Effects of predictability in food conditions on individual growth in *Daphnia magna*

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Title: Effects of predictability in food conditions on individual growth in *Daphnia magna*

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Abstract

Daphnia magna is known for living in environments were temperature and food conditions may vary a lot. Food can be a determining factor for growth and reproduction. In the presence of rapid environmental changes, ecosystems become more unpredictable, which concerns the availability of food. How increased unpredictability in food conditions affects species is not fully understood. In this study *Daphnia magna* is used to look at possible life history responses to unpredictable environments with focus on food availability. Two clones of *Daphnia magna,* with 30 individuals from each, were exposed to three different food treatments: constant food level, predictable variation, and unpredictable variation in food level. There were 10 individuals in each treatment. The constant treatment involved the same intermediate food ration given each day, while the two variation treatments involved daily variations between high and low food rations, where one treatment had predictable variations and the other unpredictable variations. Due to a general lack of reproduction and the majority of the *Daphnia* in one of the clones being males, this study is focused on how unpredictability in food affects individual growth in female and male *Daphnia magna*. ANOVA-analyses and post-hoc tests were used to indicate significance between the treatments. Individuals given the constant treatment grew significantly bigger than individuals exposed to the two variation-treatments. There was no significant size difference between *Daphnia* in the predictable variation and unpredictable variation treatments. Increased unpredictability in food variation did not seem to affect growth.

Acknowledgements

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1. Introduction

Food is an important resource for the energy allocation in animals (Glazier & Calow, 1992). If food sources are scarce, a common adaptive response is to allocate more energy into somatic maintenance than growth and reproduction. This can be explained as an attempt to delay reproduction until food conditions are good enough for offspring to survive (Masoro & Austad, 1996). The energy allocation in animals is an important part of their life history response to environmental conditions (Glazier & Calow, 1992). Population dynamics and ecosystem function can be easier understood with more knowledge on the energy allocation patterns species have adapted through life history strategies (Glazier & Calow, 1992).

Adaptive strategies to environmental conditions are becoming more crucial in the presence of rapid environmental changes. Ecosystems and species have always responded to variations and changes in their environments, but now these changes occur at a much higher pace (Philippart et al., 2011). Global warming induces mismatches between seasonal processes of different organisms due to higher mean temperatures (Betini, Wang, Avgar, Guzzo, & Fryxell, 2020). Some coexisting species respond in a similar way and remain synchronized, while others respond at different rates, resulting in asynchrony across trophic levels (Wagner et al., 2013). In the presence of these rapid changes conditions become more unpredictable, affecting the timing, variation and amount of resources (Doney et al., 2011). To optimize their fitness, animals may use environmental cues to predict future conditions so that they can modify their investments (Barbosa et al., 2015). With more unpredictable environments these cues might not give the right interpretation of the future. In this study *Daphnia magna* is used to look at possible life history responses to unpredictable environments with focus on food availability.

1.1 The study species, *Daphnia magna*

Daphnia magna (figure 1 and 2) is a species of small, filter feeding crustaceans which is often used to study life history responses in relation to environmental change (Betini et al., 2020; Giebelhausen & Lampert, 2001). They belong to the order *Cladocera* (water flees), and the family *Daphniidae*. *D. magna* are big compared to other *Daphnia* species, and has a well-known biology and life history (Martinez-Jeronimo, Villasenor, Rios, & Espinosa, 1994). They are often used as model organisms due to their short generation time, being easy to culture and manage, their direct larval development, and asexual reproduction (Martinez-Jeronimo et al., 1994). By having clonal asexual reproduction, they are ideal for studying environmental responses, as we can look at responses to different environmental conditions on the same genotype.

D. magna are primary consumers in their ecosystems and mainly feed on algae, but also bacteria and detritus (Martinez-Jeronimo et al., 1994). They live in small ponds, rock pools and shallow lakes, and are adapted to habitats with high fluctuations in both food and temperature (Giebelhausen & Lampert, 2001). Their food conditions may vary a lot and is very dependent on algae blooms (Giebelhausen & Lampert, 2001). Small habitats, such as rock pools, may have large seasonal variations in organic matter and nutrients. *Daphnia* is often the dominant zooplankton in these habitats (Wulff, 1980). Small *Daphnia*-species is often found in lakes with high pressure of fish predation while *D. magna* usually live in habitats with no fish predation (Ebert, 2005). As a species used to a variable and fluctuating ecosystem, *D. magna* is useful to study how unpredictability may affect life histories.

