Starvation effects on life history traits at fluctuating vs stable temperatures; An experimental study with *Daphnia magna*

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Master of Science Thesis

Ecology and Evolution

60 credits

Department of Biosciences

UNIVERSITY OF OSLO

November 2021

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2021

Title: Starvation effects on life history traits at fluctuating vs stable temperatures; An experimental study with *Daphnia magna*

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Acknowledgements

At first, I would like to express my gracious and sincere gratitude to my kind and very supportive supervisor Yngvild Vindenes for her continuous supervision, guidance and motivation throughout each stage of this project. Her tenacious support and practicable propositions motivated me to accomplish whatever I have accomplished in this project.

To my co-supervisor Dag O. Hessen, for his valuable suggestions and constructive comments. Thank you Dag, for all the support you have provided. To Per-Johan Færøvig, for always being there in our needs in the lab and supporting us. Thank you everyone at Daphnia lab UiO, my lab partners, Anna Olsson, Øyvind Svensen, and Colin Gaillot for your great co-ordination and co-operation during lab works and schedule adjustments.

Finally, with all my good heart, I would like to thank my family for their continuous support and selfless love. My heartfelt appreciation goes to all who have contributed for completion of this work from their end.

Abstract

Temperature and food availability are two commonly fluctuating parameters that affect organisms and can induce plasticity responses. Starvation effects has typically been studied at constant temperatures, but *Daphnia magna* are exposed to large daily thermal fluctuations and can reflect comparatively uncommon responses. The goal for this study was to find out whether starvation has more severe life history consequences in fluctuating than in constant temperatures. Individuals from two clones (genotypes) were assigned to one of two food concentrations corresponding to starvation (0.05 mg C/ind/day) and ad libitum condition (0.3 mg C/ind/day), and then assigned to a treatment of diurnally fluctuating temperatures (17°C -27°C) or constant temperature (22°C). Key life history variables were recorded. Similar to previous studies, the result indicated that individuals with high food level had higher juvenile molting rate, growth rate, earlier maturation and higher fecundity than the ones with the low food level. Similarly, individuals with high food level at constant temperature had higher adult molting rate, growth rate and fecundity than ones at fluctuating temperature. Individuals experiencing low food at fluctuating temperature reached maturation earlier than at constant temperature and had higher juvenile molting rates but there was no significant effects of temperature treatment on adult molting rates and fecundity. The results indicate that thermal fluctuations effects were inconsistent across food treatments. Food temperature interactions suggest that positive effects of thermal fluctuation, if present, can be pronounced at low food level and negative effects obvious at high food levels.

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1. Introduction

Temperature variability in both the terrestrial and aquatic environment is common both on diurnal and annual basis, and there may also be more stochastic variations. Such temperature amplitudes and variability may be even more pronounced as a result of climate change (Bernhardt, O'Connor, Sunday, & Gonzalez, 2020). The need for understanding the implications of fluctuating environments has increased as climate change predict amplified fluctuations in temperature and other environmental variables (Stocker, 2014).

Organisms have to face and deal with the several environmental fluctuations. Since they witness numerous changes throughout their lifespan, adapting to the changing conditions is one of a key challenge to them. They are exposed to environments that can vary within and across generations (Ragland & Kingsolver, 2008) and constant exposal to numerous, interacting environmental stressors affect individual fitness (Stearns, 1992; Van Doorslaer, Stoks, Duvivier, Bednarska, & De Meester, 2009) as well as population dynamics (Bruijning, ten Berge, & Jongejans, 2018). In order to sustain the changing environment by means of all possible opportunities, species undergo evolution developing characteristics or traits that help them in maintaining homeostasis (Bernhardt et al., 2020). Although environmental change is ubiquitous, and ecology to a large extent deal with organisms adaptations to such changes, comparatively fewer studies have addressed the influences of fluctuating environments on the functioning of developing organisms (Niehaus, Angilletta Jr, Sears, Franklin, & Wilson, 2012).

Temperature controls most of the physiological processes in organisms, and notably so for the ectotherms, where even a small change in surrounding temperature can cause rampant variations in their metabolism and development (Brown, Gillooly, Allen, Savage, & West, 2004). Ectotherms experience diurnal fluctuations in habitat temperatures and are considered to be susceptible to thermal fluctuations (Paaijmans et al., 2013). Hence, they need to develop behavioral, physiological and cellular adjustments to survive through such fluctuations (Schwartz, Pearson, Dawson, Allison, & Gohlke, 2016). Since temperature exhibits stochastic consequences on physiological performances and life history consequences of ectotherms, thermal fluctuation can induce variations on different life history parameters such as growth, development time and adult

body size (Hagstrum & Milliken, 1991; Huey & Berrigan, 2001; Kingsolver, Izem, & Ragland, 2004; Worner, 1992). Some studies that explicitly assessed the effects of fluctuating temperature regimes on life history traits have however concluded that growth rates and development in response to temperature variability do not always get negatively affected (Du & Feng, 2008; Kern, Cramp, & Franklin, 2015; Kingsolver, Higgins, & Augustine, 2015; Niehaus et al., 2012).

Ecotherms' physiological performance (e.g., growth, development, reproductive output) at different temperatures can be explained by a thermal performance curve (Huey & Stevenson, 1979), which elevates gradually with temperature from a lower critical level, CT_{min}, to an optimum level, T_{opt} (at which performance is maximal), and then plummets rapidly to an upper critical temperature, CT_{max} (Figure 2). Since thermal performance curves are non-linear, mean responses can be uncommon at variable and constant temperature conditions (Kingsolver et al., 2015). Relatively higher metabolic requirements prevail in daily fluctuating temperature than stable conditions that could generate energy reallocations among different traits (Niehaus et al., 2012; Niehaus, Wilson, Seebacher, & Franklin, 2011; Williams et al., 2012) but this scenario can be species specific (Dong, Dong, Tian, Wang, & Zhang, 2006; Du & Feng, 2008). Also, different outcomes in thermal fluctuation studies could rely on prevailing temperature mean and its proximity to performance tolerance of the species since fluctuating temperatures within adverse range generally slow down development than at optimum stable temperatures (Dong et al., 2006; Du & Feng, 2008; García-Ruiz, Marco, & Pérez-Moreno, 2011; Kersting, Satar, & Uygun, 1999). Gene expression studies have showed that there are differences in gene expressions of organisms in constant versus fluctuating temperature environments and to adjust with the variable environment they must maintain their cellular function (Podrabsky & Somero, 2004). However, it is still unknown about the prerequisites under which temperature fluctuations could display clear indications that generate thermal adaptability in physiological performance of organisms (Niehaus et al., 2011; Sinclair, Thompson, & Seebacher, 2006).

Adding to temperature, food availability is another commonly fluctuating parameter that affect organisms and can generate plasticity responses. Both the quantity and quality of food resource massively affects growth, development, reproduction and survival in many of ectotherms (Karowe & Martin, 1989; Pree, 2011; Rossi & Strong, 1991; Yang & Joern, 1994) whereas food depletion affects life-history traits by slow-down of development, decreases in body size, reduced fecundity

(Ellers & Van Alphen, 1997; Nylin & Gotthard, 1998) and in many cases an extension of lifespan (Pietrzak, Grzesiuk, & Bednarska, 2010).

In this study, we investigated the interaction between these two major, fluctuating parameters by a factorial design with two temperature regimes (fluctuating vs constant) and two different food concentrations (high vs. low) on *Daphnia magna*. This species is one of the largest, freshwater filter-feeding cladocerans found in ponds, rock pools and shallow lakes, commonly devoid of fish. The small water volumes promote thermal variability, and it thus lives through high daily and seasonal temperature fluctuations (Giebelhausen & Lampert, 2001). *Daphnia* species are considered as keystone species that play crucial role in food-web of freshwater habitats (Hebert, 1978). *D. magna* naturally reproduces by cyclical parthenogenesis which entails both clonal reproduction of females (during favorable environmental conditions) and sexual reproduction (under stressful situations) to produce resting eggs (Green, 1956) (Figure 1). The ability of clonal reproduction makes it appreciated as a model organism, along with other characteristics such as its relatively short life cycle, sensitiveness to environmental fluctuations and so on which provide an ideal system for studying multiple stressors (Altshuler et al., 2011). Even though *Daphnia* prefer temperatures between 18°-22°C, they can survive a much broader range (Ebert, 2005). Daphniids are highly susceptible to environmental distresses (Schindler, 1987). And once they are stressed, they respond by changing their form of reproduction (Hebert & Crease, 1983).

Food availability affects somatic growth of *D. magna* most (Giebelhausen & Lampert, 2001). Even if the resource is plentiful, the rate at which organisms can utilize it, is limited since they require a certain amount of time to process and ingest it (Pree, 2011). *Daphnia* are filter feeders and have a temperature-dependent feeding rate, making food uptake a type 1 response. The filtering rate is maximal at low food concentrations, and food uptake is proportional to the concentration up to a threshold concentration, above which the food uptake is constant (Ebert, 2005). Some studies that considered temperature and food conditions as very important environmental factors for local adaptation of *D. magna*, showed significant interaction of temperature and food concentrations on fitness of *D. magna* as the temperature response was most distinct at abundant food levels (Giebelhausen & Lampert, 2001).

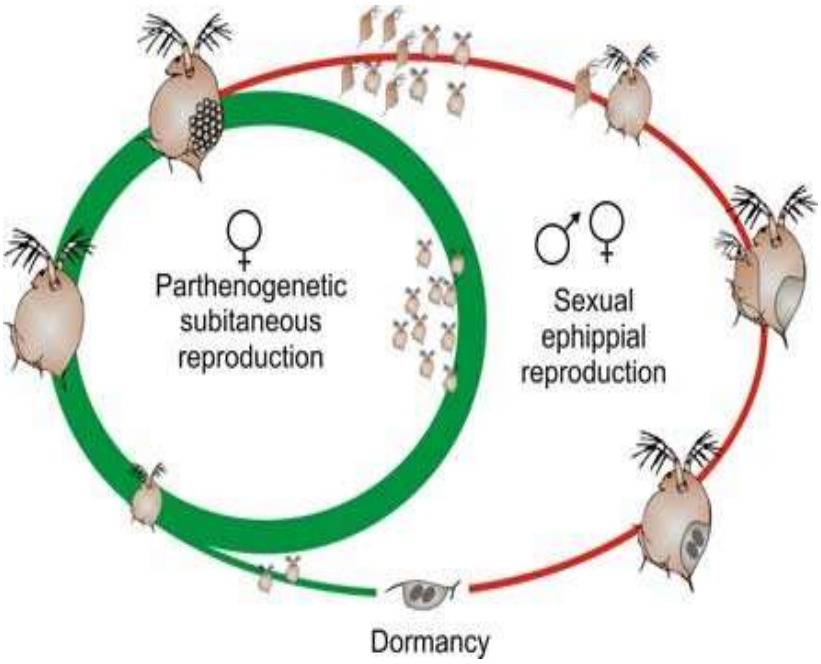

Figure 1. The diagrams illustrates the cyclically parthenogenetic life cycle of *D. magna* (Decaestecker, De Meester, & Mergeay, 2009, Figure. 15.1). *D. magna* reproduces asexually through parthenogeny under favorable conditions (green) where females produce diploid eggs that hatch into daughters whereas sexual reproduction takes place under stressful conditions, where females produce males and haploid eggs that are fertilized by the males. The resting eggs can survive long periods, and may hatch into new females once the environment turns suitable again (red).

Several studies on *D. magna* have been done at constant temperature(Giebelhausen & Lampert, 2001; Pietrzak et al., 2010; Pree, 2011) with different food levels whereas there are fewer studies on fluctuating temperatures (Reichwaldt, Wolf, & Stibor, 2005; Schwartz et al., 2016). At constant temperatures, crucial life history traits are highly influenced by food concentrations, e.g. high food level can induce early maturation, larger size at maturity and higher reproduction (Dudycha, 2003; Heugens et al., 2006; Lynch, 1989), whereas in a fluctuating temperature (19°C-27°C) more energy invested in acclimatization than reproduction or other fitness traits (Schwartz et al., 2016).

