reproduced individuals relative to the other two clones
325 combined (z = 3.154; P = 0.002).

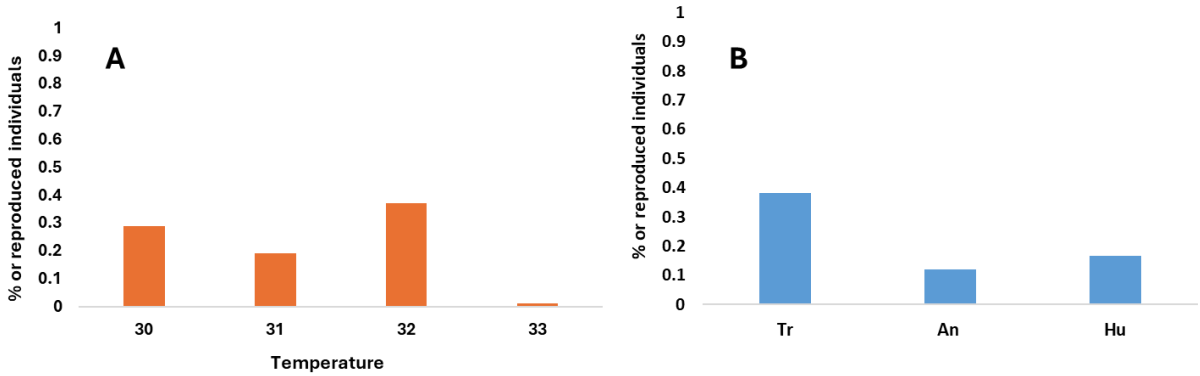

Figure 2 – Assay of the ancestral clones for the high-temperature tolerance; A) Percentage of successfully reproduced individuals per test temperature; **B)** Percentage of successfully reproduced individuals per ancestral clone.

4.2 Main evolutionary experiment

Population extinction dynamics

Populations in the warming treatment tended to go extinct earlier than controls, although this effect was marginal (Weibull regression; df = 1; χ^2 = 3.04; P = 0.08) (Figure 3A). Clone identity had a similarly marginal effect on extinction rates (Weibull regression; df = 2; χ^2 = 5.02; P = 0.08) (Figure 3B).

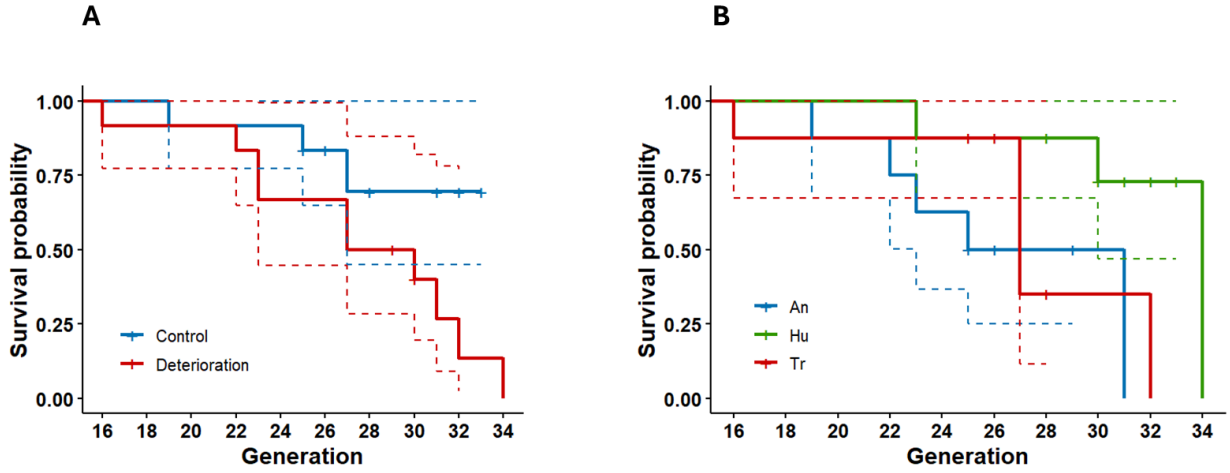

Figure 3 – Extinction dynamics of experimental populations: per treatment group (A); per clone (B); extinctions are plotted against the generations upon each extinction event; 95% confidence interval for each Kaplan-Meier survival curve is represented with the dotted lines (upper and lower boundary).

342 **Life-history trait responses**

343 ***Fecundity***

344 We saw significant overall decline in fecundity of the populations (LMM, df = 1; F = 41.33; P
345 < 0.001) (Figure 4A). The average fecundity of experimental populations was significantly
346 higher (by 12%) relative to that of the control populations (LMM, df = 1; F = 8.3; P = 0.004)
347 (Figure 4B). While we did not detect the significant main effect of the genetic component (i.e.
348 clone) (LMM, df = 2; F = 0.02; P = 0.98), there was significant clone-by-treatment interaction
349 (LMM; df = 2; F = 4.06; P = 0.02) (Figure 4D). We detected the relative advantage of Hu and
350 Tr clones with respect to An clone, manifested through their higher fecundity in the
351 deteriorating environment (An vs Hu, t = 2.58, P = 0.01; An vs Tr, t = 2.35, P = 0.02). We
352 detected a significant generation-by-treatment interaction (LMM; df = 1; F = 4.59; P = 0.03).
353 The fecundity of the experimental group was consistently higher than that of the control
354 group, except during the 24th generation – assay (which corresponded to the temperature of
355 30°C), during which we detected a 34% lower fecundity in the experimental group relative to
356 that of the control group (Figure 4C).