Increased variability in the environment is thought to decrease fitness in animals (Turelli, 1977). Some species may be better adapted than others, especially those living in habitats with high levels of fluctuations. In a previous study by Barbosa et al. (2015) increased unpredictability in temperature did not reduce fitness in *Daphnia magna* (Barbosa et al., 2015). As food and temperature is strongly connected in determining growth in *Daphnia* (Betini et al., 2020), a better knowledge on how unpredictable food variation affects growth is crucial to fully understand how these organisms may be affected by future conditions. *Daphnia* is considered as one of the best model species to study the effect of global change on freshwater ecosystems (Altshuler et al., 2011). These ecosystems are important to study in the context of climate change (Edlund et al., 2017). Aquatic ecosystems are directly affected by climate through wind, precipitation, and temperature changes. This influences processes related to the abundance of algae, which is an important food source for *Daphnia* and zooplankton in general (Edlund et al., 2017). More knowledge on how variation in food availability affect individuals is crucial to better predict the future of ecosystems (Barbosa et al., 2015).

Figure 1: Female *Daphnia magna* from the Pippi-clone.

Figure 2: Male *Daphnia magna* from the Pippi-clone.

1.2 Aim

The aim of this study was to see how predictability in food conditions affects the life history in two different clones of *Daphnia magna*. This was done by comparing the response of *D. magna* to three different food treatments consisting of a constant food level, predictable variation in food level and unpredictable variation in food level. Constant food level was the control group consisting of a favorable food level with no variation. The predictable and unpredictable variation treatments was compared to see how *D. magna* responds to unpredictability in food sources. Due to complications with the *Daphnia* cultures resulting in lack of data, my aim had to be narrowed down to only look at responses to individual growth.

My hypotheses was that the *Daphnia* would perform best, and grow the largest under constant food conditions, and worst under unpredictable food variation. I predicted the predictable food variation to have an intermediate effect on the growth. The reason for this hypothesis is that predictable variation may be easier to respond to, and has a more recognizable pattern (Barbosa et al., 2015). Increased unpredictability in an environment causes lower environmental sensitivity (Hallsson & BjÖRklund, 2012). This means that species may be unable to get enough information from the environment to come up with the most optimal responses (Hallsson & BjÖRklund, 2012). Due to this, it is reasonable to think that unpredictable environments might be a disadvantage for an organisms fitness, meaning growth and reproduction would get negatively affected.

2. Materials and methods

2.1 The experiment

The response of *Daphnia magna* to variable food availability was studied through a laboratory experiment involving different food treatments. Two clones (genotypes) of *D. magna* was used, where one originated from Morocco (Latitude 31,490714 Longitude −9,76443) and one from Sweden (Latitude 60,421733 Longitude18,51015). They were received from the University of Basel in March 2020, and has since then been held in cultures at UiO. The clone from Morocco was called AICHA, while the clone from Sweden was called PIPPI. From each clone there were 30 individuals, making a total of 60 individuals. The *Daphnia* were exposed to three different food-treatments. There were 10 individuals from each clone in each treatment. The experiment took place in a climate room with a temperature of 20°C and a 16:8 light cycle, and lasted a period of 18 days.

2.2 Treatments

The treatments used were constant food level, predictable variation, and unpredictable variation in food level, all shown in figure 3. For the constant food treatment, I used a medium ration of food (0.175 milligram carbon), and the same ration each day.

For the predictable variation I switched between high (0.3 mg C) and low (0.05 mg C) ration of food each other day. The total amount received after two days was the same as the total amount received after two days in the constant treatment (0.3+0.05=0.35 and 0.175+0.175=0.35 mg C). After the experiment the individuals from the predictable treatment and constant treatment had all gotten the same total food amount of 3.15 mg C. Half of the individuals (odd numbered) in this treatment started with a low food ration, and half of the individuals (even numbered) started with a high food ration at age 0.

In the unpredictable variation treatment, every individual had their own randomly chosen sequence of high (0.3 mg C) and low (0.05 mg C) rations of food throughout the 18 days (figure 4). This meant that the switch between high and low food had no pattern and was randomly determined. The sequence for each individual was produced in Excel with the formula: =IF(RANDBETWEEN(0;1)=0;0.05;0.3. In this treatment some individuals would get low food rations many days in a row while others got high food rations. The total amount of food received at the end of the experiment could vary for the individuals in this treatment (figure 4). However, after a longer period the total amount, variance, and mean food each day would be the similar for all individuals, as well as individuals from the other two treatments. This was not the case in a period of just 18 days.