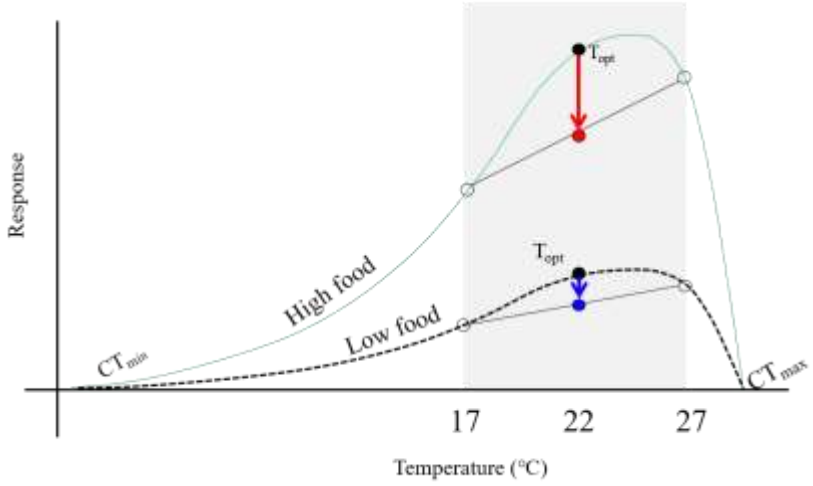

Figure 2. A typical thermal performance curve relating temperature responses with critical features highlighted (based on Huey and Stevenson 1979). $CT_{min,\ and}$ CT_{max} for lower critical and upper critical temperature respectively, black circles for thermal optimum at constant temperature, red and blue circles for thermal optimum at variable temperature (17℃-27℃) at high and low food respectively, This curve is based on thermal reaction norms due to diet effect from Giebelhausen and Lampert (2001).

Even though this species is capable of adapting to thermal fluctuations, they might need reallocation of energy to acclimatize with fluctuating temperatures demanding abundant food quantity or else remain more stressed (Figure 2). In this study, I will test how life history responses to fluctuating temperatures depend on food concentration (high vs low level). This type of study could depict the relationships between metabolic needs of *D. magna* at different thermal conditions and their interaction with diet which could exhibit some further insight on the life history plasticity.

2. Aim of study and research questions

The objectives of my master's project is to determine whether starvation has more severe life history significances in fluctuating than in constant temperatures. For this, individuals from two clones (genotypes) of the experiment animals were assigned with one of the two food concentrations corresponding to starvation (0.05 mg Carbon/ind/day) and ad libitum condition (0.3 mg C/ind/day), and were assigned to a treatment of fluctuating temperatures (17°C -27°C) or constant temperature (22°C). To achieve the objectives, following research questions were made.

Research questions

1. Do *D. magna* exhibit relatively worst performance in fluctuating temperature than constant temperatures?
2. Does food level influence the effects of temperature fluctuations on the observed life history parameters?

3. Materials and methods

3.1 Experiment animals

Two clones of *D. magna* were used as experimental animals. The *Daphnia* were received from laboratory stock cultures at University of Basel, Switzerland in March 2020 and since then they had been maintained at University of Oslo. One of the clones originates from Morocco (MA-ES-3, lat 31,490714, long -9,76443) and the other clone originate from Sweden (SE-G1-9, lat 60,421733, long 18,51015). In this study, the Moroccan clone is referred as 'Aicha' whereas Swedish clones is referred as 'Pippi'.

Before starting the main experiments, *D. magna* were kept in stock cultures in 500ml jars filled with aerated Aachener Daphnien Medium (ADaM), an artificial freshwater medium developed for culture of zooplankton (Appendix B) (Klüttgen, Dülmer, Engels, & Ratte, 1994), at 20°C temperature room. 6-8 adults were kept for each stock jar, fed with ad libitum food under 12h light cycle. New stocks were started from the third-fourth clutch, and we kept three parallel stocks per clone. The green algae *Chlamydomonas reinhardtii* cultured in batches in the laboratory in WC medium (Appendix C) (Guillard & Lorenzen, 1972) at constant light and room temperature was used as main source of food.

The medium change and feeding of the stock cultures was done at least twice a week. The stocks were reared under these conditions for several generations before their use as grandmothers for the main experiment.

3.2 Treatments

The experiment was conducted in 4226 laboratory at the University of Oslo in February 2021. The experiment included two temperature treatments, and two food treatments. Two temperature regimens were maintained in two temperature cabinets (Termaks KB8400|KB8400L) with targeted thermal setting each for fluctuating and constant temperature, as well as light. The targeted range of the fluctuating temperature was 17°C-27°C with the temperature peaking ~ 7:00 AM and at a low ~ 7:00 PM in an temperature cabinet and the constant temperature was 22°C. Temperature loggers (HOBO Pendant temp/light 64 K Data Logger, Onset) were used to monitor the temperature inside each temperature cabinet (two loggers were placed inside each cabinet, and showed highly similar values). The constant temperature treatment averaged 21.81℃ ± 0.33 (mean ±Standard deviation) through the experiment in the constant temperature environmental chamber whereas the fluctuating temperature treatment had an average of 22.1°C ±4.73. The actual temperature was found approximate to our targeted idea (Appendix A).

The food levels were identified on the basis of pilot experiments conducted by Yngvild Vindenes and Anna Olsson in May-June 2020. The chosen two food levels were high (ad libitum) food level at 0.3 mg C/ind/day and low (starvation) food level at 0.05 mg C/ind/day. The algal optical density at 800 nm was calculated by using PV4 spectrophotometer.

Four treatments of this study were abbreviated as HFCons for individuals at high food and constant temperature, HFFluct for individuals at high food and fluctuating temperature, LFCons for the ones with Low food and constant temperature and LFFluct as the ones with low food and fluctuating temperature.

3.3 Main experiment

Six neonates (newly hatched offspring) of each clone from stock cultures were randomly chosen as potential mothers and each of them were put in 100 ml jars containing aerated ADaM. Two out of six were placed into fluctuating temperature (17°C-27°C) cabinet whereas the other four were put into constant temperature (22°C) cabinet and were kept under a 16 h: 8 h light: dark cycle. Aerated ADaM was stored in each respective temperature cabinets to make sure the individuals did not experience large temperature change while changing the medium. The feeding and medium change was done every second day and individuals were monitored until the release of the second

clutch. Once they released second clutch, 10 offspring were randomly chosen from each clone and each treatment, to be experimental animals. 40 individuals (neonates) each of Aicha and Pippi, out of which 20/20 for each temperature regimen and out of those 20, 10/10 for each high and low food level, making 80 experimental individuals in total. Other than the experiment individuals, 10 extra offspring from each clone and each treatment were randomly taken for measuring length (at age zero) as representative initial length of experimental animals. Each experimental individual was collected within 24 hours of the release.

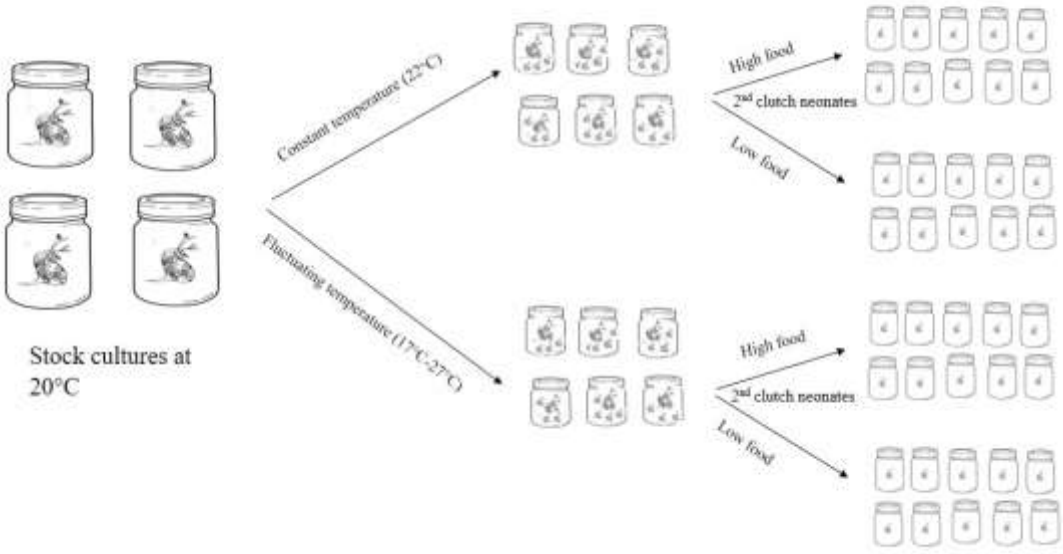

Figure 3. An illustration of the experimental setup for one set of clone. All *D. magna* mothers were taken from same clutch (third clutch), of some of the stock cultures (grandmothers) at 20°C. The mothers were transferred at two different temperature treatment (fluctuating and constant) cabinets and treated with ad libitum food. Once they released their second clutch, the main experiment was started with those offspring (the experimental individuals) and were transferred to respective temperature regimen. The column on the left presents *D. magna* grandmothers at 20°C, the middle column represent the mothers of the experimental individuals at different temperature treatments. The right column presents the experimental individuals taken from second clutch offspring at different temperature cabinets and treated with different food level within each temperature treatment.

Individuals were kept in 100ml jars in aerated ADaM. All medium (ADaM) used throughout the experiment had been aerated for at least 24 hours before use. The experiments started by using mixture of *Chlamydomonas reinhardtii* and *Nannochloropsis sp.* made from frozen *Nannochloropsis sp*.(RotiGrow Nanno; Reed Mariculture), as food. Before feeding the *Daphnia*, the absorbance or optical density (OD) of *C. reinhardtii* was measured in a PV4 spectrophotometer at 800 nm. The standard curve depicting the relationship between OD at 800 nm and carbon content helped in predicting algal biomass and desired carbon content. Carbon content was calculated by,

$$Y = (0.185 * 1.38 * OD) - 0.001$$

where 'Y' is the desired carbon content (mg C/ml) and OD is the average optical density of the algae. For high food level, the desired carbon content calculated was 0.22 mg C/*Daphnia* and for low food level was 0.04 mg C/*Daphnia*.

The required ADaM (80ml * no. of individuals approx.) and calculated amount of algae for each food concentration for all individuals of each treatment was mixed in a 2000 ml laboratory jug and after well stir of the mixture, 80 ml of the mixture were put in each individual jar for each individual of *D. magna.* Medium was changed every day for every individuals during which each of them were put to new jar using a transfer pipet. Due to problems with the live algae culture (See below), the food was switched from a mixture to Rotigrow alone in 10^{th} day of the experiment. All the individuals had released their first clutch by then. The daily observations of experiment individuals were noted until they release their third clutch. At that time, they were measured, photographed and discarded. Some of the individuals could not reach their third clutch even after many days of their second reproduction and then the experiment was ended after measuring their body size.

The experiment lasted for 19 days. During this 19 days period, the following parameters were measured or noted:

- Length at age zero (mm), measured on siblings of the experimental individuals soon after they were hatched.
- Daily moulting (Yes/No).
- Age at maturity (the time in days from their birth until the day when the eggs were visible in their brood chamber for the first time)
- Age at first reproduction (the time from birth until the day they release their first offspring ever) (in days)
- Length (mm) and age (Days) at first, second and third reproduction/death/disposal.
- Clutch size (number of offspring released at each reproduction).
- Length (mm) of the offspring at age zero.

3.4 Size measurements

Initial size (length) at age 0 of some of the representative individuals (not the ones who were used for the experiment) were measured just after they were released from the mother's using stereo microscope. The size of the individuals was measured at three points after their maturation, at first reproduction, second reproduction and at the end of the experiment (may or may not have reached third reproduction). The *Daphnia* length was measured from the top of the head to the base of the spine (Figure 3).

Figure 4. *Daphnia* measured under stereo microscope. The blue line represents the extent of body considered as length/size of the *Daphnia*.

