Master's thesis

NTNU
Norwegian University of Science and Technology
Faculty of Natural Sciences
Department of Biology

Martine Graham

Is the rate and capacity of phenotypic plasticity limited by energy availability?

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Supervisor: Sigurd Einum
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Abstract

With climate change exceeding the timeframe of evolutionary mechanisms, the ability to adapt through phenotypic plasticity might be more important now than ever. These phenotypic, within-lifetime changes, also known as *acclimation*, alter the expression of preexisting traits in response to climatic cues, without underlying genetic evolution. However, these plastic responses may be constrained by energetic costs, potentially affecting both the magnitude of the plastic response (i.e. capacity) as well as how quickly it can be achieved (i.e. rate). A commonly discussed cost of phenotypic plasticity is the energy required for maintaining the machinery for phenotypic plasticity, a fixed cost paid by all plastic organisms. However, the energy required to induce plasticity conditionally in response to a changing environment is often overlooked.

To gain further knowledge on this little studied aspect of phenotypic plasticity, I have studied the effect of energy availability during acclimation on the rate and capacity of plasticity to both salinity and temperature. I acclimated the amphipod *Echinogammarus marinus* in sub-lethal salinity and temperature for different durations, in parallel with and without food. To provide different times of acclimation, specimens were moved from 10°C to 20°C, or from 15‰ to 2‰, at different times. Survival time was then measured at a lethal high temperature (30°C) for the temperature experiment, and at a lethal low salinity (freshwater) for the salinity experiment.

I found no significant effect of acclimation time to lower salinity nor of feeding regime on freshwater tolerance of *E. marinus*. However, I revealed a significant interaction between acclimation time to a warmer temperature and feeding regime on the survival of *E. marinus* at 30°C. Both the absolute rate and the capacity of phenotypic plasticity towards warmer temperature was found to be higher for the fed treatment. These results have given valuable insight into the energetic cost associated with acclimation and may provide valuable information regarding priority rules for energy allocation.

Sammendrag

Med klimaendringer som overgår tidsrammen til evolusjonære mekanismer, kan evnen til å tilpasse seg gjennom fenotypisk plastisitet være viktigere enn noen gang. Disse fenotypiske endringene innenfor en organismes livstid, også kjent som *akklimering*, endrer uttrykket av eksisterende trekk i respons til et endret miljø, uten underliggende genetisk evolusjon. Disse plastiske responsene kan imidlertid bli begrenset av energetiske kostnader, som potensielt påvirker både omfanget til den plastiske responsen (for eksempel kapasitet), og hvor raskt den kan oppnås (for eksempel rate). En ofte diskutert kostnad ved fenotypisk plastisitet er energien som kreves for å opprettholde maskineriet for fenotypisk plastisitet, en fast kostnad betal av alle plastiske organismer. Energien som kreves for å indusere plastisitet betinget i respons til et endret miljø er imidlertid ofte oversett.

For å øke kunnskapen rundt dette lite studerte aspektet ved fenotypisk plastisitet har jeg studert effekten av energitilgang under akklimering på raten og kapasiteten av plastisitet til både salinitet og temperatur. Dette har blitt gjort ved å akklimere amfipoden *Echinogammarus marinus* til lavere saliniteter og høyere temperaturer i ulik lengde, i parallell med og uten fôr. For å gi ulik tid for akklimering ble *E. marinus* flyttet fra 10°C til 20°C, eller fra 15‰ til 2‰ på ulike tidspunkt. Overlevelsestid ble så målt i en dødelig høy temperatur (30°C) for temperatur-eksperimentet, og i en dødelig lav salinitet (ferskvann) for salinitet-eksperimentet.

Jeg fant ingen signifikant effekt av akklimerings-tid til lavere salinitet eller av fôrings-regime på ferskvannstoleranse i *E. marinus*. Jeg fant imidlertid en signifikant interaksjon mellom akklimerings-tid til varmere temperatur og fôrings-regime på overlevelsen til *E. marinus* i 30°C. Både den absolutte raten og kapasiteten av fenotypisk plastisitet for varmere temperaturer ble funnet å være høyere for behandlingen med fôr. Disse resultatene har gitt verdifull innsikt til den energetiske kostnaden assosiert med akklimering og kan gi verdifull informasjon knyttet til prioritets-regler for energifordeling.

Preface

Since a young age, the human impact on the environment has equally fascinated and scared me. I have always been very interested in exploring the nature surrounding me, which has made the realization of what we have done to the planet even harder to comprehend. Even though a worldwide, collective effort is crucial for handling climate change, I realized that studying the living world and how it will alter because of climate change could be my contribution to the puzzle. Getting the opportunity to base my master's thesis on the effect of climate change on *E. marinus* has deepened my fascination with the subject. During this period, I have learned a great deal about the scientific world, including study design, academic writing, source criticism, and statistical analysis. I hope my work and my findings can fascinate those interested in biology and climate change, and perhaps contribute to further research in the field.

Acknowledgments

I would like to thank my supervisor Sigurd Einum and my co-supervisor Tim Burton for their fantastic support during this process. The balance between guidance, help, and encouragement to figure out parts of this thesis on my own has enhanced my learning greatly. I truly appreciate the way my suggestions have been heard, and the time put into expanding my knowledge regarding the scientific world. I have felt like a part of a research team, where I have learned a lot from the earlier work and guidance of my supervisors. I would also like to express my gratitude to my friends and fellow students, Kaja Maczko Christoffersen and Nora Thelma Jevne, who have also been working on master projects related to Gammarus. Being able to learn and brainstorm together, as well as having someone to share nightly checks on the experiments with has made this period even more enjoyable. Lastly, I would like to thank my boyfriend Torgeir, for encouraging me through this whole process, and making my life a whole lot easier during long nights with experiments and writing. This thesis has taught me that struggling is part of the process, which has been a valuable lesson both personally and professionally. I have learned a great deal from this experience, and hopefully I will get the chance to do more work in the scientific field in the future.

Trondheim, June 2024

Martine Graham

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1. Introduction

Alterations in a species' environment, both naturally and anthropogenically induced, are potential sources of new or modified selection of traits important for fitness (Gienapp et al., 2008). Populations can respond to new selection pressures in three ways; 1) by dispersing to a more suitable habitat, 2) through phenotypic plasticity without altering the genetic constitution, or 3) by genetic changes through the process of evolution (Gienapp et al., 2008). Climate change has led to quicker alterations in the environment, and the ability to adapt through phenotypic plasticity might be more important now than ever before (DeWitt et al., 1998). These phenotypic, within-lifetime processes, also known as *acclimation*, can be induced by the stress an individual experiences due to a changing environment (Horowitz, 2001). Acclimation can improve the fitness of the individual to tolerate that stress, usually involving alterations in the expression of preexisting traits (Horowitz, 2001). It is presumed that acclimation plays a significant role in a species' ability to cope with within-lifetime changes to the environment, and the ability to induce plasticity is likely under strong selection (Noer et al., 2022).