357 ***Span between the clutches***

358 We saw no overall change in span between clutches (LMM, df = 1; F = 1.33; P = 0.25). While
359 neither the treatment nor the genetic background showed a significant main effect, we
360 detected a marginally significant clone-by-treatment interaction (LMM, df = 2; F = 2.96; P =
361 0.05) on the span between the clutches. A pairwise comparison indicated a significant
362 decline of the span between clutches of Tr clone when subjected to the deteriorating vs
363 control conditions (t = 2.29, P =0.02) (Figure 5A).

364

365

366 ***Neonate body size***

367 We saw no overall change in mean neonate body size (LMM, df = 1; F = 1.27; P = 0.26).
368 Likewise, neither the treatment (LMM, df = 1; F = 1.84; P = 0.18) nor the genetic background
369 (LMM, df = 2; F = 1.22; P = 0.3) have affected the mean neonate body size.

370 ***Adult body size***

371 We saw a significant overall decline in mean body size (LMM, df = 1; F = 17.63; P < 0.001)
372 (Figure 5B), which was not affected by the treatment (LMM, df = 1; F = 1.48; P = 0.23). We
373 detected a significant effect of the genetic background (LMM, df = 2; F = 3.37; P = 0.04) on
374 the mean adult body size, reflected in a higher decline in An clone relative to the other two
375 clones (Figure 5C). We detected a significant clone-by-treatment interaction (LMM, df = 2; F
376 = 3.12; P = 0.047) (Figure 5D), as the relative advantage of Tr clone with respect to the other
377 two clones (higher mean body size) increased in the deteriorating environment (Tr vs Hu, t =
378 2.29, P = 0.02; Tr vs An, t = 1.99, P = 0.048).

379 ***The development time***

380 We detected a significant overall increase in development time (LMM, df = 1; F = 12.56; P =
381 0.005) (Figure 6A). The development time of the populations depended on the treatment.
382 The experimental populations developed 3 days earlier on average relative to the control
383 populations (23% difference) (LMM, df = 1; F = 7.66; P = 0.006) (Figure 6B). While the
384 overall effect of the genetic background on development time was only marginally significant
385 (LMM, df = 2; F = 2.93; P = 0.05), post hoc comparison revealed a significant difference
386 between An and Tr clone (t = 2.41, P = 0.02) (Figure 5C).

387

388

389 ***Somatic growth rate***

390 We detected a significant overall decline of somatic growth rate (LMM, df = 1; F = 23.05; P <
391 0.001) (Figure 6D). The somatic growth rate of the populations depended on the treatment.
392 The experimental populations had a 14% faster somatic growth rate relative to that of the
393 control populations (LMM, df = 1; F = 6.23; P = 0.01) (Figure 6E). We detected a marginally
394 significant effect of the genetic background on somatic growth rate (LMM, df = 2; F = 2.43; P
395 = 0.09). However, pairwise comparison indicates a significant difference between the growth
396 rate of An and Tr clones (t = - 2.14, P =0.03) (Figure 6F).

397 ***Lifespan***

398 We saw a significant overall decline of the lifespan (LMM, df = 1; F = 35.59; P < 0.001)
399 (Figure 7A). The elevated temperatures significantly reduced the lifespan of experimental
400 populations (by 21%) relative to that of control (LMM, df = 1; F = 24.92; P < 0.001) (Figure
401 7B). We detected a significant interaction between the treatment and generation (LMM, df =
402 1; F = 10.21; P = 0.002), manifested through the highest difference in the lifespan between
403 the treatment groups during the 12th generation assay (28°C) (Figure 7C). We detected a
404 significant main effect of the genetic component (LMM, df = 2; F = 7.43; P < 0.001), reflected
405 in the longer lifespan of An clone relative to that of both Tr clone (t = - 3.83; P < 0.001) and
406 Hu clone (t = - 3.16; P = 0.002) (Figure 7D).