3.5 Statistical Analysis

All the data was analyzed in R software version 3.6.3 (R core Team, 2020). Data visualizations was done by making mean ± standard deviation plots for treatments versus responses using the packages, "ggplot2" and one way Anova has been done for most of the analytical part.

Since the length of experimental animals were measured at three events; first, second and third reproduction respectively. The growth rates G1, G2 and G3 were calculated accordingly. For growth rates,

G1 = (Length at first reproduction – Length at age zero)/Number of days between the events

G2 = (Length at second reproduction-Length at first reproduction)/Number of days between the events

G3 = (Length at third reproduction-Length at second reproduction)/Number of days between the events

Where G1 is the body growth rate of the individuals from age zero to their first reproduction,

G2 is the rate growth of the individuals from first to second reproduction and G3 is the growth rate from second to third reproduction.

The effects of all four treatments on growth rates, age at maturity and both the juveniles molting rates [number of molts occurred until maturation/ age (days)] and adult molting rates [number of molts occurred after maturation to survival/number of days] were determined by using (ANOVA) through aov()function and if significant, pairwise comparisons using Tukey's HSD (honestly significant difference) test was done by using TukeyHSD() function to determine which groups varied. Since the length of experiment individuals were measured at each clutch release, the differences of all four treatments on length during each clutch release was also determined by ANOVA, followed by pairwise comparisons using Tukey's HSD. Similarly, the effects of treatments on age at first reproduction and clutch size were also analyzed by using ANOVA and was followed by pairwise comparisons using Tukey's HSD.

On ANOVA table, ‘source’ is the one responsible for variation in the data, ‘Df’’is degree of free dom of the data, ‘Sum Sq’ is the sum of squares of data, ‘Mean Sq’ is mean sum of square of dat a, ‘F-value’ is ratio of two mean square values and Pr (>F) - is P-value associated with the F statist ic of a given source, where significance codes ‘***’ represents ‘ 0.001’ ‘**’ represents ‘0.01’ and ‘*’ represents ‘0.05’.

3.6 Challenges

Algae collapsing: There were several challenges faced throughout the experiments. The algae cultured in the laboratory was not growing well since the few days of starting experiment and kept collapsing several times. After first week (approx.), we had to use the supplementary algae “RotiGrow” alone as the main source of diet for the rest of the experiment.

Issues with Pippi clones: Within the experimental animals of Pippi clone, there were 70% of male individuals on HFFluct treatment, 10% and 40% of males on LFCons and LFFluct treatments respectively. Since they had no eggs and comparatively smaller in size than other individuals, they were run under stereo microscope and discovered to be males on the 7^{th} day of their life and were discarded from rest of the experiment and also were excluded during statistical analysis. Also, Pippi individuals treated with high food level at both temperature regimen could not reach third reproduction. No eggs were seen even after molting occurred and the experiment was ended.

Bacterial infection: Weeks after the end of our experiment, it was discovered that stocks were likely infected by an unknown bacteria and this could be the reason of them being physiologically in a bad state and remained stressed. Since the experiment animals were selected from same stocks and assigned with the same treatments, the comparisons were assumed to be valid.

4. Results

4.1 Growth rates

Aicha

Treatments had significant impacts on the mean growth rates of *Daphnia* (Table 1). Although there was no significant impact of temperature regimen on G1, food levels showed substantial effect depicting that individuals treated with high food level had higher growth rate (G1) than the ones with low food level (Table 2). The mean (±Standard Deviation) growth rate G1 was highest (0.297±0.015 mm per day) at high food level at constant temperature and lowest (0.198±0.008 mm per day) at low food level at same temperature (Figure 5, Table 13). Individuals seemed to grow fast at early stages and reduced the growth after first reproduction and there was no statistical significance of treatments on G2 (Table 1). For G3, only food treatments at stable temperature had displayed differences in *Daphnia* i.e. only high food level enabling higher growth rate and no impact of other treatments were observed (Table 2).

Pippi

The mean (±Standard Deviation) growth rate G1 of Pippi individuals was highest (0.268 mm per day) at HFFluct treatment and lowest (178±0.006 mm per day) at LFCons treatment (Figure 5 Table 13). Similar to Aicha, there was no effect of temperature regimen but high food level had positively affected G1 at both temperature conditions (Table 2). Although effect of treatments on G2 was not statistically important but on G3 there was substantial impact of temperature regimen on well fed individuals indicating that individuals grew better at constant temperature than varied state (Table 2).

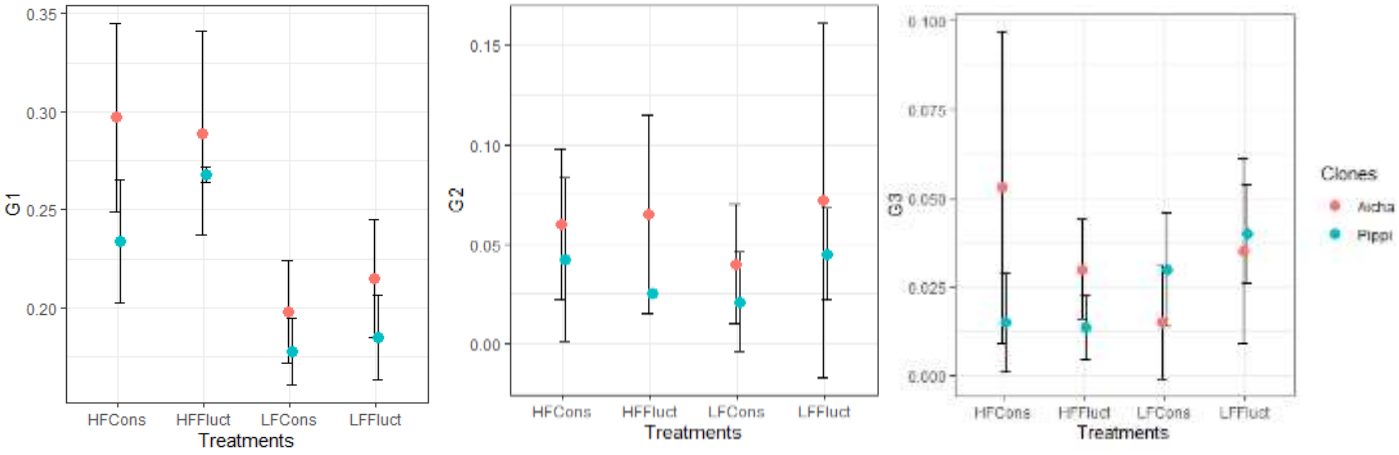

Figure 5. Mean(± Standard Deviation) growth rates, (G1, G2 and G3 from age zero to first clutch release, first to second clutch release and second to third clutch release respectively) of both the clones throughout different treatments.

Table 1. One way ANOVA table for growth rates (G1, G2 and G3) for both Aicha and Pippi

Growth rates	Clones	Source	Df	Sum Sq	Mean Sq	F value	Pr (>F)
G1	Aicha	Treatment	3	0.07506	0.025018	15.18	1.72e-06***
		Residuals	35	0.05769	0.001648		
	Pippi	Treatment	3	0.029	0.0095	17.53	3.04e-06 ***
		Residuals	24	0.013	0.0005		
G2	Aicha	Treatment	3	0.00572	0.001906	0.625	0.603
		Residuals	35	0.10671	0.003049		
	Pippi	Treatment	3	0.003054	0.0010180	1.076	0.378
		Residuals	24	0.022712	0.0009463		
G3	Aicha	Treatment	3	0.01231	0.004104	4.053	0.0142 *
		Residuals	35	0.03544	0.001013		
	Pippi	Treatment	3	0.002657	0.0008855	4.29	0.0152 *
		Residuals	23	0.004747	0.0002064		

Table 2. Tukey's HSD table showing multiple comparisons of means of growth rates (G1, G2 and G3) of both Aicha and Pippi at different treatments with 95% family-wise confidence level**.** Here, 'diff' is mean difference value between two groups, 'lwr' and 'upr' and lower and upper end point of interval respectively and 'P adj'is adjusted p-value for multiple comparisons.

Growth rates	Clones	Treatments	diff	lwr	upr	P adj
G1	Aicha	HFFluct-HFCon	-0.008	-0.057	-0.041	0.97
		LFCon-HFCon	-0.099	-0.148	-0.05	0.00002
		LFFluct-HFFluct	-0.074	-0.0124	-0.0235	0.002
		LFFluct-LFCon	0.017	-0.034	0.067	0.805
	Pippi	HFFluct-HFCon	0.034	-0.0084	0.076	0.15
		LFCon-HFCon	-0.056	-0.085	-0.03	0.0001
		LFFluct-HFFluct	-0.083	-0.128	-0.038	0.0002
		LFFluct-LFCon	0.0063	-0.028	0.0401	0.954
G2	Aicha	HFFluct-HFCon	0.0095	-0.06	0.08	0.98
		LFCon-HFCon	-0.016	-0.82	0.051	0.92
		LFFluct-HFFluct	0.007	-0.61	0.08	0.99
		LFFluct-LFCon	0.033	-0.36	0.1	0.5
	Pippi	HFFluct-HFCon	-0.017	-0.073	0.039	0.83
		LFCon-HFCon	-0.021	-0.06	0.018	0.46
		LFFluct-HFFluct	0.02	-0.41	0.08	0.81
		LFFluct-LFCon	0.02	-0.21	0.069	0.49
G3	Aicha	HFFluct-HFCon	-0.04	-0.075	0.0021	0.07
		LFCon-HFCon	-0.05	-0.086	-0.009	0.01
		LFFluct-HFFluct	0.009	-0.03	0.049	0.92
		LFFluct-LFCon	0.02	-0.02	0.06	0.52
	Pippi	HFFluct-HFCon	-0.001	-0.03	0.03	0.02
		LFCon-HFCon	0.014	0.005	0.032	0.44
		LFFluct-HFFluct	0.027	-0.003	0.06	0.08
		LFFluct-LFCon	0.012	-0.01	0.034	0.46

4.2 Body length at each clutch release

Aicha

Individuals treated with high food level at constant temperature attained the highest body length (3.033 mm ±0.17) at 15.1 days ±3.14 seconded by individuals with same food level at fluctuating temperature (2.71mm ±0.13) at 14.6 days±2.27 whereas lowest body length was observed in low food at fluctuating temperature (2.49mm ±0.125) at 14.5 days ±2.34 (Figure 6).

At each measurement (clutch 1, 2 and 3), it was observed that temperature regimen had significant effects on length at high food level implying that individuals had greater length at constant temperature than fluctuating temperature whereas no effect on length was seen on low food level at second and third clutch release (Table 3 and 4). That means, starved individuals at both temperature had insignificant differences in size. Also, food level showed significant effect on length of *D. magna* within each temperature regimen indicating that high food resulted greater length than low food (Table 3 and 4).

Pippi

In Pippi, well fed individuals at both constant and fluctuating temperature regimens had attained the highest body length (2.76mm ± 0.04) at 18 days and (2.75mm ±0.034) at 16.33 days ± 1.2 respectively whereas starved ones at the both the temperature regimen had the lowest body length (2.69mm ±0.02) at 18.1 days ±1.17 and (2.67mm ±0.012) and at 16.5 days ± 1.22 respectively (Figure 6). Temperature treatment had substantial impact on length at high food level only indicating that well fed individuals at fluctuating temperature were bigger than the ones at constant temperature but there was no effect of temperature at low food level (Table 3 and 4). Surprisingly, there was no effect of food level at constant temperature but individuals treated with high food level at fluctuating temperature were bigger than the ones treated with low food at first and second clutch release but no significant difference between food levels was observed at third clutch (Table 4).