Even though the ability to phenotypically adapt is known to have many benefits, no organism is ideally or infinitely plastic (DeWitt et al., 1998). It is therefore suggested that the evolution of phenotypic plasticity must have limits, or that plasticity may have inherent costs (Murren et al., 2015). A commonly discussed cost of the ability to induce plasticity is the energy and material expenses required for maintaining the sensory and regulatory machinery (DeWitt et al., 1998). This fixed cost applies to all plastic organisms, regardless of the environmental changes experienced. In addition to the cost of maintaining the ability to phenotypically adapt, actually inducing a phenotypic response conditionally to a changing environment requires energy (DeWitt et al., 1998). Both the fixed and the conditional costs could constrain the phenotypic response, affecting both capacity and rate of plasticity (Figure 1).

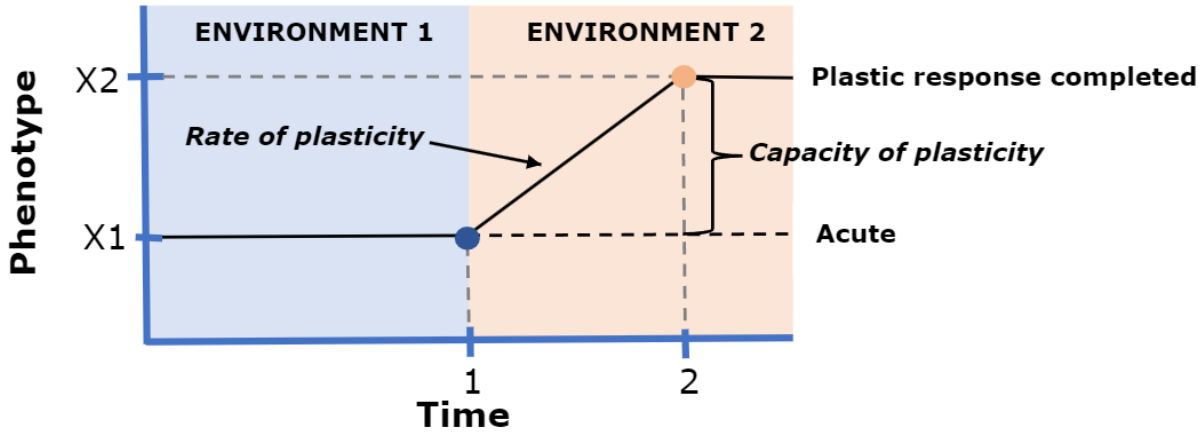

Figure 1 (After Einum & Burton, 2023). Illustration of the two parameters of phenotypic plasticity; rate and capacity. The rate of plasticity represents the time it takes for the phenotype of a specimen to become fully adjusted when exposed to a new environment. The capacity of plasticity represents the difference in phenotype between the acute response and the acclimated response.

The *capacity* of a genotype to produce different phenotypes in response to a changing environment has been thoroughly studied, and is thought to be crucial for a better understanding of evolution and the maintenance of biodiversity (Forsman, 2015). The *rate* at which organisms can respond plastically should also be taken into account, a factor which is often overlooked (Burton et al., 2022). It is not certain that all environmental changes will give an organism enough time to mount the phenotypic change required for persistence, reflecting a potential limit to plasticity (Burton et al., 2022). Studying the rate of a plastic response can provide insight into these limits (DeWitt et al., 1998), and how rapidly organisms can truly change their phenotype (Burton et al., 2022).

In the face of global warming, a lot of research has been put into understanding the impact of phenotypic plasticity on thermal tolerance. Human activities have unequivocally caused big alterations in global thermal patterns, resulting in a rise in global temperatures of over 1.1°C from the late 1800s until today (Lee et al., 2023). Both the mean environmental temperatures, and the frequency of extreme thermal events have increased (Diffenbaugh & Field, 2013; Gunderson & Stillman, 2015). Evidence is also building that anthropogenic changes to the global thermal regime are changing the global water cycle, affecting precipitation patterns (Terray et al., 2012; Zhang et al., 2007). These changes can in turn lead to alterations in salinity levels (Terray et al., 2012; Trenberth, 2011), where studies performed by the World Climate Research Program indicate that the subtropical oceans could become more saline, while the tropics and the high latitudes could freshen up (Sathyanarayanan et al., 2021; Terray et al., 2012).

Aquatic ectotherms are thought to be particularly affected by changes to climate regimes, because their main route for regulating body temperature is through heat exchange with the environment through conduction and convection. Consequently, their body temperature closely follows the temperature of the surrounding waters (Narum et al., 2013). Both temperature and salinity can also affect osmoregulation in aquatic ectotherms, influencing processes such as water and ion influx and efflux, blood-cell interchange of water and ions, and urine production rate (Dorgelo, 1981; Normant & Lamprecht, 2006). Ectotherms living in the intertidal zone can experience huge alterations in temperature and salinity within a lifetime (Crickenberger et al., 2020; Studer & Poulin, 2012). To cope with these alterations, specimens could either have an evolved, broad tolerance, or a plastic tolerance that changes with the environment (Yampolsky et al., 2014), but the relative contributions of these two factors are poorly understood (Jensen et al., 2019). If a species copes only by phenotypic plasticity, it is assumed that fluctuations in temperatures within the plastic tolerance range will not threaten the survival of local populations (Yampolsky et al., 2014). However, if populations exhibit signs of adaptation to local temperatures, the onset of global warming will put local populations under stress (Yampolsky et al., 2014).

Since the 1960s there has been a significant increase of interest in phenotypic plasticity among a wide range of taxa, testing whether the plastic response is an adaptive solution to an unpredictable, heterogeneous environment (Coquillard et al., 2012; Relyea, 2002). However, few studies exist to evaluate the energetic cost of phenotypic plasticity, more specifically the energetic cost of inducing a response when exposed to a changing environment. With limited food availability, the functional scope of an organism becomes constrained, reducing the energy excess used to support life-sustaining performances (Bozinovic & Pörtner, 2015). To survive and thrive, the allocation of resources to different traits is important (Perinchery-Herman, 2020), with theoretical and empirical studies

suggesting that organisms may have priority rules for energy allocation (Jokela & Mutikainen, 1995). This hierarchical rule might also include the ability to phenotypically adapt, where a constrained energy availability could reduce the resources put into maintaining the machinery of phenotypic plasticity, as well as the energy required to adjust the phenotype when experiencing a changed environment. Depending on the duration and the intensity of energy deficiency, both the rate and the capacity of plasticity could be affected.

To increase our understanding of phenotypic plasticity, and the impact of energy availability during acclimation, I have studied how plastic responses in a new environment depend on access to food. This has been done using the amphipod species *Echinogammarus marinus* (Gammaridae). Gammaridae are often considered keystone species because of their high abundance, major role in processing organic matter, and their role as a food source for fish and invertebrate predators (Hieber & Gessner, 2002; Semsar-Kazerouni & Verberk, 2018). *E. marinus* inhabits the intertidal zone of higher latitudes and experiences a variable environment in terms of temperature and salinity (Canale & Henry, 2010). Taking this into account, together with the fact that these are important abiotic factors likely to change noticeably due to climate change, I have researched how energy availability during acclimation affects the rate and capacity of phenotypic plasticity of *E. marinus* to both salinity and temperature (Gunderson & Stillman, 2015; Terray et al., 2012). Groups of *E. marinus* were exposed to changes in temperature and salinity for different durations, providing different timescales of reaching acclimation. This was achieved by transferring specimens from a colder to a warmer environment, or from a more saline to a less saline environment, for varying lengths of time, and in the presence or absence of food. Studying how *E. marinus* responds to different acclimation periods and feeding regimes may provide valuable insight into the effect of energy availability during acclimation on the rate and capacity of phenotypic plasticity.