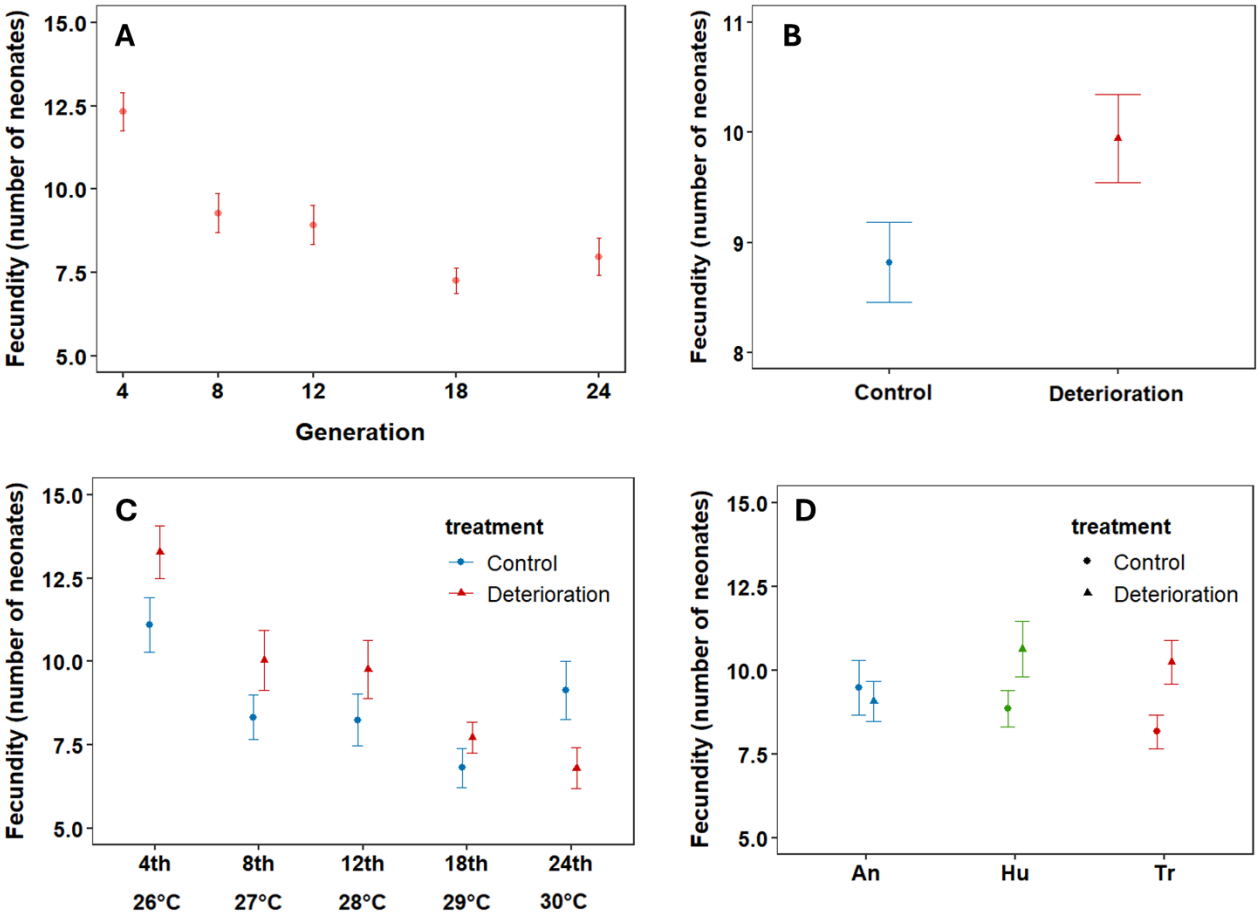

Figure 4 – Average fecundity (the number of neonates within the first two clutches) of the populations: per generation (A); per treatment group (B); per treatment group during each assay (C): the X axis contains the number of generations that each assayed population underwent, with the temperature degree that the experimental populations experienced during the assay stated below; per clone, per treatment group (D); the bars represent standard error of the mean.

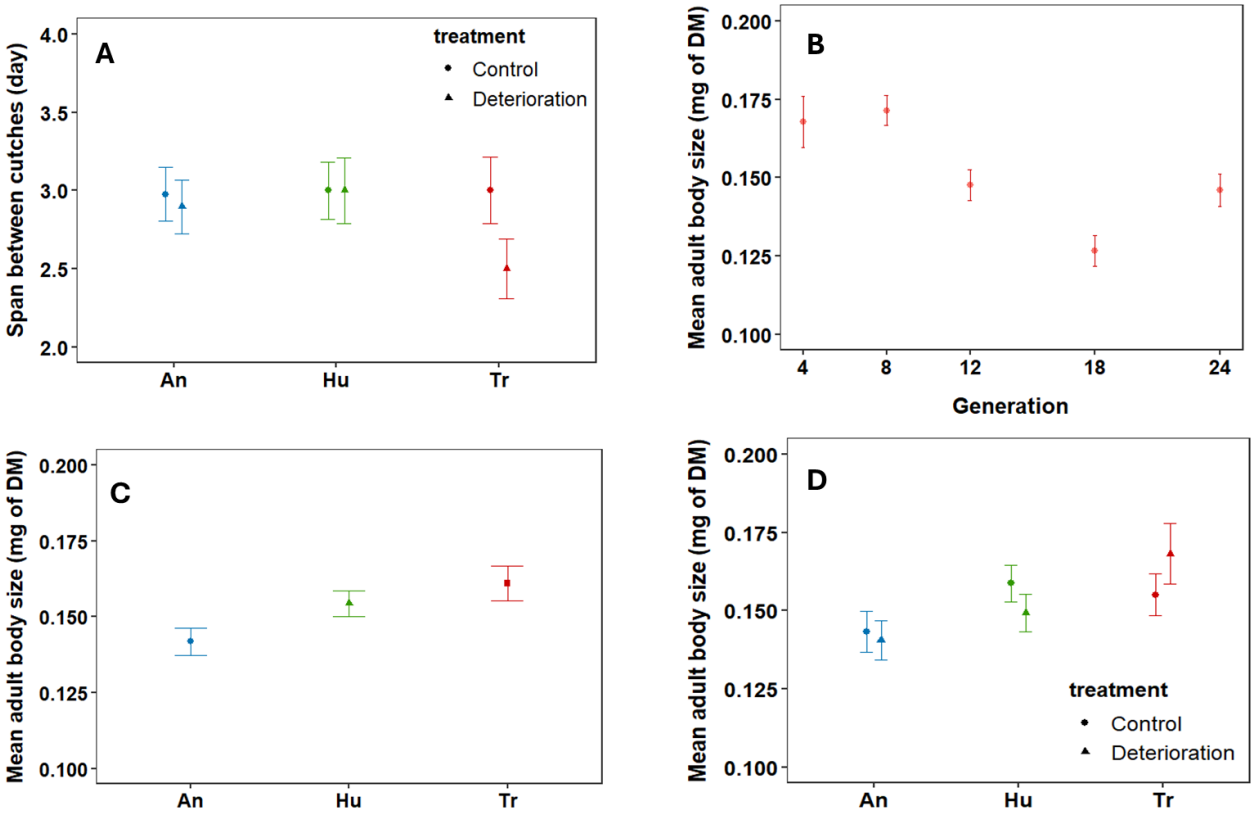

Figure 5 – Average span between the first two clutches (day) of the populations (A); Average adult body size (mg od DM) of the populations (B-D): per generation (B); per clone (C); per clone per treatment group; the bars represent standard error of the mean.