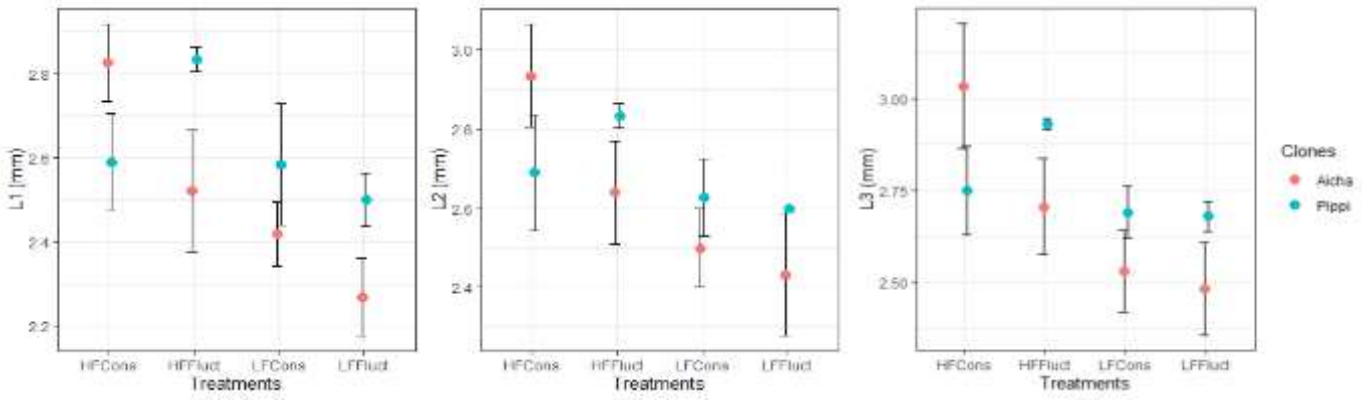

Figure 6. Mean (± Standard Deviation) body length (L1, L2 and L3 at first, second and third clutch release respectively) of both the clones throughout different treatments.

Table 3. One way ANOVA table for Aicha and Pippi for length at first, second and third clutch release (L1, L2 and L3 respectively)

Length	Clones	Source	Df	Sum Sq	Mean Sq	F value	Pr (>F)
L1	Aicha	Treatment	3	1.6520	0.5507	50.86	5.01e-13 ***
		Residuals	36	0.3897	0.0108		
	Pippi	Treatment	3	0.2260	0.07534	6.56	0.00214**
		Residuals	24	0.2757	0.01149		
L2	Aicha	Treatment	3	1.4482	0.4827	29.68	9.99e-10***
		Residuals	35	0.5692	0.0163		
	Pippi	Treatment	3	0.1842	0.06140	5.615	0.00461**
		Residuals	24	0.2625	0.01094		
L3	Aicha	Treatment	3	2.0241	0.6747	34.15	1.25e-10***
		Residuals	36	0.7113	0.0198		
	Pippi	Treatment	3	1.844	0.6146	3.965	0.0153*
		Residuals	36	5.581	0.1550		

Table 4. Tukey's HSD table showing multiple comparisons of means of length at each clutch release of both Aicha and Pippi at different treatments with 95% family-wise confidence level. Here, 'diff' is mean difference value between two groups, 'lwr' and 'upr' and lower and upper end point of interval respectively and 'P adj'is adjusted p-value for multiple comparisons.

Length	Clones	Treatments	diff	lwr	upr	P adj
L1	Aicha	HFFluct-HFCon	-0.31	-0.43	-0.18	0.0000007
		LFCon-HFCon	-0.41	-0.53	-0.281	0.0000000
		LFFluct-HFFluct	-0.25	-0.38	-0.125	0.0000276
		LFFluct-LFCon	-0.15	-0.27	-0.024	0.014
	Pippi	HFFluct-HFCon	0.243	0.049	0.44	0.015
		LFCon-HFCon	-0.007	-0.143	0.13	0.99
		LFFluct-HFFluct	-0.33	-0.542	-0.124	0.001
		LFFluct-LFCon	-0.083	-0.24	0.072	0.47
L2	Aicha	HFFluct-HFCon	-0.295	-0.45	-0.141	0.00005
		LFCon-HFCon	-0.44	-0.59	-0.281	0.000
		LFFluct-HFFluct	-0.207	-0.37	-0.049	0.006
		LFFluct-LFCon	-0.067	-0.225	0.091	0.67
	Pippi	HFFluct-HFCon	0.193	0.003	0.38	0.045
		LFCon-HFCon	-0.06	-0.192	0.073	0.603
		LFFluct-HFFluct	-0.28	-0.49	-0.079	0.0042
		LFFluct-LFCon	-0.030	-0.182	0.1220	0.95
L3	Aicha	HFFluct-HFCon	-0.33	-0.496	-0.16	0.00005
		LFCon-HFCon	-0.501	-0.67	-0.332	0.00
		LFFluct-HFFluct	-0.261	-0.430	-0.092	0.001
		LFFluct-LFCon	-0.09	-0.26	0.082	0.52
	Pippi	HFFluct-HFCon	-0.51	-0.99	-0.037	0.031
		LFCon-HFCon	-0.16	-0.63	0.314	0.80
		LFFluct-HFFluct	0.035	-0.44	0.51	0.99
		LFFluct-LFCon	-0.316	-0.79	0.158	0.29

4.3 Development

4.3.1 Age at maturity (days) across different treatments

Aicha

Age at maturity data indicated that there was significant impact of treatments (Table 5). Although there was no statistical difference on maturation between temperature treatments at high food but was significantly different at low food level (Table 6). At low food level, individuals at fluctuating temperature reached maturation earlier than constant temperature (Figure 7, Table 6 and 13). Moreover, there was significant effects of food levels within temperature treatments which showed that individuals treated with high food level at both temperature treatments matured earlier than at low food level (Figure 7, Table 6 and 13).

Pippi

Treatments showed strong effect on age at maturity (Table 5). Like in Aicha, temperature regimen showed no effect on maturation in individuals treated with high food level but was statistically significant on individuals treated with low food level (Table 6). At low food level, the ones at the fluctuating temperature reached maturation earlier than the individuals at constant temperature (Figure 7; Table 6 and 13). Moreover, there was no significant effect of food level on individuals within fluctuating temperature but individuals at constant temperature were affected by food level where the ones with the high food reached maturation earlier than the ones with low food (Table 6 and 13).

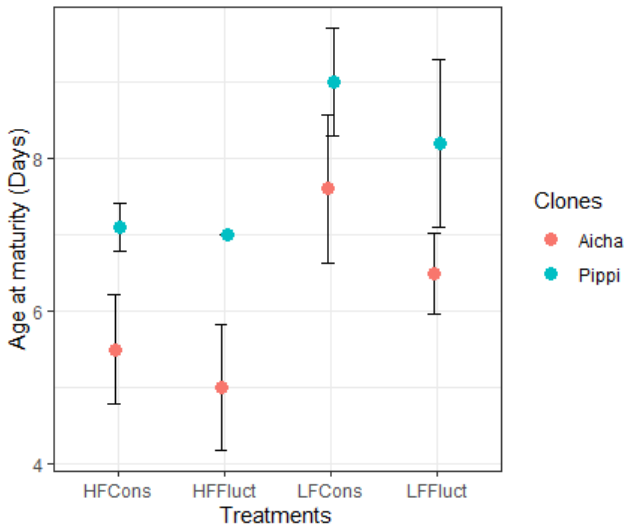

Figure 7. Mean (± Standard Deviation) age at maturity (Days) of both the clones throughout different treatments.

Table 5. One way ANOVA table for Age Maturity for both Aicha and Pippi

Clones	Source	Df	Sum Sq	Mean Sq	F value	Pr (>F)
Aicha	Treatment	3	39.7	13.233	22.26	2.51e-08***
	Residuals	36	21.4	0.594		
Pippi	Treatment	3	19.78	6.593	14.52	1.34e-05 ***
	Residuals	24	10.90	0.454		

Table 6. Tukey's HSD table showing multiple comparisons of means of age at maturity of both Aicha and Pippi clones at different treatments with 95% family-wise confidence level. Here, 'diff' is mean difference value between two groups, 'lwr' and 'upr' and lower and upper end point of interval respectively and 'P adj'is adjusted p-value for multiple comparisons.

Clones	Treatments	diff	lwr	upr	p adj
Aicha	HFFluct-HFCon	-0.5	-1.43	0.43	0.48
	LFCon-HFCon	2.1	1.17	3.03	0.00
	LFFluct-HFFluct	1.5	0.57	2.43	0.00
	LFFluct-LFCon	-1.1	-2.03	-0.17	0.01
Pippi	HFFluct-HFCon	-0.1	-1.32	1.12	0.99
	LFCon-HFCon	1.9	1.05	2.75	0.00
	LFFluct-HFFluct	1.0	-0.31	2.31	0.18
	LFFluct-LFCon	-1.0	-1.98	-0.02	0.04

4.3.2 Molting rates at different treatments

Aicha

In Aicha, treatments had significant impact on juvenile and adult molting rates (Table 7). During juvenile stage, food levels had significant effect at both the temperature regimen but there was no significant difference of temperature treatments indicating that high food levels had higher juvenile molting rates than low food levels at both temperature regimen (Figure 8. Table 8 and 13). During adult stage, individuals treated with high food level at fluctuating temperature had higher molting rate than the ones at constant temperature (Figure 9, Table 8 and 13). And there was no effect of food levels (Table 8).

Pippi

In Pippi, treatments had significant impact on both juvenile and adult molting rate (Table 7). During juvenile stage, temperature treatments had no significant effects on the individuals at high food level but had affected individuals with low food level (Table 8). Starved individuals at fluctuating temperature had higher JMR than at constant temperature (Figure 8, Table 8 and 13). Similarly, food level showed statistical significance at constant temperature only indicating that well-fed individuals at constant temperature had higher juvenile molting rate than starved ones (Table 8). At both the temperature regimen, individuals with high food level had higher molting rate than the individual with low food level. During adult stage, temperature treatments showed significant difference but only on the individuals treated with low food levels (Table 8). Low food individuals at constant temperature had higher adult molting rate than fluctuating ones (Figure 9). No significant differences were seen within food levels (Table 8).

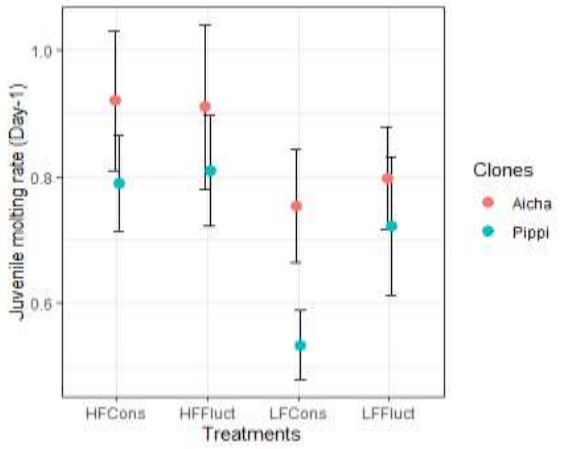

Figure 8. Mean (± Standard Deviation) juvenile molting rate (per day) of both the clones throughout different treatments.

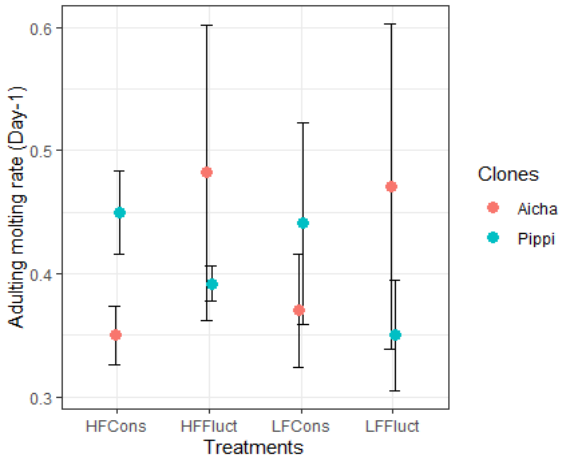

Figure 9. Mean (± Standard Deviation) adult molting rate (per day) of both the clones throughout different treatments.