2. Method

2.1 Specimen collection and cultivation

Specimens of *E. marinus* were collected in Korsvika in Trondheimsfjorden, Norway (63°26'58.7"N 10°25'54.2" E) in November 2023 for the salinity experiment and in January 2024 for the temperature experiment. The amphipods were collected from underneath rocks in the intertidal zone during low tide. Upon collection, the specimens were transferred to the lab and kept in a 50L cultivation tank, maintained at a constant temperature of 10°C and a salinity of 15‰, under a 24h:0h L:D regime. The cultivation tank was equipped with an aquarium air pump providing aeration, and rocks from the collection site to provide shelter. Fish feed (tetra cichlid colour) was provided three times per week, while the tank water was replaced completely once per week.

2.2 Acclimation to lower salinity and survival assessment in freshwater

The salinity experiment aimed to study how access to food during acclimation affects the rate and capacity of phenotypic plasticity in tolerance to freshwater exposure. This was done by quantifying the survival of specimens exposed to freshwater following acclimation to a low salinity for different durations, either in the absence or presence of food. A total of 300 individuals were used in the experiment, with 30 individuals in 10 different treatments. For the whole experiment (including both acclimation and freshwater exposure), each treatment group was kept at 10°C in dim lighting (24h) in a beaker containing 0.8 L of water, a rock from the collection site and an aquarium air pump. The treatments included 5 different acclimation periods, in parallel with and without food. To provide different acclimation periods, the treatments were transferred from 15‰ to 2‰ at 96, 48, 24, 12, and 0 hours before the freshwater exposure (Figure 2). All the treatments with food, regardless of the acclimation period, were given food for four hours every day for the 96 hours prior to exposure to freshwater. The rocks and the air pumps were removed during feeding for both fed and starved treatments. The water in all beakers was changed following each feeding period.

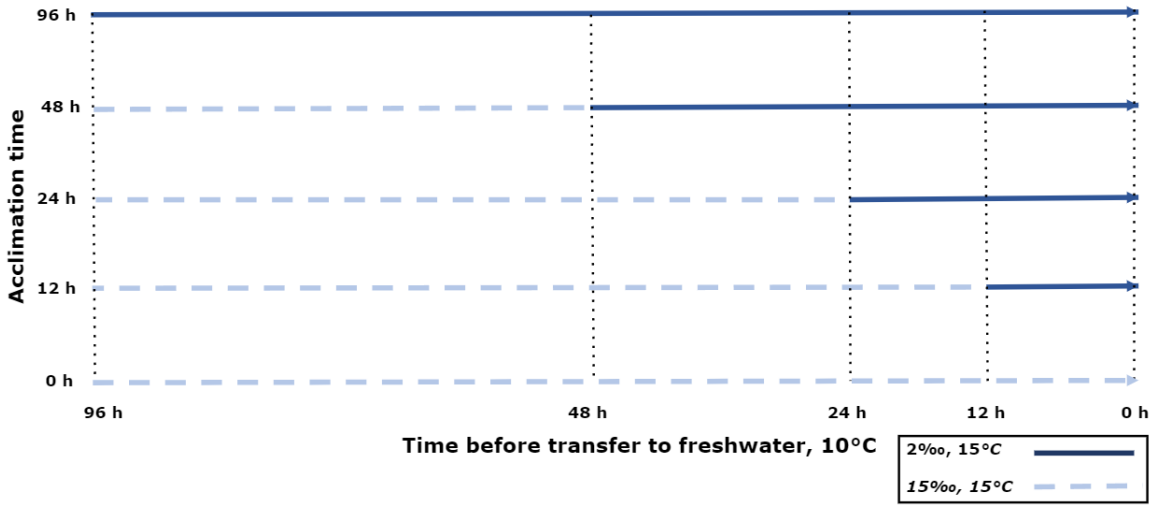

Figure 2. Acclimation regimes for different salinity treatments of *Echinogammarus marinus,* run in parallel with and without food. The specimens were moved from 2‰ to 15‰ at 96, 48, 24, 12, and 0 hours prior to exposure to freshwater. The fed treatments received fish food (tetra cichlid colour) for four hours each day.

After transfer to freshwater, survival was monitored every hour for the initial 9 hours, then every 5-10 hours for the next 116 hours (ca. 5 days in total).

2.3 Acclimation to higher temperature and monitoring survival at 30°C

The temperature experiment was conducted to examine whether food availability during acclimation to a sublethal high temperature (20°C) influences the rate and capacity of phenotypic plasticity in time to immobility when exposed to a lethal high temperature (30°C, hereafter referred to as T_{imm} or thermal tolerance). Acclimation was conducted in glasses containing 0.2 L of 15‰ saltwater in constant dim light, with daily water replacement. To provide different acclimation periods, the specimens were moved from 10°C to 20°C at different times prior to exposure to 30°C (96, 48, 24, 12, and 0 hours before exposure, Figure 3). The experiment was run in parallel with and without food, where the fed treatments were given fish feed (tetra cichlid colour) for four hours every day for 96 hours prior to exposure to 30°C. Thermal tolerance was quantified in 6 runs, with 5 individuals from each of the 10 treatments in each run, for a total of 30 individuals for each treatment.

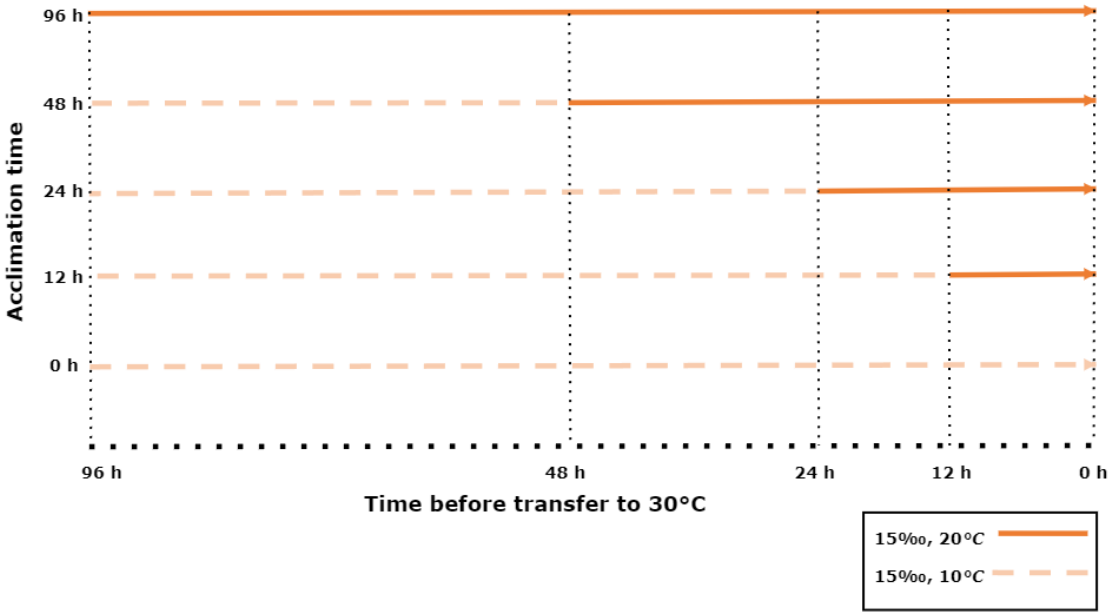

Figure 3. Acclimation regimes for different temperature treatments of *Echinogammarus marinus*, run in parallel with and without food. The specimens were moved from 10°C to 20°C at 96, 48, 24, 12, and 0 hours prior to exposure to 30°C. The parallel with food was provided fish feed (tetra cichlid colour) for four hours each day.