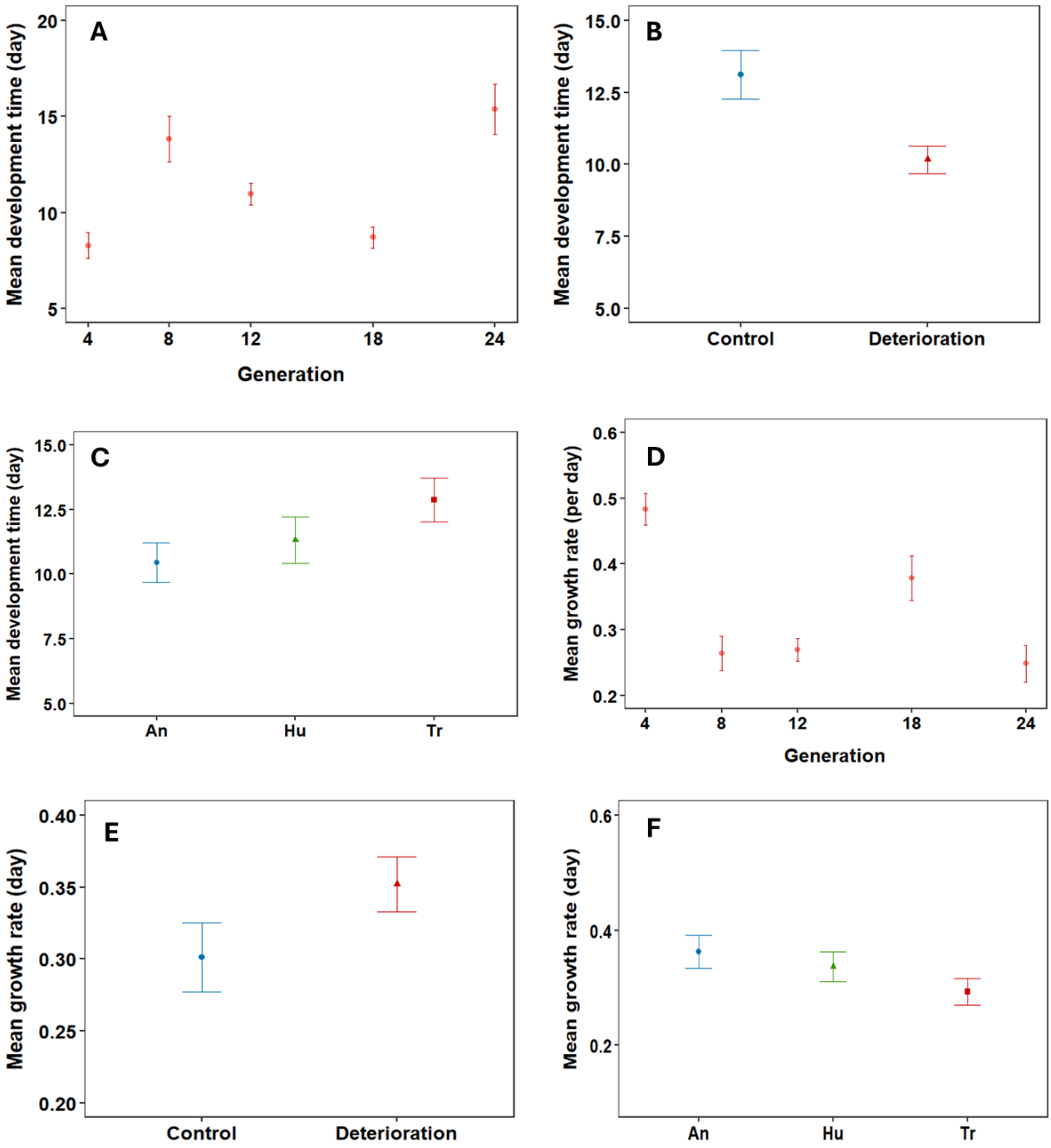

Figure 6 – Average development time (day) of the populations (A - C): per generation (A); per treatment group (B); per clone (C); **Average growth rate (per day) of the population (D - F)**: per generation (D); per treatment group (E); per clone (F); the bars represent standard error of the mean.