Table 7. One way ANOVA table for Juvenile and adult molting rate for both Aicha and Pippi clones

Stage	Clones	Source	Df	Sum Sq	Mean Sq	F value	Pr (>F)
Juvenile	Aicha	Treatment	3	3.6	1.2000	9	0.00014***
		Residuals	36	4.8	0.1333		
	Pippi	Treatment	3	0.3659	0.12197	19.82	1.1e-06 ***
		Residuals	24	0.1477	0.00615		
Adult	Aicha	Treatment	3	0.1351	0.04504	5.242	0.00418**
		Residuals	36	0.3093	0.00859		

	Pippi	Treatment	3	0.04361	0.014538	4.686	0.0103*
		Residuals	24	0.07446	0.003103		

Table 8. Tukey's HSD table showing multiple comparisons of means of juvenile and adult molting rates of both clones at different treatments with 95% family-wise confidence level. Here, 'diff' is mean difference value between two groups, 'lwr' and 'upr' and lower and upper end point of interval respectively and 'P adj'is adjusted p-value for multiple comparisons.

Stage	Clones	Treatments	diff	lwr	upr	p adj
Juvenile	Aicha	HFFluct-HFCon	2.220446e-16	-0.44	0.44	1.0
		LFCon-HFCon	-6.000000e-01	-1.04	-0.16	0.004
		LFFluct-HFFluct	-6.000000e-01	-1.04	-0.16	0.0041
		LFFluct-LFCon	2.775558e-16	-0.44	0.439	1.0
	Pippi	HFFluct-HFCon	0.021	-0.121	0.163	0.98
		LFCon-HFCon	-0.255	-0.355	-0.156	0.000002
		LFFluct-HFFluct	-0.088	-0.241	0.065	0.401
		LFFluct-LFCon	0.188	0.074	0.302	0.0007
Adult	Aicha	HFFluct-HFCon	0.129	-0.121	0.241	0.018
		LFCon-HFCon	0.017	-0.095	0.128	0.98
		LFFluct-HFFluct	-0.011	-0.123	0.1004	0.993
		LFFluct-LFCon	0.102	-0.01	0.213	0.086
	Pippi	HFFluct-HFCon	-0.0543	-0.155	0.047	0.463
		LFCon-HFCon	-0.005	-0.076	0.065	0.997
		LFFluct-HFFluct	-0.043	-0.152	0.065	0.693
		LFFluct-LFCon	-0.092	-0.173	-0.011	0.021

4.4 Reproduction

4.4.1 Age at first reproduction

Treatments had substantial effect on time to first reproduction on both Aicha and Pippi (Table 9). In Aicha, temperature regimen had no effects on age at first reproduction at high food level but was significant at low food (Table 10). Starved individuals at fluctuating temperature reached first reproduction earlier than ones at the constant temperature (Figure 10, Table 10 and 13). Similarly, food level had significant impact on constant temperature only, pointing that well-fed individuals reached first reproduction earlier than the starved ones only at constant temperature (Figure 10, Table 10 and 13). In Pippi, temperature treatment had no effect on first reproduction whereas food level had significant impact (Table 10). This implied that at both temperature regimen well-fed individuals reached first reproduction earlier than starved ones (Figure 10).

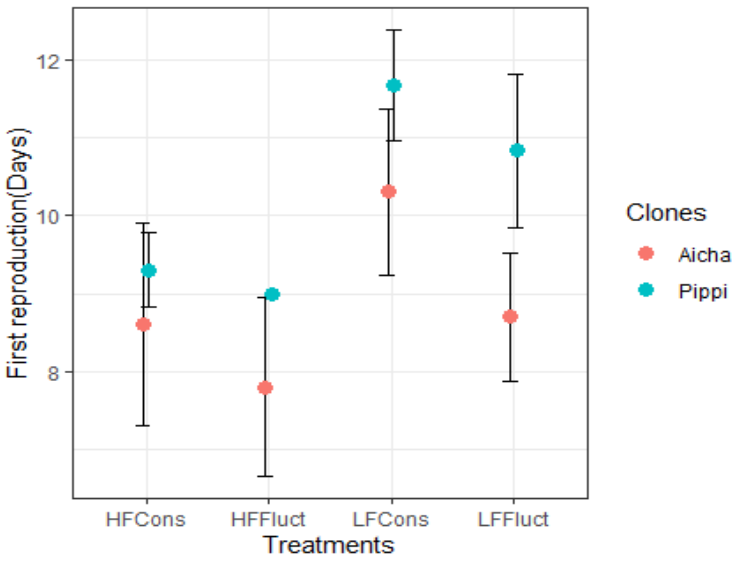

Figure 10. Mean (± Standard Deviation) age at first reproduction (Days) of both the clones throughout different treatments.

Table 9. One way ANOVA table for age at first reproduction for both Aicha and Pippi clones

Clones	Source	Df	Sum Sq	Mean Sq	F value	Pr (>F)
Aicha	Treatment	3	32.9	10.967	9.355	0.000104 ***
	Residuals	36	42.2	1.172		
Pippi	Treatment	3	33.50	11.165	24.51	1.75e-07 ***
	Residuals	24	10.93	0.456		

Table 10. Tukey's HSD table showing multiple comparisons of means of age at first reproduction at of both clones at different treatments with 95% family-wise confidence level. Here, 'diff' is mean difference value between two groups, 'lwr' and 'upr' and lower and upper end point of interval respectively and 'P adj'is adjusted p-value for multiple comparisons.

Clones	Treatments	diff	lwr	upr	p adj
Aicha	HFFluct-HFCon	-0.8	-2.1	0.51	0.36
	LFCon-HFCon	1.7	0.4	3.0	0.01
	LFFluct-HFFluct	0.9	-0.4	2.2	0.26
	LFFluct-LFCon	-1.6	-2.9	-0.3	0.011
Pippi	HFFluct-HFCon	-0.3	-1.53	0.93	0.91
	LFCon-HFCon	2.37	1.51	3.2	0.0000004
	LFFluct-HFFluct	1.83	0.52	3.15	0.0041
	LFFluct-LFCon	-0.83	-1.81	0.15	0.12

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4.4.2 Clutch size

Aicha

In Aicha, treatments had significant effects on clutch size at each clutch (Table 11). At clutch 1, temperature had significant effect on clutch size indicating that well fed individuals at constant temperature had bigger clutch size than the fluctuating temperature (Table 12). Also, individuals treated with high food had attained bigger clutch size than the starved ones at both temperature regimen (Figure 11; Table 12-13). Unlike Clutch 1, at clutch 2, temperature regimen had no significant effect on individuals at high food level (Table 12). Like in clutch 1, food level also had significant impact on clutch size at clutch 2 within each temperature regimen. The ones with high food level had bigger clutch size than the ones with low food (Figure 11, Table 12-13). In clutch 3 there was no significant effect of treatments depicting that individuals at all treatments attained clutch size with insignificant differences (Table 12).

Individuals with high food level at constant temperature had the biggest clutch size along the first (14.7±1.28) and second (21.4 ±0.95) clutch whereas high food level at fluctuating temperature had the biggest clutch size on third clutch (17.9±4.042) (Table 13). Individuals with low food level at constant temperature had the smallest clutch size along first (5.2±0.49), second (8.2±0.467) and third clutch (6.4±0.73) (Table 13).

Pippi

For Pippi, treatments had significant effects on clutch size at each clutch (Table 11) .At Clutch 1, like Aicha, only food level had significant effects on cluch size whereas temperature regimen had no effect (Table 12). This indicated that well fed individuals within each of the temperature regimen had bigger clutch size at clutch 1 than the starved ones (Figure 11, Table 12 -13). At clutch 2, temperature had significant effect on high food level but no effect on low food which implied that individuals treated with high food, at fluctuating temperature had bigger clutch size than the ones at constant temperature (Figure 11; Table 12-13). Like clutch 1, food level was highly significant on clutch size at clutch 2 as well and individuals having high food had bigger clutch size than the ones with low food within each temperature regimen (Figure 11, Table 12-13). At clutch 3, individuals treated with high food at constant temperature couldn't reach third clutch and there was no significant effect of any treatment on clutch size (Figure 11). HFFluct has biggest

clutch size at first (14.67±0.33) and second (11.67±4.84) clutch. At HFCons, clutch size at first was 12.1±0.92 and second was 9.8±0.696 (Table 13). Individuals at low food at fluctuating temperature had the bigger clutch size at second (6.17± 1.07) and third (4.4 ±0.245) clutch than the second clutch (4.11±0.423) and third clutch (4.11 ±0.423) at constant temperature (Table 13).

The average length of offspring of experimental animals of both clones at were comparatively bigger at low food conditions than at high food (Table 13).

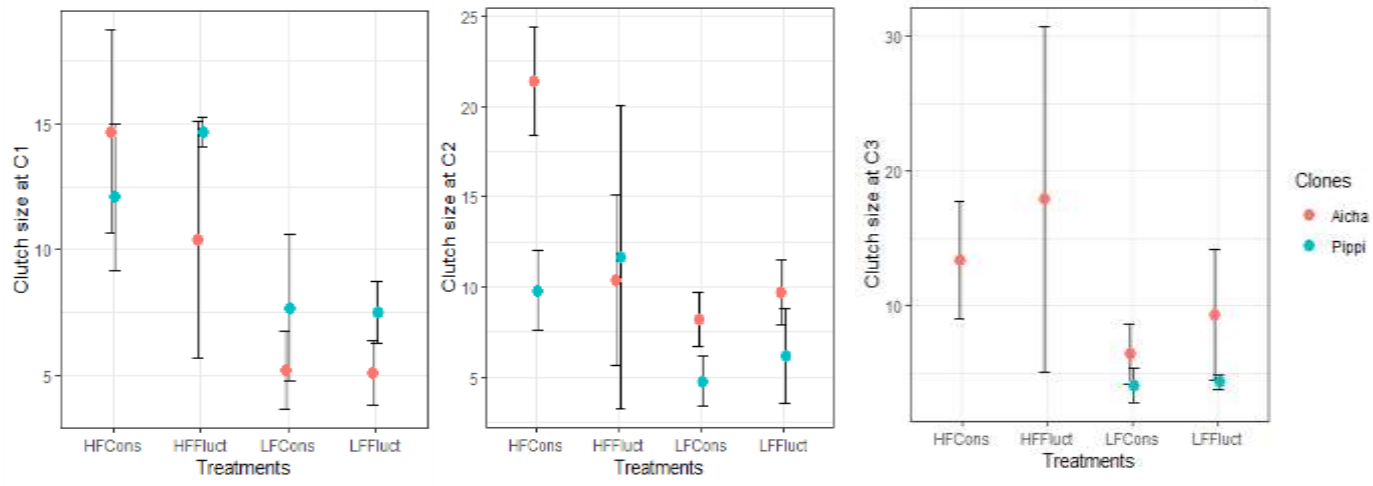

Figure 11. Mean (± Standard Deviation) number of offspring at each clutch (C1, C2 and C3 at first, second and third clutch release respectively) of both the clones throughout different treatments.

Table 11. One way ANOVA table for clutch size at clutch one, two and three for both Aicha and Pippi clones

Clutch	Clones	Source	Df	Sum Sq	Mean Sq	F value	Pr (>F)
One	Aicha	Treatment	3	688.1	229.37	22.82	1.88e-08 ***
		Residuals	36	361.8	10.05		
	Pippi	Treatment	3	195.9	65.30	10.24	0.000158 ***
		Residuals	24	153.1	6.38		
Two	Aicha	Treatment	3	1345.1	448.4	46.8	2.44e-12***
		Residuals	35	335.3	9.6		
	Pippi	Treatment	3	464.2	154.74	23.99	2.11e-07***
		Residuals	24	154.8	6.45		
Three	Aicha	Treatment	3	276.1	92.04	2.901	0.049*
		Residuals	34	1078.7	31.73		
	Pippi	Treatment	2	18.02	9.009	3.428	0.0498*
		Residuals	23	60.44	2.628		

Table 12. Tukey's HSD table showing multiple comparisons of means of clutch size at first, second and third clutch of both clones at different treatments with 95% family-wise confidence level. Here, 'diff' is mean difference value between two groups, 'lwr' and 'upr' and lower and upper end point of interval respectively and 'P adj'is adjusted p-value for multiple comparisons.