The thermal tolerance test was conducted after the method of Burton et al. (2020). Specimens of *E. marinus* were put into individual wells in a custom-built, aluminum and glass thermostatic well plate with 5x9 individual wells containing 3 ml of 15‰ saltwater at 30°C. The individuals were transferred to the wells by pipetting 0.5 ml of medium from the well to the Eppendorf containing the individual, before pouring them into the well. The well number and time elapsed (in seconds) from the first individual was placed in a well were recorded for each individual. After the last individual had been transferred into the well, the well plate was filmed from above with a digital camera (Basler aCA1300-60gm, fitted with 5–50 mm, F1.4, CS mount lenses). Backlighting from an LED light board (Huion A4 LED light pad, set to maximum intensity) was used to provide contrast between the specimens and the background. To ensure that all 45 individuals were still mobile when the camera started (it took between 7 and 9 min to transfer all 45 individuals to the well plate), the longest acclimated individuals were put into the wells first. Video recording was stopped when a visual inspection indicated that all individuals were motionless. The specimens were then frozen overnight, before the wet weight of each individual was measured.

2.4 Calculating LT50-values and T_{imm}

For the freshwater experiment, LT50-values were estimated to evaluate differences in survival time. LT50-values refer to the time it takes for 50% of the population to die under specific conditions, in this case in freshwater. For each treatment, survival was modeled as a function of exposure time using the glm-function in R (R Core Team,2023), and LT50-values were estimated as:

$$LT50 = -\frac{\beta 0}{\beta 1}$$

Where β0 is the intercept (the outcome when the predictor variable (exposure time) is equal to 0), and β1 is the coefficient of the predictor variable (the change in the log-odds associated with a one-unit change in exposure time). The standard error of the LT50 estimate was then calculated:

$$SE_{LT50} = \sqrt{\frac{var(\beta 1)}{\beta 1^2}}$$

Based on SE_{LT50} and the LT50 estimate, the 95% confidence interval was calculated, using the critical value (1.96) for a 95% confidence level:

$$CI_{95\%} = LT50_{estimate} \pm 1.96 \times SE_{LT50}$$

To assess thermal tolerance, the resulting video files were processed in Ethovision (version XT 11.5, Noldus Information Technology, The Netherlands) to produce a time series of velocity data (in mm s^{-1}, traveled by the center-point of each individual). T_{imm} was then estimated from the video-derived tracking data, using a modified version of an algorithm in the R computing environment (R Core Team,2023) which objectively identifies the loss of locomotory function (Burton et al. 2020). T_{imm} was calculated based on the threshold swimming velocity (1.0 mm s^{-1}) identified in a pilot study, to exclude movement under the baseline level of 'noise'.

2.5 Estimating linear models to evaluate significance of variables

In the salinity experiment, an analysis of the interaction effect between acclimation time (covariate) and food (factor, two levels) on the LT50-estimate was conducted through a two-way ANOVA analysis. The same analysis was also conducted looking solely on the main effects of the variables.

For the temperature experiment, the lmer-function in R was used to fit a linear mixed-effects model to the data with maximum likelihood (Bates et al.,2014). This was done by looking at changes in T_{imm} in response to the fixed effects of feeding regime, acclimation time and weight of the specimens, and the random effect of run. Both the main effects and the interaction effects of the predictor variables were studied, with T_{imm}, acclimation time and weight as continuous covariates, feeding regime as a factor with two levels, and run as a random factor with six levels. To identify the most appropriate model based on AICc, a model comparison was performed using the dredge function in the MuMIn package (Barton, 2022). To evaluate the significance of the predictor variables feeding regime, acclimation time and weight, and their interaction effect on survival in 30°C, as

well as the random effect of run, the lmer-function was fitted with restricted maximum likelihood on the model with the lowest AICc.

2.6 Calculating rate and capacity of plasticity

The relative rate of plasticity (λ) in thermal tolerance was calculated following the method of (Einum & Burton, 2023). First, the proportion of the full plastic response remaining to be achieved after an acclimation time *t* for individual *i* ($D_{t,i}$) was calculated as:

$$D_t = \frac{Z_{t,i} - \overline{Z_\infty}}{\overline{Z_0} - \overline{Z_\infty}}$$

$\overline{Z_0}$ is the mean phenotype at 0h acclimation, while $\overline{Z_\infty}$ is the mean phenotype at 96h acclimation. $Z_{t,i}$ is the phenotype of individual *i* after acclimation time *t*.

D_t can then be used to estimate the relative rate of plasticity, where D_t has a value of 1 at t=0 before decaying exponentially towards 0 with a rate λ, calculated through the following equation:

$$D_t = e^{-\lambda t}$$

This exponential decay model was fitted separately for the fed and the starved treatment, using nonlinear least squares regression through the nls-function in R (R Stats package).

To estimate the capacity of plasticity for the fed and the starved treatment, the log2-fold change in T_{imm} in response to the difference between specimens without acclimation and fully acclimated specimens was calculated, using the values of $\overline{Z_0}$ and $\overline{Z_\infty}$:

$$Capacity\ of\ plasticity = log2\frac{\overline{Z_\infty}}{\overline{Z_0}}$$

The relative rate of plasticity provides a numerical estimate of how long it takes to reach the given change in phenotype (determined by capacity), but it does not reflect how large this change is per unit of time. Thus, a high relative rate could reflect a low capacity if it takes a shorter time to make a smaller change. I therefore also calculated the absolute rate of plasticity. This was done by looking at how much the trait has changed per unit time when half of the change has occurred. This is given as:

$$Absolute\ rate\ of\ plasticity = \frac{0.5 \times capacity\ of\ plasticity}{\left(\frac{\log 2}{\lambda}\right)}$$

The denominator in this equation represents the half-time of the plastic response, or how long it takes for the trait to reach 50% of the total response. This provides a standardized absolute rate, which can be used across different experimental conditions and systems.

3. Results

3.1 Freshwater tolerance is not improved by acclimation time and feeding

Inspection of the different LT50 estimates shows no clear pattern of the effect of acclimation time nor feeding regime on survival in freshwater for *E. marinus* (Figure 4). I found no significant differences in LT50 estimates among feeding regimes, acclimation times and their interaction effect. This was shown through high p-values for all effects; p= 0.322 (F-value =1.166) for the effect of feeding regime, p=0.188 (F-value=2.290) for the effect of acclimation time, and p=0.951 (F-value = 0.004) for the feeding regime by acclimation time interaction. By removing the interaction from the model, the p-value was found to be p=0.282 (F=1.359) for acclimation time, and p=0.146 (F=2.675) for feed, still showing no significant effect of the main effects. With all p-values greater than 0.05 for all variables and interactions, there is not enough evidence to conclude that there are significant differences in the LT50 estimates among different acclimation times or between feeding regimes.