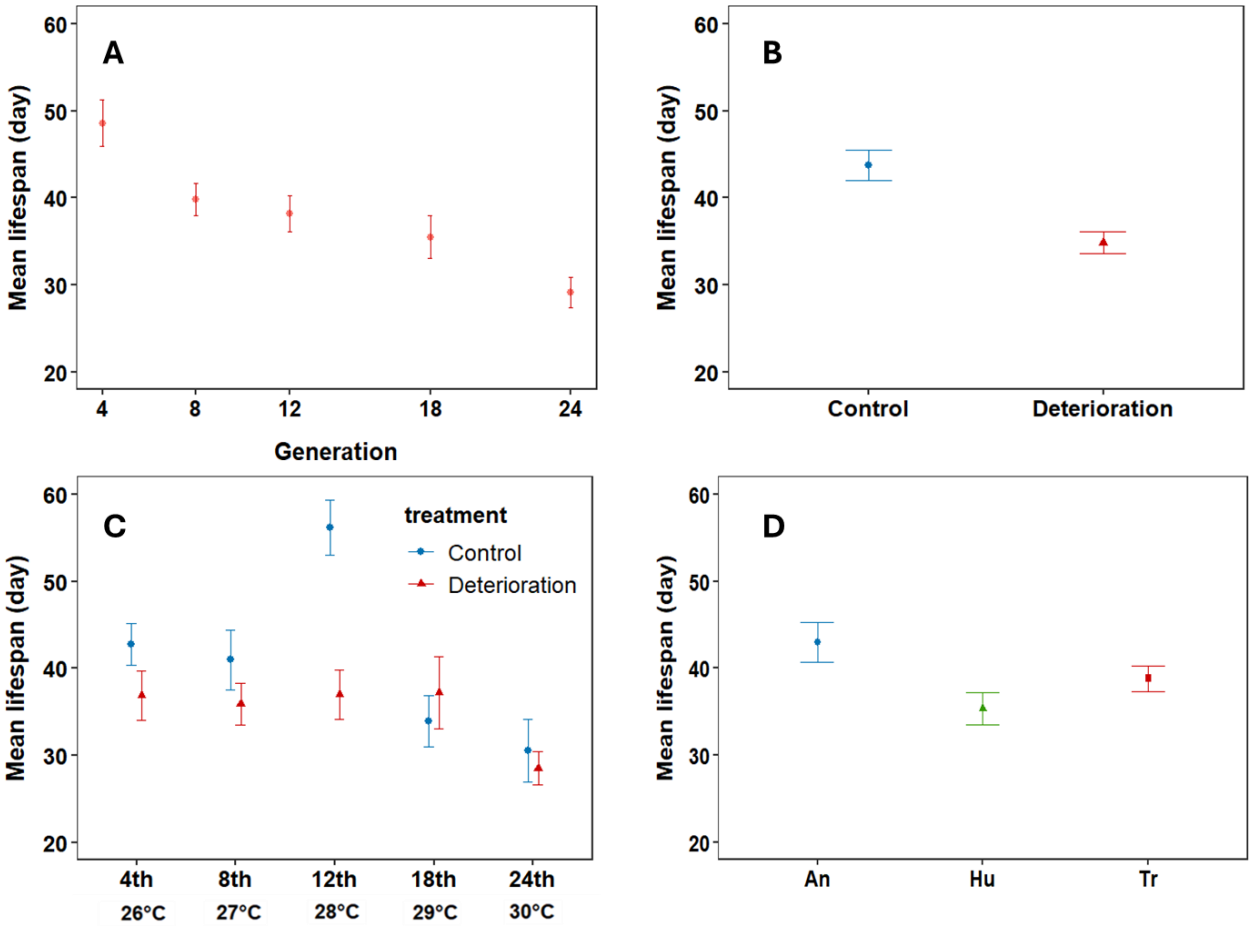

Figure 7 – Average lifespan (day) of experimental populations: per generation (A); per treatment group (B); per treatment group during each assay (C): the X axis contains the number of generations that each assayed population underwent, with the temperature degree that the experimental populations experienced during the assay stated below; per clone (D); the bars represent standard error of the mean.

Covariation between the life history traits of experimental populations

We detected the difference in the relative contribution of relative growth (size increment) and development time on somatic growth rate. Pearson's correlation test revealed a strong negative correlation between somatic growth rate and development time ($r = -0.86$; $t(60) = -13.41$; $P < 0.001$) (Figure 7B), and no significant correlation between somatic growth rate and relative growth ($r = -0.05$; $t(60) = -0.38$; $P = 0.7$) (Figure 7A).

We detected significant negative correlation between somatic growth rate and neonate body size ($r = -0.31$; $t(60) = -2.52$; $P = 0.01$) (Figure 7C), and a significant negative correlation between somatic growth rate and adult body size ($r = -0.49$; $t(60) = -4.38$; $P < 0.001$) (Figure 7D).

We detected significant positive correlation between development time and adult body size ($r = 0.64$; $t(60) = 6.42$; $P < 0.001$) (Figure 7E). Likewise, we saw a positive correlation between fecundity and adult body size ($r = 0.43$; $t(67) = 3.92$; $P = 0.002$) (Figure 7F).

We found no significant correlation between lifespan and development time ($r = -0.04$; $t(79) = -0.34$; $P = 0.73$); lifespan and somatic growth rate ($r = 0.02$; $t(33) = 0.09$; $P = 0.93$); lifespan and fecundity ($r = 0.06$; $t(69) = 0.52$; $P = 0.61$); fecundity and span between clutches ($r = -0.28$; $t(272) = -0.29$; $P = 0.78$); adult body size and span between clutches ($r = -0.03$; $t(67) = -0.26$; $P = 0.79$); somatic growth rate and fecundity ($r = -0.08$; $t(60) = -0.6$; $P = 0.55$).

4.3 The reciprocal transplant experiment

Mean adult body size of the populations depended on the treatment (LMM, df = 1; $F = 9.05$; $P = 0.004$). The tested control populations had a 17% larger mean body size relative to that of experimental populations. The size difference did not depend on the environment, as we detected no environment-by-treatment interaction (LMM, environment X treatment; df = 1; $F = 0.32$; $P = 0.57$) (Figure 8A).