Clutch	Clones	Treatments	diff	lwr	upr	P adj
First	Aicha	HFFluct-HFCon	-4.7	-8.52	-0.88	0.107
		LFCon-HFCon	-9.9	-13.72	-6.08	0.00
		LFFluct-HFFluct	-5.3	-9.12	-1.48	0.003
		LFFluct-LFCon	-0.1	-3.92	3.72	0.99
	Pippi	HFFluct-HFCon	2.57	-2.02	7.15	0.43
		LFCon-HFCon	-4.43	-7.63	-1.23	0.004
		LFFluct-HFFluct	-7.17	-12.09	-2.24	0.002
		LFFluct-LFCon	-0.17	-3.84	3.51	0.99
Two	Aicha	HFFluct-HFCon	-1.50	-5.23	2.23	0.70
		LFCon-HFCon	-13.1	-16.83	-9.37	0.00
		LFFluct-HFFluct	-10.13	-13.97	-6.29	0.00
		LFFluct-LFCon	1.47	-2.37	5.30	0.73
	Pippi	HFFluct-HFCon	7.60	2.99	12.21	0.00
		LFCon-HFCon	-5.62	-8.84	-2.40	0.00
		LFFluct-HFFluct	-11.83	-16.79	-6.88	0.00
		LFFluct-LFCon	1.39	-2.30	5.08	0.73
Three	Aicha	HFFluct-HFCon	2.66	-4.33	9.65	0.74
		LFCon-HFCon	6.56	-0.62	13.73	0.08
		LFFluct-HFFluct	-2.90	-9.70	3.90	0.66
		LFFluct-LFCon	-6.80	-13.79	0.19	0.06
	Pippi	LFFluct-HFFluct	-0.94	-2.92	1.03	0.47
		LFFluct-LFCon	1.11	-0.80	3.03	0.33

Table 13. Mean values ± standard deviations of the growth rates (mm/day) during first, second and third reproduction, length (mm) at time of disposal, age at maturity (days), juvenile and adult molting rate molts/day, age at first reproduction (days), clutch size (number of offspring) and size of offspring (mm) at clutch 1, 2 and 3 for the two clones, Aicha and Pippi.

	Treatments			
	HFCons	HFFluct	LFCons	LFFluct
Growth rate at first reproduction				
Aicha	0.297 ± 0.048	0.289 ± 0.052	0.198 ± 0.026	0.215 ± 0.03
Pippi	0.234 ± 0.031	0.268 ± 0.004	0.178 ± 0.017	0.185 ± 0.0214
Growth rate at second reproduction				
Aicha	0.06 ± 0.038	0.065 ± 0.05	0.04 ± 0.03	0.072 ± 0.089
Pippi	0.042 ± 0.041	0.025 ± 4.25E-18	0.021 ± 0.025	0.045 ± 0.023
Growth rate at third reproduction				
Aicha	0.053 ± 0.044	0.03 ± 0.014	0.015 ± 0.016	0.035 ± 0.026
Pippi	0.015 ± 0.014	0.0135 ± 0.009	0.03 ± 0.016	0.04 ± 0.014
Length at disposal				
Aicha	3.033 ± 0.17	2.71 ± 0.13	2.532 ± 0.11	2.483 ± 0.125
Pippi	2.75 ± 0.121	2.93 ± 0.015	2.691 ± 0.07	2.68 ± 0.041
Age at maturity				
Aicha	5.5 ± 0.71	5 ± 0.82	7.6 ± 0.97	6.5 ± 0.53
Pippi	7.1 ± 0.32	7	9 ± 0.71	8.2 ± 1.1
Juvenile molting rate				
Aicha	0.92 ± 0.11	0.91 ± 0.13	0.754 ± 0.09	0.798 ± 0.081
Pippi	0.79 ± 0.076	0.81 ± 0.087	0.534 ± 0.054	0.722 ± 0.11
Adult molting rate				
Aicha	0.35 ± 0.024	0.482 ± 0.12	0.37 ± 0.046	0.471 ± 0.132
Pippi	0.45 ± 0.034	0.392 ± 0.0144	0.441 ± 0.082	0.35 ± 0.045
Age at first reproduction				
Aicha	8.6 ± 1.3	7.8 ± 1.14	10.3 ± 1.06	8.7 ± 0.823
Pippi	9.3 ± 0.48	9	11.67 ± 0.71	10.83 ± 0.983
Clutch Size-Clutch 1				
Aicha	14.7 ± 4.06	10.4 ± 4.72	5.2 ± 1.55	5.1 ± 1.29
Pippi	12.1 ± 2.923	14.67 ± 0.58	7.67 ± 2.92	7.5 ± 1.225
Clutch Size-Clutch 2				
Aicha	21.4 ± 2.99	10.4 ± 4.72	8.2 ± 1.48	9.67 ± 1.803
Pippi	9.8 ± 2.2	11.67 ± 8.4	4.78 ± 1.394	6.17 ± 2.64
Clutch Size-Clutch 3				
Aicha	13.4 ± 4.31	17.9 ± 12.8	6.44 ± 2.19	9.33 ± 4.85
Pippi	-	-	4.11 ± 1.27	4.4 ± 0.55
Offspring Size- Clutch 1				
Aicha	0.577 ± 0.342	0.585± 0.031	0.696 ±0.0304	0.624 ±0.026
Pippi	0.684±0.017	0.675±0.0412	0.687±0.057	0.692±0.0461
Offspring Size-Clutch 2				
Aicha	0.677±0.038	0.619±0.021	0.716±0.044	0.692±0.068
Pippi	0.676±0.0354	0.621±0.057	0.793±0.0399	0.759±0.024
Offspring Size-Clutch 3				
Aicha	0.65±0.034	0.615±0.034	0.734±0.0412	0.671±0.04
Pippi	-	-	0.765±0.039	0.772±0.03

5. Discussion

The objectives of this master's thesis was to find if starvation has more severe life history significances in fluctuating than in constant temperatures. The impacts of temperature regimen on the measured life history parameters were inconsistent within and between food levels and the clones. As per our first research question, fluctuating temperature had noteworthy impact on the body length, development and reproduction of *Daphnia*. But the responses differed on the basis of food concentrations as per our second research question. Fluctuating temperature had negative effects on body length but positively affected maturation time and juvenile molting rate (only at low food level), adult molting rates (only at high food of Aicha) and age at first reproduction (only at low food level). For clutch size, then again, the effect of fluctuating temperature was negative on the first clutch (only at high food of Aicha) than the second and third clutch. But two clones responded differently throughout the treatments. Pippi was observed in performing better at fluctuating temperature than at constant as body length and clutch size were relatively bigger. Since, only 30% of Pippi individuals were considered in fluctuating temperature at high food level, sample size might have influenced the overall result.

Our data indicated that both temperature regimens and food levels were affected body length and growth rates. At all the temperature food combinations, growth during early life was rapid but gradually decreased with age. Body length depended on temperature condition only where the food resource was abundant. In this condition, the *Daphnia* grew better at stable temperature while thermal fluctuations had comparatively negative effects on growth and body length. Individuals mature at a size that resulted from maximal fitness at the expense of one or more trade-offs (Berrigan & Charnov, 1994). Answering our first research question to some extent, we noticed that the energetic costs of the fluctuating temperatures were evident in body length at first reproduction, which was smaller in the fluctuating temperature regimen than the constant, and the effect was more pronounced at high food level. Giebelhausen and Lampert (2001) also found a more pronounced effect of temperature (considering at a range of 15-30 °C) at high food concentrations (0.1-1.0 mg C l-1) on growth. Reichwaldt et al. (2005) also observed that under a fluctuating temperature regime (considering at a range of 12℃-19℃), the somatic growth rate of *D. hyalina* was even as low as at stable cold temperature indicating high expenses of a fluctuating temperature which means even though the temperature range (12℃-19℃) is comparatively lower

than our study, responses towards thermal fluctuations could count on performance tolerance of the species (Dong et al., 2006; Du & Feng, 2008).

In our study, food concentrations had strong impacts on body length regardless of temperature treatments. Well-fed individuals within each temperature treatment were bigger in size than the starved ones. Low food might compel individuals to reallocate their energy/resources in survival and reproduction or other life-history parameters rather than body length/size (Betini, Wang, Avgar, Guzzo, & Fryxell, 2020).

Previous works stated that molting occurs frequently in the juvenile phase, but more slowly and less regularly in adult life (Bottrell, 1975; Green, 1956). My results indicated few impacts of temperature regimen in developmental stages (molting rate and maturation time) of the *Daphnia* (Figure 7-9). The direction of the effect was dissimilar between the clones. At low food level, thermal fluctuation relatively had positive effects on the molting rate at juvenile stage than adult stage in Pippi. In line with our findings, Giebelhausen and Lampert (2001) also observed that low food concentrations could have lower temperature effects. There are some documentations which also illuminate positive effects of thermal fluctuations on insect development (Kaufmann, 1932) and the growth rate of *Daphnia* (Orcutt & Porter, 1984) and which is often referred as the 'Kaufmann effect' (Cossins & Bowler, 1987), according to which sometimes, non-optimal temperatures can either speed up or delay development that can be deviated from the expectations when insects are subjected to thermal fluctuations (Capinera, 2008). In our study, although, no food level effect was observed on adult molting rate of both the clones, molting rates at adult stage in low food level was negatively influenced by thermal fluctuations. The negative impact of thermal fluctuations may have been enhanced by pressure (Cossins & Bowler, 1987), since the fluctuating temperature regimen of our experiment slightly had warmer range and low food itself being stressful for adult individuals.

Fluctuating temperature had shortened the maturation time on both the clones, assigned with low food level but did not affect high food level. Since only the starved individuals at fluctuating temperature attained maturity earlier than the starved ones at constant temperature, it could mean that starvation was handled well in fluctuating temperature than starvation, answering some extent of my first and second research questions. Chen et al. (2019) found that in *Aldrichina grahami*, a forensic and cold tolerant insect, at fluctuating temperatures, the developmental rate was relatively lower than in stable temperatures over the entire developmental cycle, and most developmental periods had substantial differences between thermal regimens An account for different findings in thermal fluctuation studies on development could rely on existing temperature average and its proximity to developmental tolerance of the species since fluctuating temperatures within unfavorable range generally slow down development than at optimal stable temperatures (García-Ruiz et al., 2011; Kersting et al., 1999).

The food effect could be more pronounced on reproduction than the temperature effect (Lampert & Trubetskova, 1996). In this study, fluctuating temperature has positively affected age at first reproduction only at low food in Aicha. Individuals could have enhanced their thermal tolerance and decreased their metabolic plasticity (Chen & Stillman, 2012) allocating energy for reproduction. On the contrary, Schwartz et al. (2016) highlighted that in *D. pulex* thermal fluctuations have higher impacts on reproduction e.g. late reproduction on low food treatment. In that case, food limitation might have reduced energy budget that delayed reproduction as seen in other *Daphnia* studies as well (Kim, Ansell, & Dudycha, 2014).

Clutch size was observed to be shaped by food level; high food resulted in relatively bigger clutch size and low food relatively smaller clutch size in both temperature regimen. In line with our studies, Pietrzak et al. (2010) also observed strong reproductive effort, under high food level but found a strong negative association between investments in the early reproduction and life span indicting that high early reproductive investment could be costly. Since the experiment of our study was ended after third clutch release, we could not check such costs of early investment. Moreover, temperature regimen affected the clutch size only at high food level at clutch 1 of Aicha clone, i.e. constant temperature had relatively positive effects on clutch size than fluctuating temperature. Since, body length of Aicha individuals at constant temperature was bigger than the ones at fluctuating temperature, the larger body could have supported higher number of offspring.

Xie et al. (2000) had found that brood/clutch size of *Daphnia rosea* was positively related to the size of the mother. The performance is better at good food conditions and (Xie, Iwakuma, & Fujii, 2000).

The overall reproduction (no. of offspring considered) in this experiment seemed to be very low in comparison to previous experiments conducted in University's laboratory with the same clones (unpublished) and may be the *Daphnia* were physiologically in a more stressed condition due to possible infection and the food quality change.