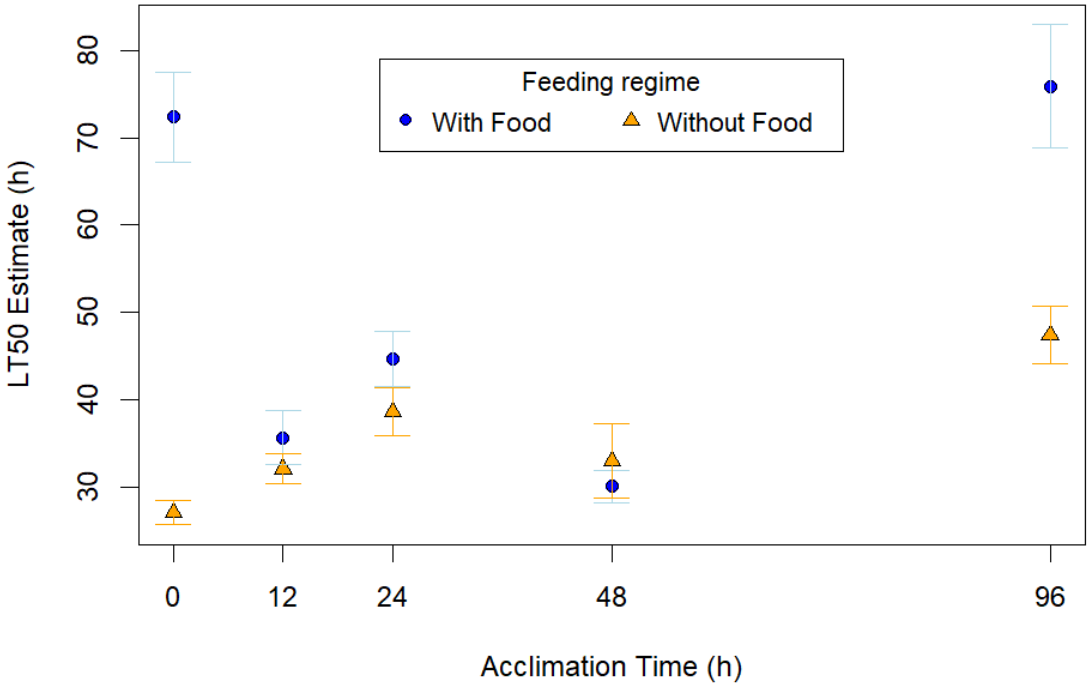

Figure 4. Estimated LT50 values (lethal time in hours for 50% of the population to die) with a 95% confidence interval for *Echinogammarus marinus* at different acclimation times and feeding regimes in freshwater treatment. Different acclimation times represent how long the specimens were kept at a lower salinity (2‰) before exposure to freshwater.

3.2 Acclimation time and feeding improve survival in warmer temperature

To explain variance in T_{imm} at 30°C for *E. marinus* among the predictor variables acclimation times, feeding regimes, and weight of the specimens, as well as the random effect of run, different model structures and their ΔAICc were evaluated (Table 1). By looking at the different models and their respective AICc, I found evidence for a strong effect on T_{imm} by the interaction between acclimation time and feeding regime, as well as the weight of the specimens. Looking at the different model structures with low ΔAICc, there was strong evidence for an interaction between acclimation time and feeding regime, evidenced by being represented in the four models with the lowest AICc.

An analysis of the fitted linear mixed-effects model with the lowest AICc revealed low p-values for the feeding regime by acclimation time interaction, as well as the main effect of weight (Table 2). The interaction effect of acclimation time by feeding regime on T_{imm} is also shown in Figure 5, where the effect of food on T_{imm} increases with longer acclimation time. By looking at the random effects in the model with the lowest AICc, I found some variances in the intercept of T_{imm} between the 6 runs and some variance that is not explained by the fixed and the random effects in the model (Table 3).

Table 1. Top candidate models (ΔAIC_c <5.00) explaining variance in survival time of *Echinogammarus marinus* among different acclimation periods and different feeding regimes when exposed to 30°C. "Acc_tim" represents different acclimation times, being moved from 10°C to 20°C at different times prior to exposure to 30°C. "Trt" represents different treatments in terms of feeding (starved vs. fed). "Wgh" is the individual body mass.

Model-structure	**AICc**	**ΔAICc**
Acc_tim*Trt+Wgh	2975.1	0.00
Acc_tim*Trt+Acc_tim*Wgh	2975.7	0.61
Acc_tim*Trt+Trt*Wgh	2977.2	2.14
Acc_tim*Trt+Acc_tim*Wgh+Trt*Wgh	2977.8	2.74
Acc_tim+Trt+Wgh	2978.2	3.05
Acc_tim*Trt*Wgh	2979.1	3.95
Acc_tim*Wgh+Trt	2979.1	4.03

Table 2. Summary of the analysis of a fitted linear mixed-effects model on the fixed effect of variables on survival time at 30°C for the amphipod *Echinogammarus marinus*. The different variables considered are the continuous covariate acclimation time, representing different times of being moved from 10°C to 20°C prior to exposure to 30°C, feeding regime as a factor, where the different acclimation times had parallels with and without feed, and weight, representing the individual body mass of each specimen as a continuous covariant.

Fixed effects	**Parameter estimates**	**SE**	**Df**	**t-value**	**p-value**
(Intercept)	105.91	19.53	61.970	5.423	<0.001
Acclimation time (h)	1.54	0.24	239.657	6.466	<0.001
Treatment Starved	3.12	17.17	239.947	0.182	0.86
Weight (g)	-790.51	271.46	106.817	-2.912	0.0044
Acclimation time: Treatment Starved	-0.81	0.36	240.816	-2.259	0.025