By contrast, the mean growth rate of the populations did not depend on the treatment (LMM, df = 1; $F = 0.05$; $P = 0.82$). We detected no significant environment-by-treatment interaction (LMM, environment X treatment; df = 1; $F = 0.96$; $P = 0.33$) (Figure 8B).

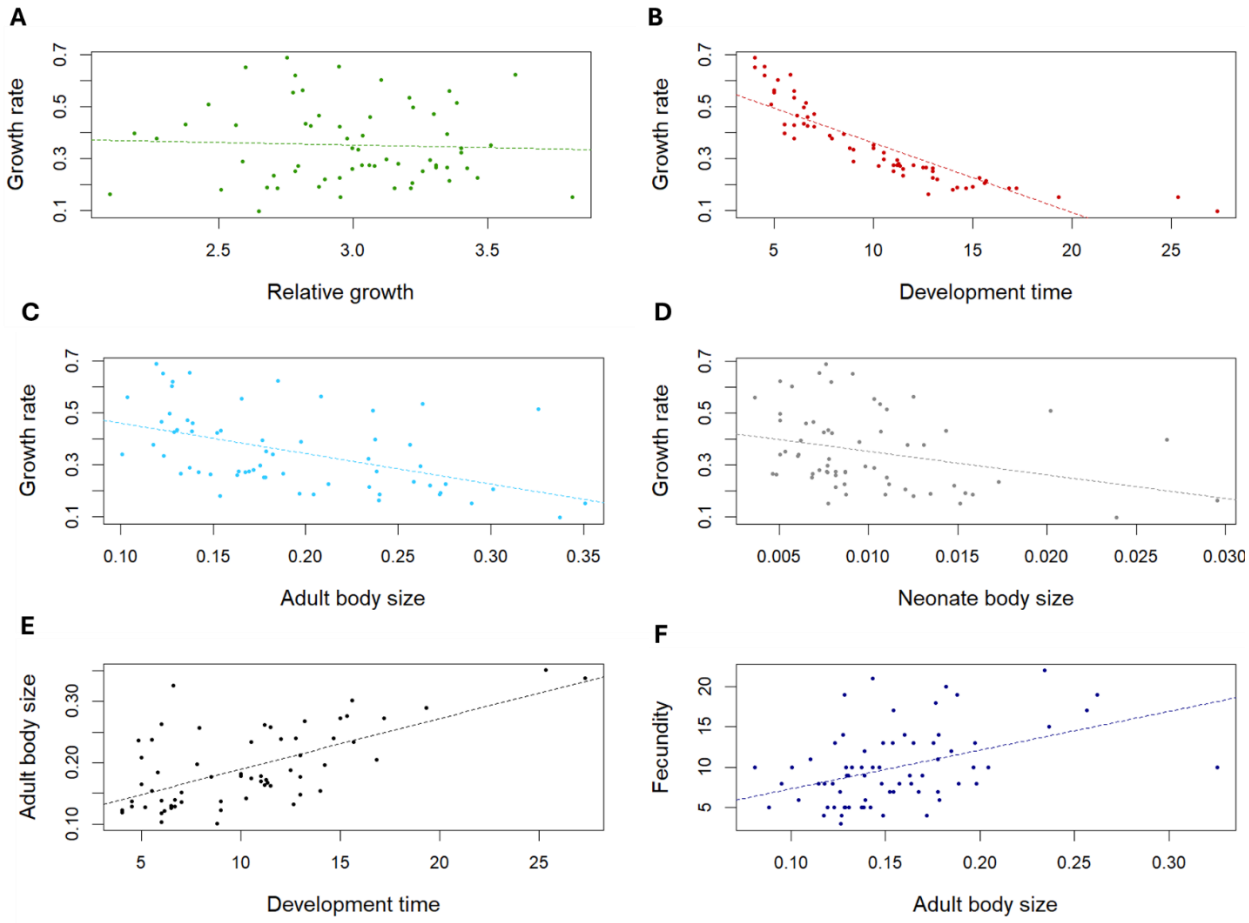

Figure 7 – Significant correlations between the pairs of the life history traits of experimental populations; A) Somatic growth rate (per day) plotted against relative growth (mg of DM); **B)** Somatic growth rate (per day) plotted against development time (day); **C)** Somatic growth rate (per day) plotted against adult body size (mg of DM); **D)** Somatic growth rate (per day) plotted against neonate body size (mg of DM); **E)** Adult body size (mg of DM) plotted against development time (day); **F)** Fecundity (number of neonates within the first two clutches) plotted against adult body size (mg of DM).