6. Conclusion

Since in real world, thermal fluctuations is evident and it is important to be able to predict how organisms adapt to and respond to such fluctuations. This study suggests that thermal fluctuations can have deviating effects on important life history traits impacting *Daphnia*s' life history strategies depending upon other environmental parameters like food availability, i.e. relatively negative effects (growth and reproduction) on high food availability and somewhat better performance (development) at low food concentrations.

In *Daphnia magna*, since thermal fluctuations effects were inconsistent across the food treatments, it can be suggested that they can perform cellular adjustments through energy reallocation. Identifying the basis of such adjustment through laboratory studies can help us predict the real structure that occurs in natural populations.

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Appendix

A. Temperature outline throughout the experiment

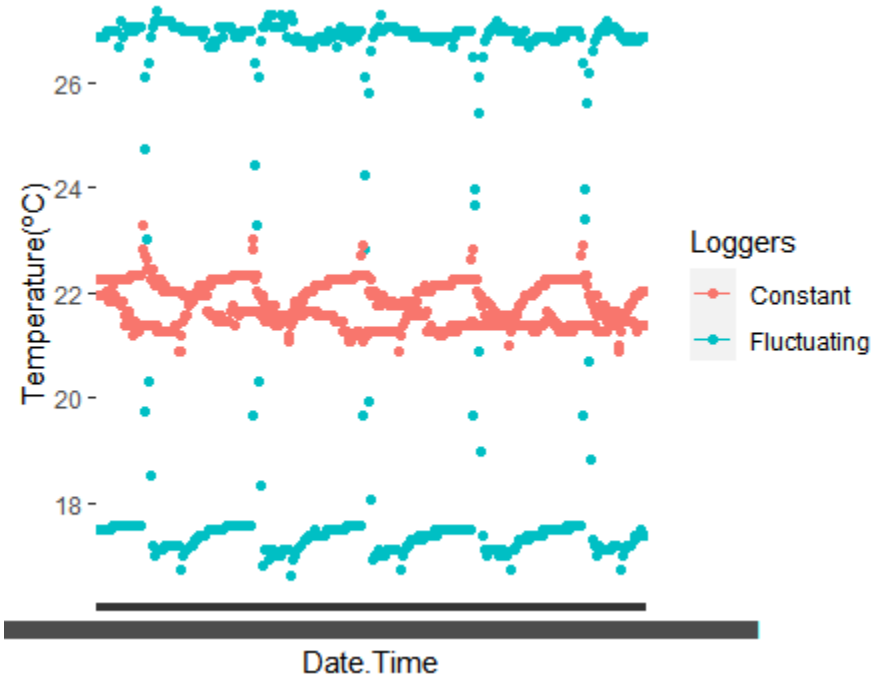


Figure 12. Daily temperature profiles for the constant and fluctuating temperature chambers for over four days.

B. Recipe for ADaM medium

The experimental *Daphnia* were cultured in artificial medium. Artificial *Daphnia* Medium (ADaM) protocol was provided by Ebert et al. (2013). The original formula for the medium was developed by Klüttgen, B., Dülmer U., Engels, M. & Ratte H. T. 1994. ADaM, an artificial freshwater for the culture of zooplankton. Water Research 28:743-746. However, as per the University of Basels' description, we used a modified version of this medium i.e. altered Seleniumoxide concentration. We used 60 liter plastic tanks to prepare the medium. The required amount of sea salt (Table I) was taken for 60 litres of distilled water and three stock solutions (A,B and C) were added using electric pipettes (Table II). We made sure the medium was made 24 hours in advance to keep them aerated.

Table I. Recipe for different amounts of ADaM (using the concentrations of stock solutions illustrated below):

Amount of ADaM	Sea salt (g)	Stock A (mL)	Stock B (mL)	Stock C (mL)
5L	1.67	12.5	12.5	0.5
10L	3.34	25	25	1
20L	6.68	50	50	2
30L	10	75	75	3
40L	13.4	100	100	4
50L	16.7	125	125	5
60L	20	150	150	6

Table II. Concentrations for three stock solutions:

Stock solutions	Component	Amount (g/L)
A	Calcium chloride dihydrate ($CaCl_2$ $2H_2O$)	108.2
B	Sodium bicarbonate ($NaHCO_3$) /($CHNaO_3$)	22.2
C	Selenium dioxide (Seo_2)	0.07

C. Recipe for WC medium for algae cultures

WC medium for algae cultures was made in the laboratory following the formula given by Guillard RRL & Lorenzen CJ (1972) Yellowgreen Algae with Chlorophyllide C. J. Phycol. 8, 1014.

5 L recipe (without autoclaving)

- 0.575 grams (575 mg) of the TES buffer was added to 5-liter Erlenmeyer flask and the flask was filled with 4.96 litres of distilled water and stirred well using stirrer plate with ethanol/distilled water rinsed magnet.
- 5 ml of each of stock solutions (1-6, Table. III) was added.

- 10 ml of stock solution 7 (trace elements) and 5 ml of stock solution 8(vitamins) were added to the medium (Table. IV and V). The medium was stirred for some minutes and was filled into clean flasks and were stored in cold and dark.

Table III. Components and amount of stock solutions (1-6)

Stock solutions	Component	Molar Weight g/mol	Quantity stock solution g/L	Quantity stock solution g/ml
1	CaCl2 * 2 H2O	147.01	36.80	3.68
2	MgSO4 * 7 H2O	246.47	37.00	3.70
3	NaHCO3	84.01	12.60	1.26
4	K2HPO4 * 3 H2O	228.22	11.40	1.14
5	NaNO3	84.99	85.00	8.50
6	Na2 O3 Si* 5 H2O	605.19	21.20	2.12
7	Combined trace elements			
8	Vitamin mix			

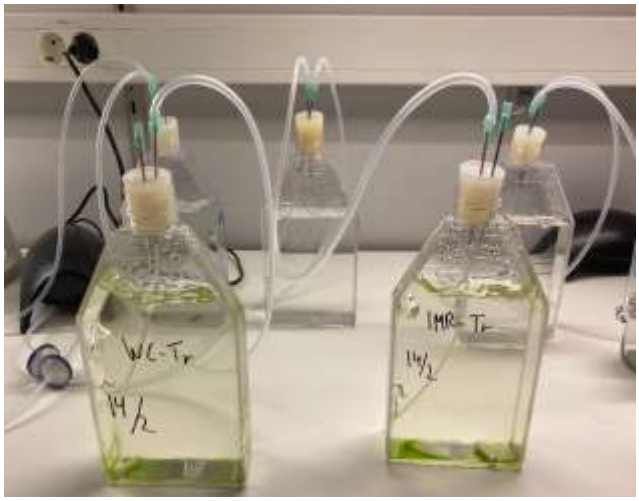
Table IV. Components and quantity of stock solution 7 (Combined trace elements)

	Component	Molar weight g/mol	Quantity stock solution g/l	Quantity stock solution g/500ml	Primary stock solution g/l	Primary stock solution g/ml
7.1	Na2EDTA	372.24	4.36	2.18		
7.2	FeCl3 * 6 H2O	270.32	3.15	1.575		
7.3	CuSO4 * 5 H2O	249,69	0.01	0.005	10	1
7.4	ZnSO4 * 7 H2O	287.54	0.022	0.011	22	2.2
7.5	CoCl2 * 6 H2O	237.93	0.01	0.005	10	1
7.6	MnCl2 * 4 H2O	197.9	0.18	0.09	180	18
7.7	NaMoO4 * 2 H2O	241.95	0.006	0.003	6	0.6
7.8	H3BO3	61.83	1	0.5		

Table V. Components and quantity of Stock solution 8 (Vitamin mix)

#	Component	Molar Weight g/mol	Quantity stock solution g/l	Primary stock solution g/100ml
8.1	Thiamine Hydrochloride	337.27	0.1	1
8.2	Biotin	244.31	0.0005	0.005
8.3	Cyanocobalamin	1355.37	0.0005	0.005

D. Pictures taken during experiments



Chlamydomonas reinhardtii collapsed during experiment

Male Pippi (HFFluct)

Aicha before disposal (HFFluct)

Pippi before disposal (HFFluct)

Temperature cabinets used in our experiment

D. Individual length at different age

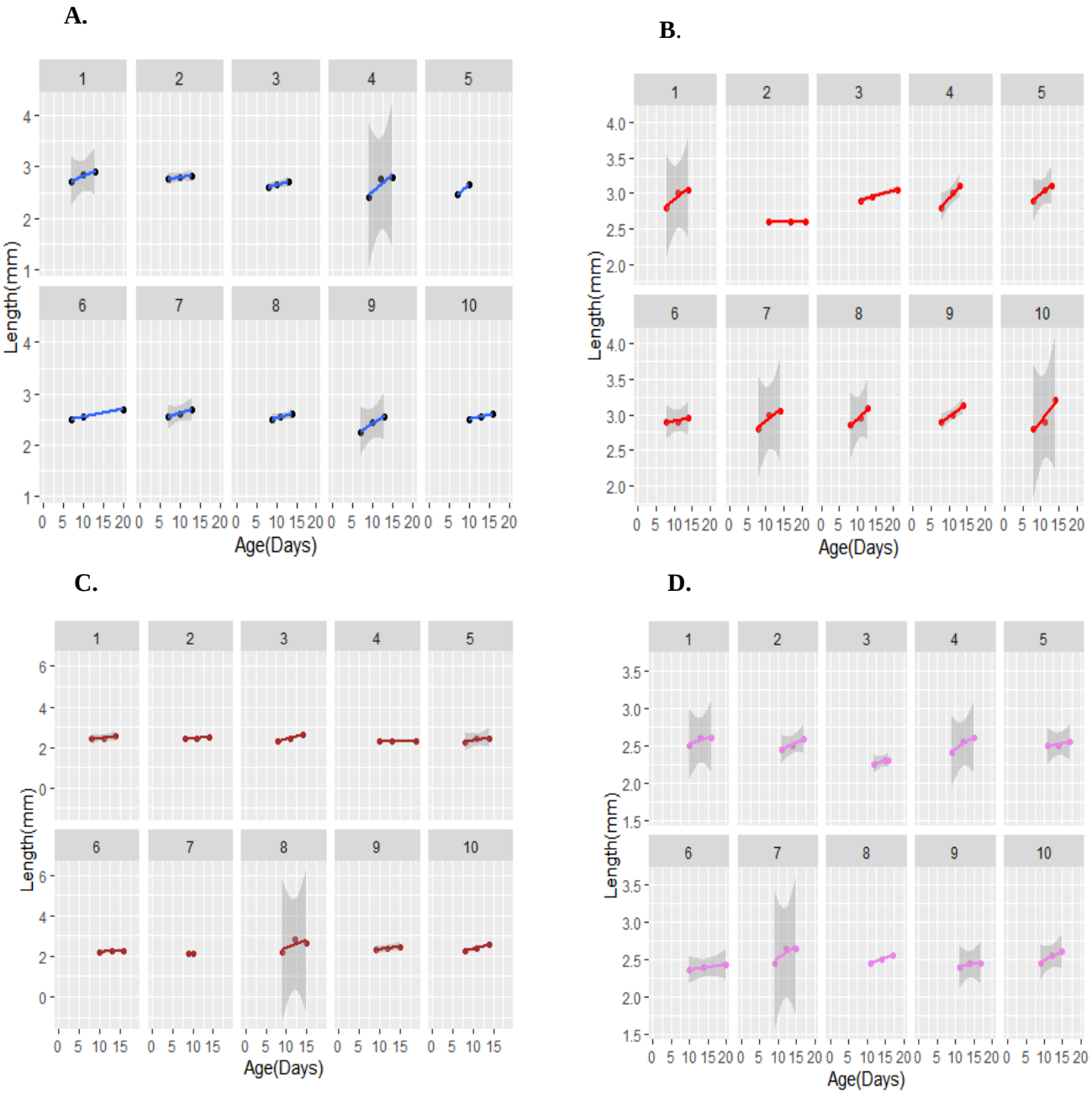


Figure A. Relationship between age and length of Aicha clones at HFFluct **(A)**, HFCons **(B)**, LFFluct **(C)** and LFCons **(D)** treatments