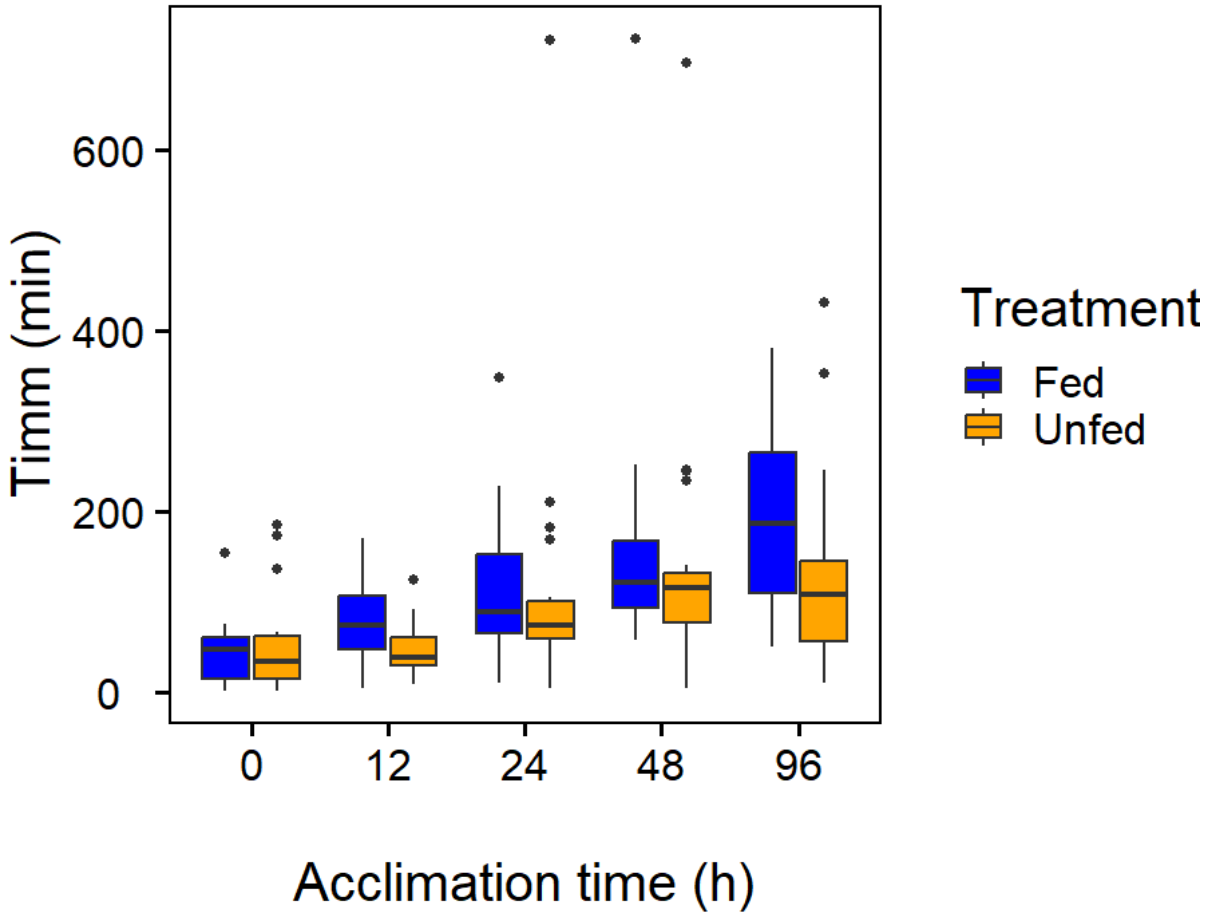

Figure 5. Time to immobilization of *Echinogammarus marinus* when exposed to 30°C as a function of acclimation time and feeding regime.

Table 3. Variance of the random effects of run and residuals on T_{imm} of *Echinogammarus marinus* in 30°C.

Groups	Variance
Run	124.7
Residual	8618.8

The relative rate of plasticity (λ) was found to be higher for the starved treatment, with a mean ± SE value of 0.040 ±0.021 for the starved treatment, and 0.028±0.005 for the fed treatment. This translates into half-times of 25.0 hours for the fed treatment, and 17.3 hours for the starved treatment. The slope of the exponential decay function, and the difference between the fed and the starved treatments revealed a significant interaction between acclimation time and feeding regime on the rate of phenotypic plasticity (Figure 6). The capacity of plasticity was found to be higher for the fed treatment, with a value of 2.247 for the fed parallel, and 1.228 for the starved parallel. By using the values from capacity and rate of plasticity, the absolute rate of plasticity was found to be 0.0449 for the fed treatment and 0.0356 for the starved treatment.

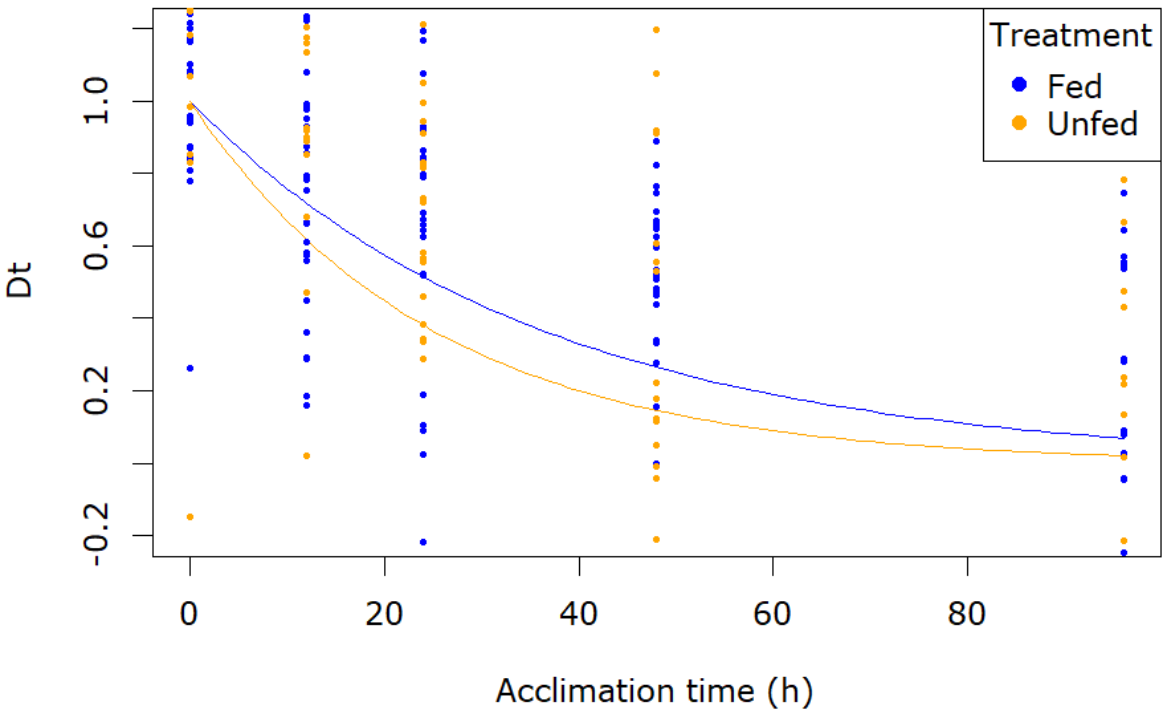

Figure 6. Exponential decay function, showing the relative rate of plasticity for *Echinogammarus marinus* for the treatment with and without food throughout different acclimation times. D_t for all individuals within the range of –0.2 to 1.2 are shown as individual dots.

4. Discussion

To survive in a rapidly changing environment, the ability to adapt through phenotypic plasticity is considered the most important route (DeWitt et al., 1998). Both capacity and rate of plasticity should be considered when evaluating the effect of climate change on the survival of species. Maintaining the machinery for phenotypic plasticity, as well as inducing a plastic response to a changing environment requires energy, where energy limitation potentially can affect both rate and capacity of plasticity. If a change in the environment is outside the limits of the capacity to phenotypically adapt, or if the speed of the change exceeds the rate of the plastic response, the organism will not be able to adapt plastically (Burton et al., 2022; Forsman, 2015). In the present study, I have evaluated how energy availability during acclimation affects the rate and capacity of phenotypic plasticity. This has been done by studying *E. marinus,* an amphipod from the family Gammaridae, exposing them to lower salinity and higher temperature. I found no significant effect of acclimation time nor feed on survival in freshwater, but a significant acclimation time by feed interaction on the survival at 30°C. My results show that T_{imm} at 30°C can be dependent on energy availability during acclimation, acclimation time, and size of the specimen, while survival in freshwater might be influenced by the relative contribution of inherent tolerance and phenotypic plasticity.