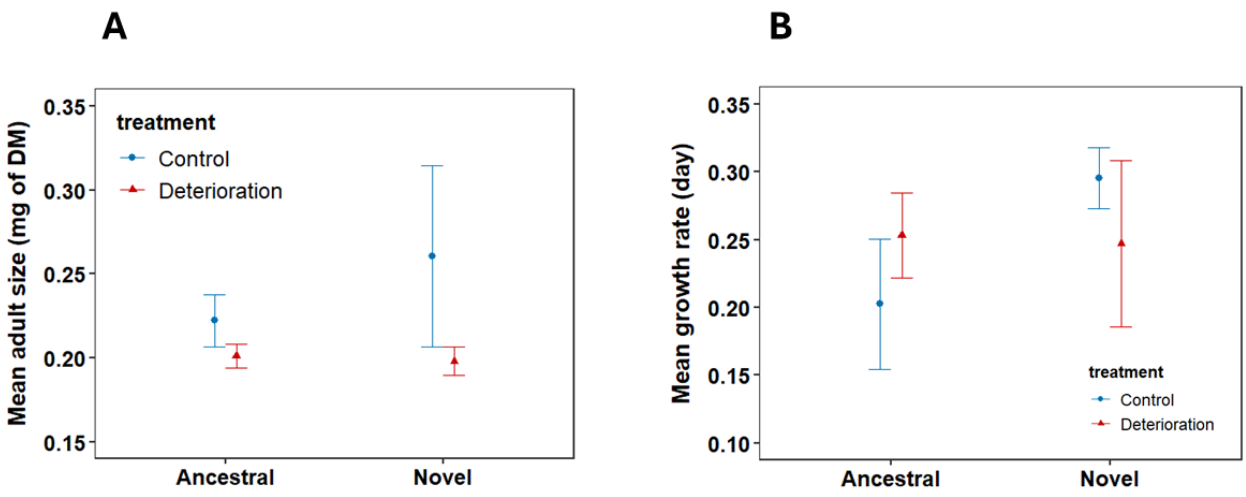

Figure 8 – The reciprocal transplant experiment; A) Mean adult size of control and experimental populations kept in their ancestral and novel environment; **B)** Mean growth rate of control and experimental populations kept in their ancestral and novel environment; the bars represent standard error of the mean.

Discussion

The main objective of our study was to quantify the responses of the seven life history traits under the gradually deteriorating thermal conditions, and to identify the mechanism(s) underlying these responses. Overall, experimental populations showed increased fecundity, matured earlier and showed a faster growth rate, whilst suffering a reduction in their lifespans. The populations showed the plastic response (manifested through variation of the trait values among the clones in the deteriorating vs control environment) with respect to fecundity, adult body size and span between clutches.

The assay of the ancestral clones for the high-temperature tolerance revealed the detrimental effect of the temperatures between 30°C and 32°C on the reproductive ability of the clones, constituting their upper tolerance limit. This corroborates the results of Seefeldt and Ebert (2019), who found that the maximal reproduction temperature of 123 *D. magna*

clones tested ranged between 26 and 32°C. The extinction rate of our populations during the main experiment parallels the results of the high-temperature tolerance assay. Two thirds of our populations went extinct whilst being subjected to temperatures of 30°C and 31°C. Furthermore, the initial difference in the high salt tolerance detected among the three clones was not a good predictor of their extinction rate in the main experiment. Taken together, these results confirm that elevated temperatures entailed a stressful effect on our populations, manifested through the decline in both reproductive output and survival probability.

Experimental populations increased fecundity at elevated temperatures relative to the control populations, except at the highest temperature of 30°C, characterized by the shift of the advantage between the treatment groups. The positive correlation of the number of eggs or hatched offspring with the temperature has been reported in a number of studies, including the ones using both insects (Musolin et al., 2010; Ciota et al., 2014; de Leon et al., 2023;) and plankton species (Van Doorslaer et al., 2007; Giovannini et al., 2023; Wang et al., 2023) as model organisms (but see Cambronero et al., 2018, for the opposite pattern detected). In natural conditions, an increase in fecundity in *D. magna* with temperature is advantageous as it maximizes fitness in a resource rich window (where high temperature correlates with food availability) (Lampert, 2011). Moreover, of all life history traits monitored, phenotypic plasticity in fecundity (clone X treatment interaction) was the most pronounced, as two clones showed an increase in the relative advantage under elevated temperature.

The main effect of an increasing temperature on the span between clutches was limited (a 7% reduction relative to the control populations). This contrasts with previous studies which detected a significant decline of an interclutch span as a response to elevated temperatures (Betini et al., 2019; Bowman and Post, 2023). However, these studies did not directly compare the effect of a temperature across multiple clones. In our study, we detected a

519 significant clone-by-treatment interaction, as a single clone (Tr) has significantly reduced the
520 span between clutches in elevated temperatures. This indicates that a decrease in the span
521 between clutches might be a relevant response in the context of a temperature increase,
522 however additional studies using a higher number of clones are required.

523 We detected an overall reduction in mean adult body size with the temperature, observed in
524 a number of studies using various invertebrate species (for example, Musolin et al., 2010;
525 Laughton et al., 2017; Walczynska et al., 2017), including *D. magna* (for example,
526 Cambronero et al., 2018; Wang et al., 2023; Albini et al., 2025), However, we saw no
527 difference between the treatment groups. A plausible explanation is that this temperature
528 window (the 26 - 30°C range) lies near the upper end of the thermal performance curve for
529 *Daphnia* (Seefeldt and Ebert, 2019; Wang et al., 2023), where body size plasticity may
530 already be constrained. Furthermore, there is evidence that resource limitations become
531 stronger at higher temperatures and larger body size, causing growth to decelerate at a
532 smaller size at warmer temperatures (Hoefnagel et al., 2018).

533 Despite an overall reduction in mean adult body size in both treatment groups, there is
534 evidence that a larger body size was advantageous at elevated temperatures, through its
535 effect on fecundity (a strong positive correlation). Moreover, we detected a significant plastic
536 response in both traits, manifested through a simultaneous increase of both mean body size
537 and fecundity of Tr clone when subjected to the elevated temperature.