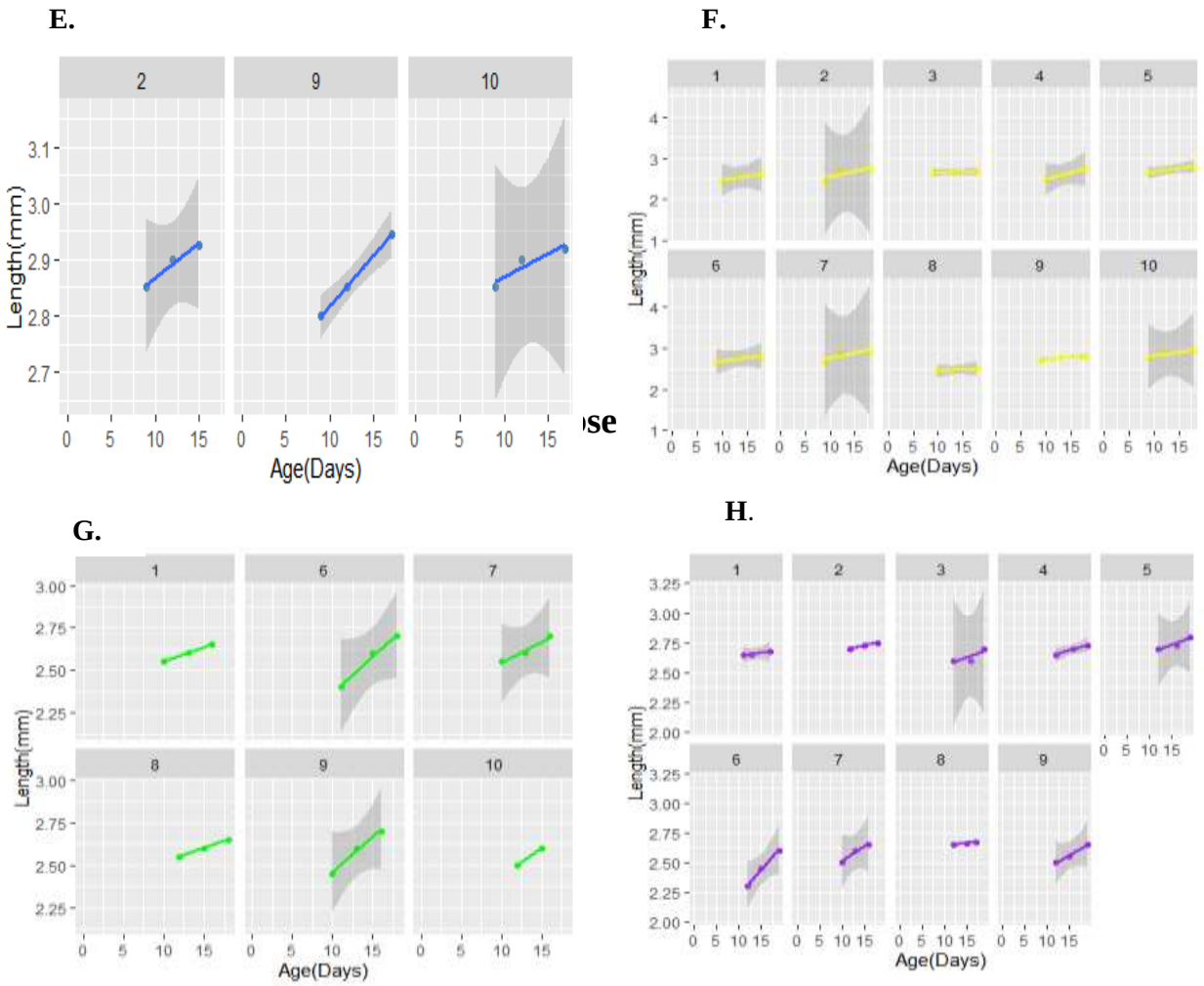


Figure B. Relationship between age and length of Pippi individuals at HFFluct **(D)**, HFCons **(E)**, LFFluct**(F)** and LFCons **(G)** treatments. Male individuals were excluded (missing ones).

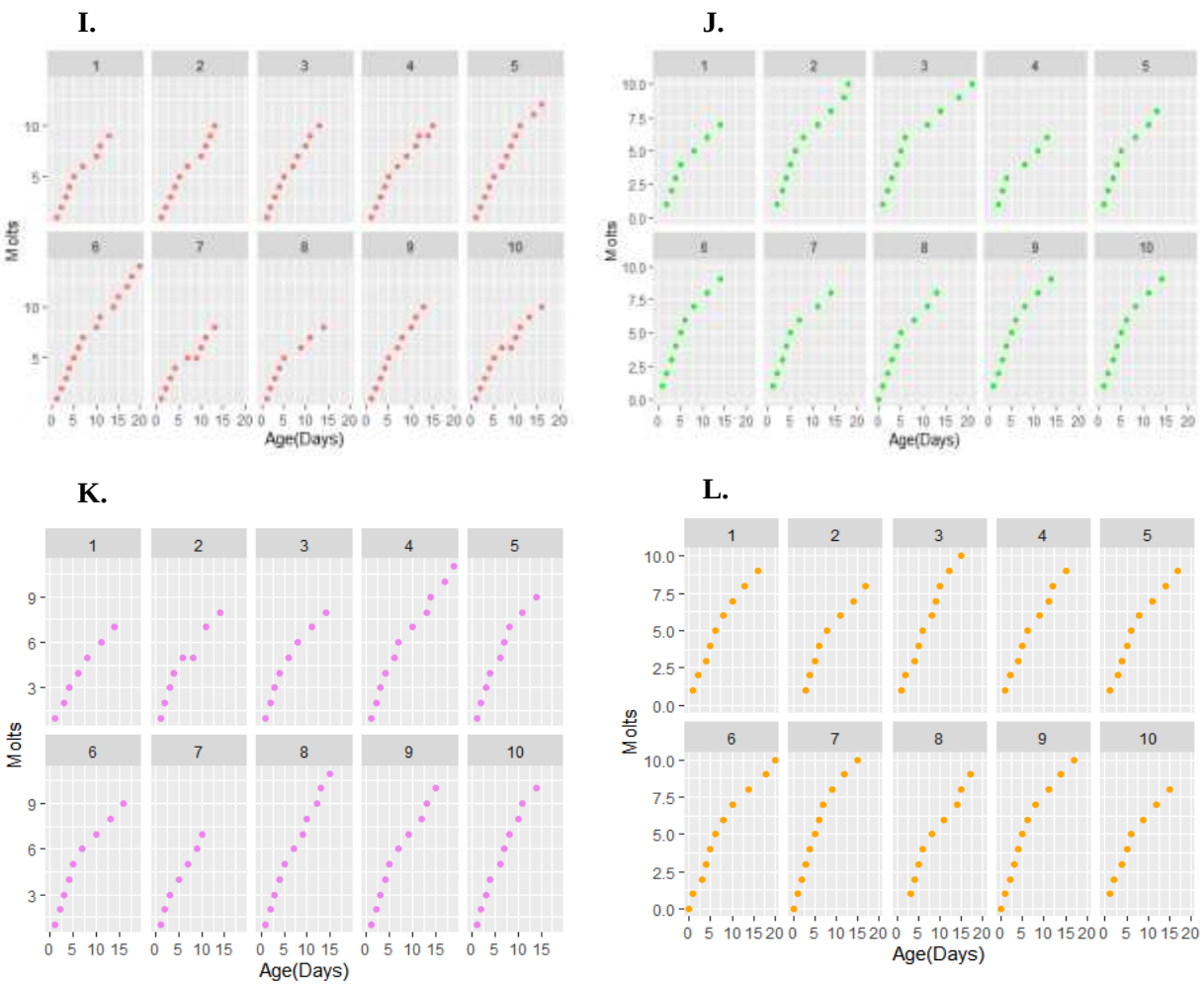


Figure C. Individual molting profile (cumulative number of molts) of Aicha individuals at HFFluct (**I**), HFCons **(J),** LFFluct **(K)** and LFCons **(L)** treatments.

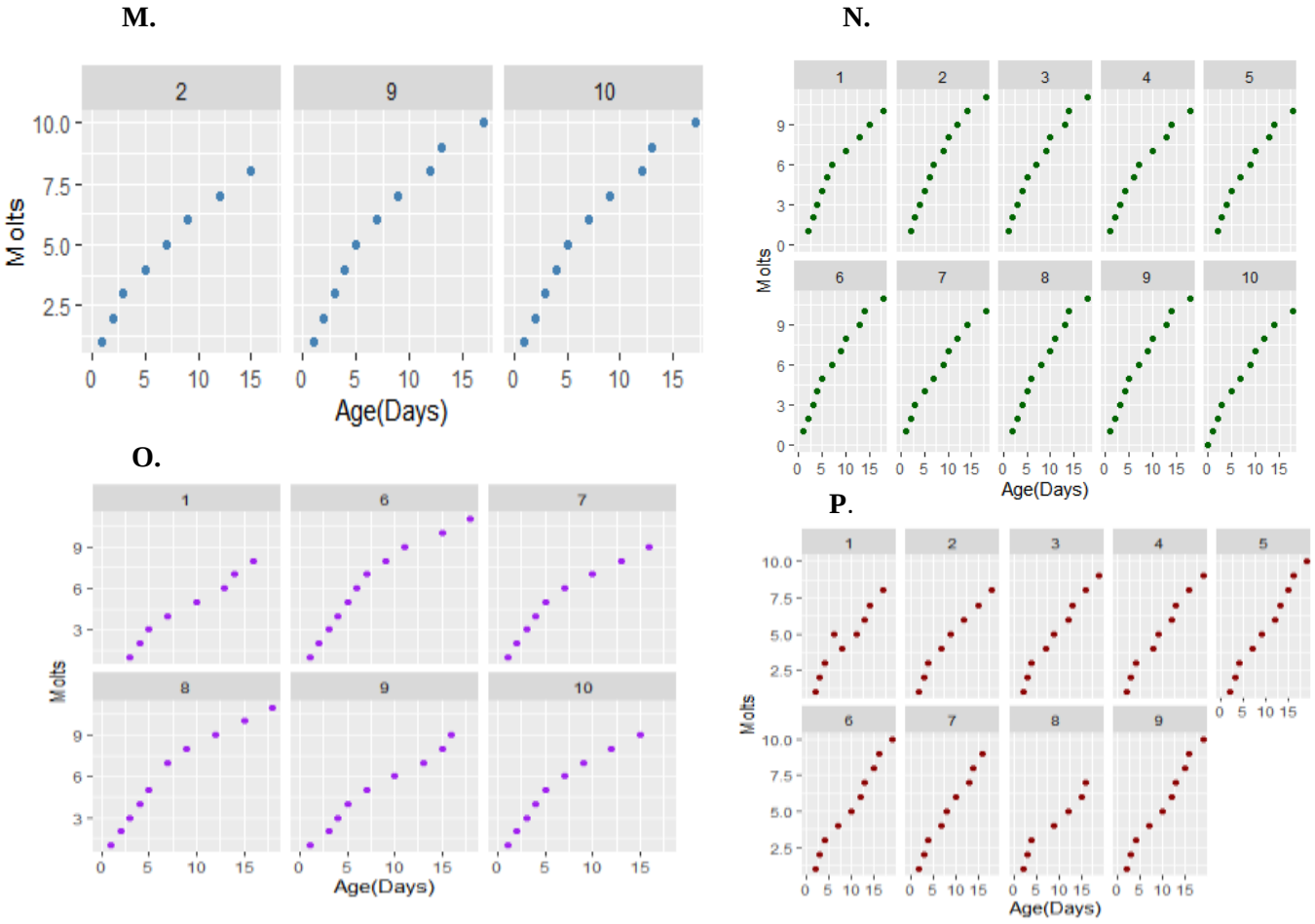


Figure D. Individual molting profile (cumulative number of molts) of Pippi individuals at HFFluct (**M**), HFCons **(N),** LFFluct **(O)** and LFCons **(P)** treatments. Male individuals were excluded (missing individuals).