I observed no significant effect of acclimation time to lower salinity nor of feeding regime on survival in freshwater for *E. marinus*. If the survival time had improved with longer acclimation time, but not with a clear difference between fed and starved specimens, the result could have been discussed further concentrating on the energetic cost of phenotypic plasticity. However, with no evidence of better survival with longer acclimation time, it is not certain that the tolerance of *E. marinus* to different salinities is plastic. To cope with quick alterations in the environment, species can cope through both local

adaptation and phenotypic plasticity (Yampolsky et al., 2014). However, the relative contribution of these two forms of evolutionary adaptation is poorly understood (Jensen et al., 2019). With no evidence for a plastic response with longer acclimation time, inherent tolerance could play an important role in the survival time of *E. marinus* in freshwater. Studies have found that many organisms with a high inherent tolerance towards an environmental factor also have a low tolerance plasticity (Gunderson & Stillman, 2015; Somero & DeVries, 1967; Stillman, 2003). This trade-off in the use of resources could explain some of the results shown in the salinity experiment. Ectotherms living in the intertidal zone at higher latitudes experience high variance in salinity, from low tide to high tide, rain and snow, and run-off from streams and rivers (Sunday et al., 2011). This could result in a trade-off in resource use, where an inherent tolerance is more effective than expressing plasticity with respect to freshwater tolerance. This could also be true for *E. marinus,* where specimens in the family Gammaridae are known to have a broad salinity tolerance (Costa et al., 1998).

The inability to demonstrate a clear acclimation response could also come from measurement error, or from biological variation caused by differences in mean individual characteristics among treatments. The experiment was run with only one replicate for each treatment, and I observed large variance among replicates which appeared unrelated to treatments. Furthermore, the weight of the specimens was not measured, which is an important individual difference that could have explained some of the variance in survival. Finally, the acclimation period could have had a negative impact on the survival of the specimens. Although being in a sub-optimal environment is expected to induce an adaptive plastic response, such treatments can also affect the specimens negatively, for example due to increased oxygen consumption or changes in the osmoregulatory stress (Dorgelo, 1981; Normant & Lamprecht, 2006; Semsar-Kazerouni & Verberk, 2018). If the environment the specimen is acclimating to is too harsh, the specimen could potentially be worn out instead of acclimating. This could be the case explaining some of the random survival times shown in the salinity experiment (Figure 4), where individual fitness could affect whether the specimen can acclimate, or if they rather get worn out during the acclimation period in a suboptimal environment.

Another factor that should be considered when looking at the difference in survival between fed and starved treatments in the salinity experiment is the effect of acclimation on nutritive absorption. A study performed on *Gammarus oceanicus*, testing physiological performance across a broad range of salinity (5-30‰), showed decreasing rates of feeding with increasing salinity, whereas the nutritive absorption efficiency increased (Normant & Lamprecht, 2006). In my study, some specimens were acclimated to 2‰ for 96 hours, while the other treatments stayed longer at 15‰. The specimens staying at 2‰ for longer could potentially have a lower nutritive absorption, thereby decreasing the effect of the feeding regime on the difference in survival with and without food. The difference in nutritive absorption, and thereby the difference in functional scope between fed and starved treatments could therefore in reality be smaller than intended. This could give results that do not show the true effect of energy availability on tolerance to lower salinities.

To investigate the salinity tolerance of *E. marinus* further, an experiment with longer and shorter acclimation periods would be of interest, to exclude a plastic tolerance that is outside the timeframe studied in this experiment. If there still is no clear pattern showing better survival with longer acclimation time, a study on salinity tolerance in *E. marinus* as a case of inherent tolerance could be of interest. If the species show adaptation to local

salinities and no signs of plasticity, a lowering of salinity due to climate change could put the population under stress (Sathyanarayanan et al., 2021; Yampolsky et al., 2014).

In the temperature experiment, I found a significant interaction between acclimation time to higher temperature and feeding regime on survival at 30°C. The small difference in T_{imm} between fed and starved at 0h acclimation, in contrast to a bigger difference in T_{imm} with longer acclimation time could indicate that starvation alone does not have a pronounced effect on T_{imm}. However, it may negatively impact the energy available for acclimation. Dietary conditions are known to be a major factor contributing to plasticity in longevity of *Drosophila* (Arking et al., 1996; Vigne & Frelin, 2007). If food availability is limited, the functional scope of an organism becomes constrained (Bozinovic & Pörtner, 2015), and the organism will have to prioritize which physiological processes to allocate energy to (Jokela & Mutikainen, 1995). My results could reveal a constraint in the energy invested in acclimation, potentially giving insight into the priority rules for energy allocation during acclimation to warmer temperatures. The impact of starvation on the organism will be affected by both intensity and duration of energy limitation. Specimens that are chronically starved, with low nutrient supply throughout their life could to a smaller extent invest resources into the machinery allowing a high rate of plasticity. However, with acute starvation like in this experiment, it seems more likely that the reduced energy affected the production cost, rather than the cost of maintaining a machinery allowing high rates of plasticity. The production cost will be higher for a higher rate, but the machinery required to acclimate quickly is still intact, perhaps a result of the relatively short duration of starvation.

A factor that could impact the survival of an individual is the size of the specimen, which was shown by the significant effect of weight on T_{imm} in the temperature experiment. Here, it is shown that smaller individuals have a significantly higher T_{imm} than larger individuals. Several studies have shown the same negative correlation between acute thermal tolerance and body size for other species. This includes studies on fish, mollusk, arthropod, amphibian and reptile species (Peralta-Maraver & Rezende, 2021; Recsetar et al., 2012).

The capacity of phenotypic plasticity in thermal tolerance was found to be higher for the fed treatment, while the relative rate of plasticity was highest for the starved treatment. Einum & Burton (2023) has calculated the relative rates of plasticity towards warmer temperatures across different taxa, including crustaceans. By comparing their relative rate for crustaceans (0.019) to my relative rates, both my estimate for the starved treatment (0.040 ±0.021) and for the fed treatment (0.028±0.005) was found to be higher. By looking at the absolute rate of plasticity, the rate was found to be highest for the fed treatment. This reversal of the treatment effect when going from relative to absolute rate shows how capacity is treated differently by the two calculations of rate. The relative rate is higher for the starved treatment, showing that these approach their capacity faster. However, their capacity is smaller, and the absolute rate, which considers both capacity and relative rate, is therefore higher for the fed treatment. Thus, having access to food allows them to change their phenotype to a larger extent in a shorter time. From an ecological perspective, absolute rates may therefore provide a better understanding of the true ability to cope with fluctuating temperatures.

Another factor that should be discussed is the duration of acclimation in the experiment. In my study, the specimens were exposed to short acclimation periods, for a maximum of 96 hours. However, the beneficial effects of acclimation could have been larger with longer exposure. Studies have found that a prolonged acclimation period, studying acclimation time from minutes to months, will increase the beneficial effects notably (Semsar-Kazerouni & Verberk, 2018). Looking at the difference in T_{imm} between acclimation times, it appears that the fed treatment could have reached an even higher capacity of phenotypic plasticity than what was obtained in 96 h of acclimation, evidenced by no plateau shown in Figure 5. Based on the same figure, the starved treatment seems to have reached a plateau, and the difference between the capacity of fed and starved could therefore have been even bigger if the acclimation time was longer.