538 We saw an overall increase in mean development time and a decline in growth rate of the
539 populations. However, experimental populations showed both a significant decline of
540 development time and acceleration of somatic growth rate relative to those in control
541 populations. The combination of these two responses at elevated temperatures has been
542 detected in several studies (for example, Henning-Lucass et al., 2016; Hoefnagel et al.,
543 2018). Moreover, these traits showed a high negative correlation, driven by the difference in

the response of An clone (a decrease in development time and acceleration of growth rate) vs Tr clone (an increase in development time and a decline in growth rate)

We saw a significant positive correlation between development time and adult body size; a significant negative correlation between somatic growth rate and adult body size. This suggests an advantage of a delayed reproduction, as the clone which reached maturation later (Tr) not only had a higher mean fecundity (via increase in body size), but also a shorter span between clutches. By contrast, the clone with the fastest growth rate (An) showed the slowest development, coupled with the lowest average body size and fecundity.

While elevated temperature elicited a general shift in life history traits – namely reduced development time, accelerated somatic growth rate, and smaller adult body size – these changes appear maladaptive – given the positive correlations between adult body size and fecundity, and between development time and adult body size. However, not all clones (genotypes) followed this population-level trend. One clone, in particular (Tr), exhibited a markedly different response, with increased development time under high temperature, leading to larger adult size and ultimately higher fecundity. This suggests a strong genotype-by-environment interaction (G X E), where genetic variation shapes the plastic response to environmental stress (Via and Lande, 1985; Nussey et al., 2007). The presence of such adaptive variants amidst a generally maladaptive mean response underscores the importance of intra-population variation in plasticity (Ghalambor et al., 2007; Murren et al., 2015). Moreover, it points to the potential for evolutionary rescue (Gomulkiewicz and Holt, 1995) through selection on genotypes whose trait responses align more closely with fitness optima under novel environmental conditions (Chevin et al., 2010). This highlights the need to consider not only average plastic responses but also the distribution and evolutionary potential of genotype-specific trajectories in the face of climate change.

568 The elevated temperature has significantly reduced the lifespan of experimental populations
569 relative to control populations. Moreover, we saw a significant effect of the genetic
570 background on the lifespan. The negative correlation between temperature and longevity has
571 previously been reported (for example, Musolin et al., 2007; Ciota et al, 2014; Giovaninni et
572 al., 2023), including the studies with *D. magna* (for example, MacArthur and Baillie, 1929;
573 Hoefnagel et al., 2018). Hoefnagel et al. (2018) demonstrated a marked temperature-
574 dependent decline in *D. magna* lifespan, with mean values decreasing from approximately
575 90 days at 10°C to around 15 days at 30°C. While they reported lifespan data across a
576 temperature gradient, only the extreme values were published, limiting the resolution for
577 accessing changes at intermediate temperatures. In contrast, our study provided detailed
578 lifespan data across a narrower but ecologically critical thermal window (26 - 30°C),
579 capturing a sharp decline relative to control populations during the first three assays (26 -
580 28°C), followed by the equalization of mean longevity between the two treatment groups at
581 the two highest temperatures.

582 The detected patterns of a response of fecundity and lifespan to elevated temperatures
583 might indicate a phenotypic canalization or a trade-off between these traits, the latter having
584 been reported in previous studies (for example, Myatake, 1997). During the first three assays
585 (26 - 28°C), we detected a clear advantage in fecundity of experimental populations relative
586 to control populations, which diminished during the subsequent assays at the highest
587 temperatures. Simultaneously, the control populations had a clear advantage in lifespan
588 during the same temperature window, which diminished during the final two assays.
589 However, we saw no significant covariation of fecundity and the lifespan within experimental
590 populations. In addition, we did not detect significant covariation of their lifespan and both
591 development time and somatic growth rate. This indicates that reduction in the lifespan

592 detected in our experimental populations can be attributed to the stressful effect of the
593 temperature alone, rather than the trade-offs with the other life history traits.

594 The reciprocal transplant experiment revealed no evidence of a long-term adaptation of
595 experimental populations (genetic change), as both experimental and control populations
596 retained the same values of the traits in their respective novel environments. The results
597 indicate that a single step of a temperature change imposed during this experiment (from 26
598 to 30°C for the control populations; from 30 to 26°C for the experimental populations)
599 between the two consecutive generations had no significant effect on the life history traits
600 tested. In addition, a limited number of generations that the populations underwent prior to
601 the reciprocal transplant experiment (25) may have been insufficient to detect an
602 evolutionary response.

603 Taken together, our results demonstrate that gradual thermal deterioration may entail the two
604 distinctive patterns of the response in life history traits: adaptive and maladaptive. Moreover,
605 it highlights the importance of adaptive plasticity which may countereffect the maladaptive
606 response in development time, somatic growth rate and adult body size, through an increase
607 in reproductive output. To the best of our knowledge, this pattern of interactions among the
608 life history traits has not been reported in experimental studies which involved an abrupt
609 temperature increase. It is possible that a slower pace of environmental change, coupled
610 with a wider range of stressful temperatures (closer to the upper limit of *D.magna*'s
611 temperature tolerance) used in our study, allowed for a more efficient acclimatization and/or
612 plastic response, previously undetected under a faster rate of thermal increase. The
613 limitations of our study include low genetic diversity and short timeframe in terms of the
614 number of generation that our populations underwent. Investigating whether adaptive
615 plasticity can potentially influence adaptive evolution requires an increase in standing genetic
616 variation and a longer-term experiment.

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