To study the phenotypic plasticity of *E. marinus* to warmer temperatures further, an interesting approach would be to study the acclimation response and the effect of energy availability with a more gradual rise in temperature. By studying this over a longer period, the effect of a gradual warming of the environment on the acclimation process could be studied in a more realistic timeframe. However, an organism does not have infinite time to alter the phenotype in response to a changing environment. Combining studies looking at the effect of acute changes with studies looking at the effect of gradual changes could provide a better picture of the effect on rate and capacity of plasticity, and the true harm of climate change on species.

The study of energy availability during acclimation to both temperature and salinity for *E. marinus* has given valuable insight into the energetic cost of acclimation. My findings show that salinity tolerance does not improve with acclimation time nor feed. However, the thermal tolerance of *E. marinus* improves with acclimation time and does so to a larger extent and more rapidly (in absolute term) in the presence of food. This result could reveal a constraint in the energy put into acclimation and provide information about priority rules for energy allocation. It is worth mentioning that the fitness of a species is not only dependent on survival, but also on reproduction. Examining how reproduction is affected by higher temperatures and lower salinities could give valuable insight into how climate change will affect biodiversity and the abundance of species. Analyzing the potential impact of climate change on species is a challenging task, considering several parameters, and combining information from experiments, historical data, and predictions about the future. This study on the energetic cost of acclimation to two important abiotic factors, likely to change noticeably due to climate change, indicate that the ability of *E. marinus* to manage fluctuations in salinity does not rely on energy availability, while their ability to manage fluctuations in temperature does. These findings highlight how environmental factors and energy availability interact to influence survival, and adds valuable information to the broader effort of predicting the effects of climate change on various species.

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Appendix A: R-citations

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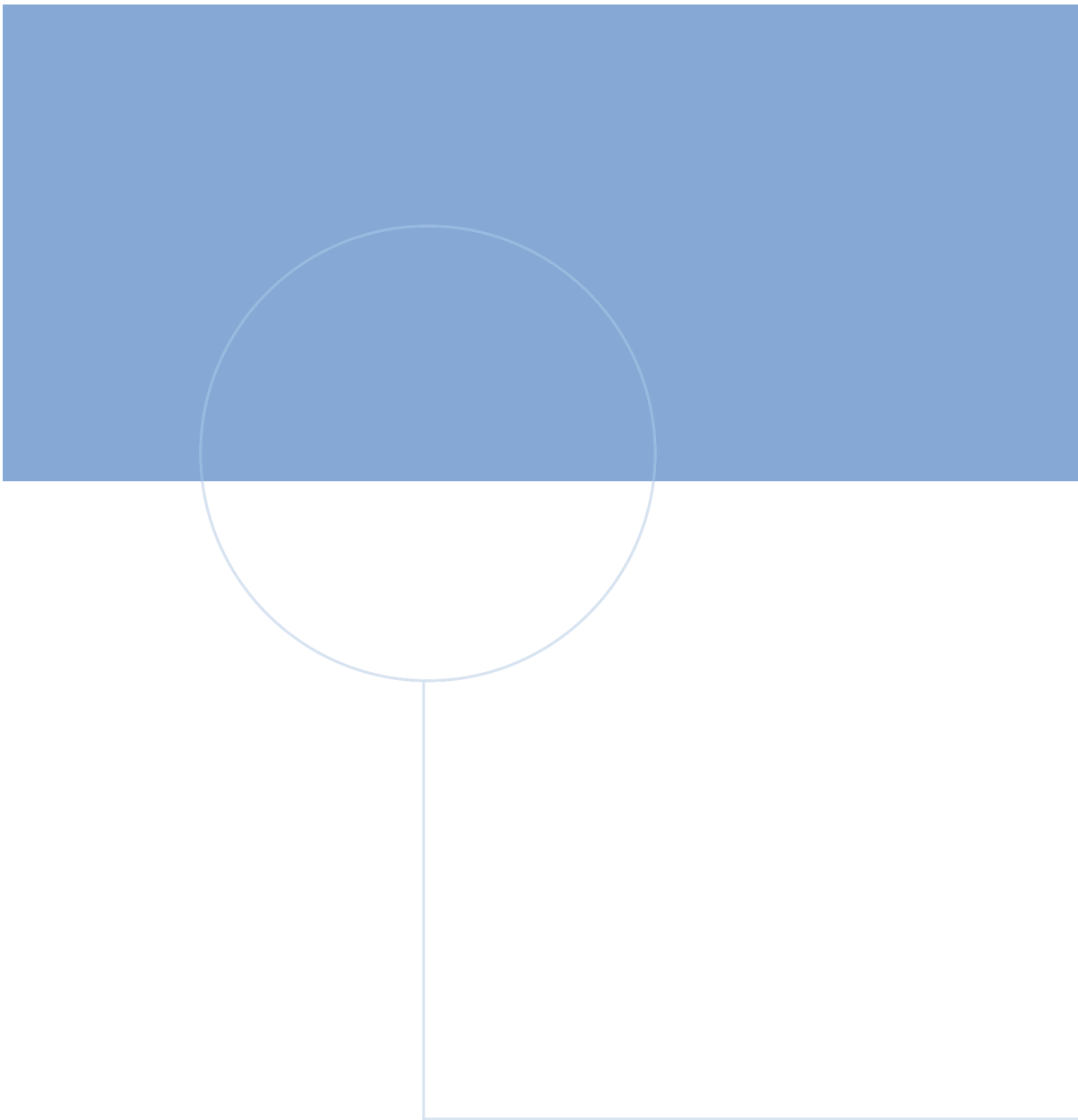
Appendix B: Professional relevance and sustainable development

Working with this master's thesis has given me valuable insight into the world of science, including how to study living organisms, source criticism, and how to best disseminate a message. By connecting knowledge and skills from previous courses, I have had the opportunity to use this on a bigger project, following the entire process from start to finish. This has included figuring out how the specimens should be collected and reared, running pilots to check their tolerance, finding a fitting study design, and learning how to use new tools to monitor survival. All of this has been done in collaboration with other students and supervisors, making this a good practice in communication through a project over a longer period, and the balance between working with others and working alone. The work with this master thesis has given me an area of expertise I most definitely can use in my profession as a teacher, especially when it comes to fieldwork, experiments, climate change, sustainable development, and connecting the curriculum to the real world in an engaging way.

During this process, I have been introduced to multiple ways of using my experiences from this project in my pedagogical practice. I have learned how a local, easily accessible species can be used to study climate change, and even more importantly increase students' curiosity regarding the nature surrounding them. Being out in nature with an academic purpose, connecting observations and findings to research in the lab, and immersion in a field is an excellent way of learning how the scientific field works, building on the existing curiosity of the students. Working with species in the family Gammaridae, connecting them to sustainable development can easily be linked up with The United Nations sustainable development goals. This includes “life below water,” “life on land,” “climate action,” “responsible consumption and production” and “partnerships for the goals.” Having a local example showing how warmer temperatures can affect organisms in the future is an engaging, realistic way of learning about sustainable development and The United Nations sustainability goals.



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