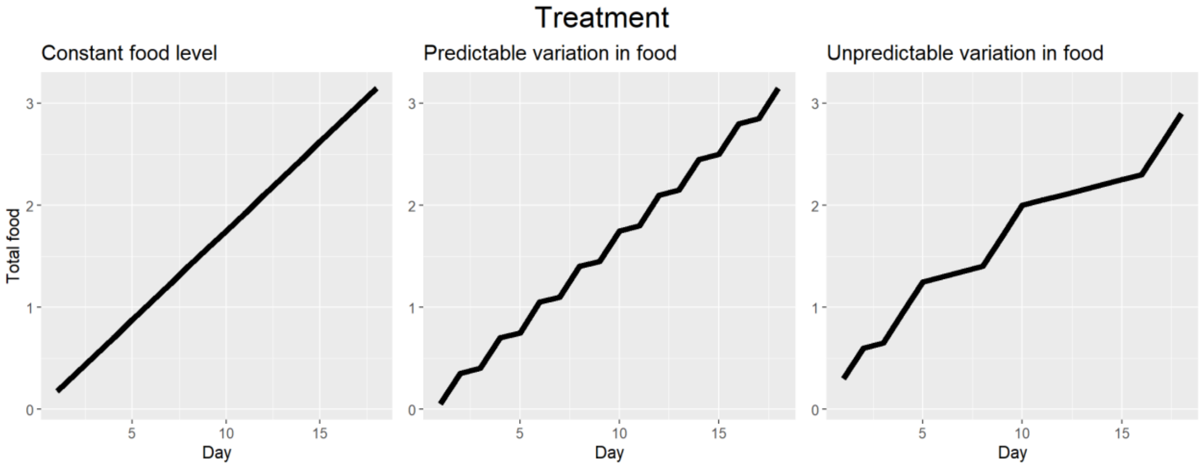

Figure 3: The food variation in each treatment: Constant food level, Predictable variation, and Unpredictable variation. In the unpredictable treatment the fluctuations throughout the experiment varied across individuals, this is graph is just an example.

I had all the individuals in separate jars, marked with individual number, clone and treatment. The jars contained 80 mL ADAM-medium (Klüttgen et al., 1994), which was changed each day for all the 60 individuals. The *Daphnia* were transferred by the use of a pipette to new jars containing fresh ADAM-medium every day. The ADAM-medium was stored in a 20°C room.

Every day it was noted down which individuals had molted, obtained eggs, and produced offspring. Body size was measured for the individuals three times during the experiment: at birth (age 0), at age 11 and at age 18. The size was measured in body length of the *Daphnia* from the start of the tail to the top of the head, in millimeters. A stereo microscope with a visual ruler was used for the measurements. The size of the *Daphnia* at age 0 for each clone was measured by using a representative group from the clones, as small individuals easily can be harmed when put under a microscope. 10 individuals of each clone was taken from the stock cultures at age 0 to be measured. The mean of the 10 individuals was calculated and set as the size at age 0 for each clone.

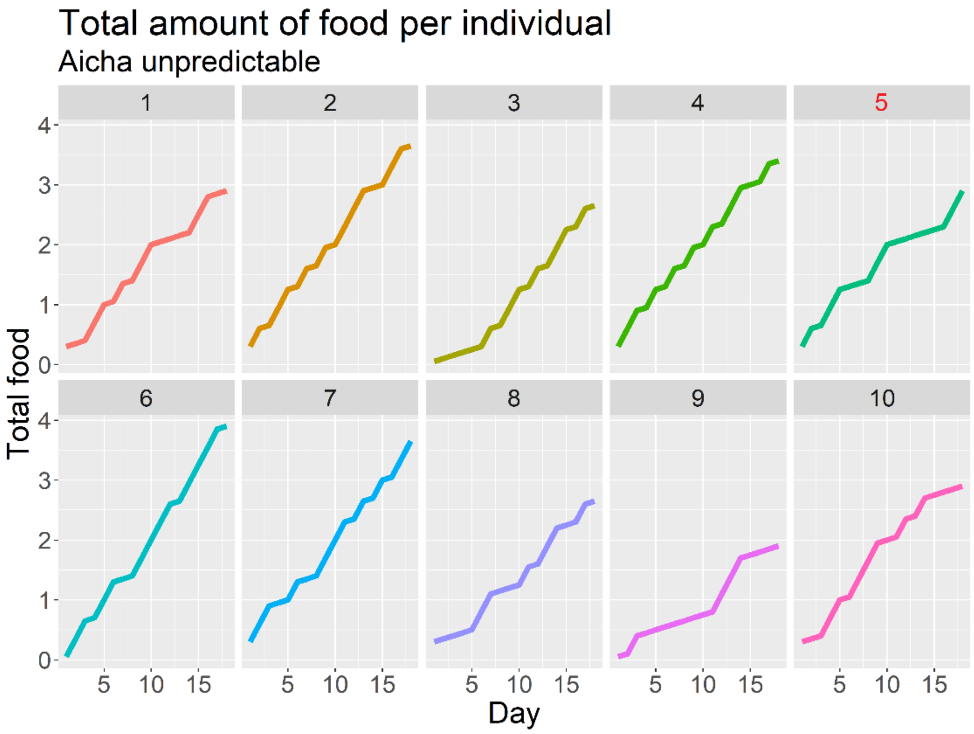

Figure 4: The total amount of food each Aicha-individual received throughout the unpredictable variation treatment. There are clear differences in the fluctuations between high and low food ration. Individual 5 is the one that got excluded from the statistical analysis.

2.3 Calculating food rations

A mix of the two green algae *Chlamydomonas reinhardtii* and *Nannochloropsis sp.* was used as food for the *Daphnia*. The *C. reinhardtii* was alive and cultured in the laboratory (with the use of WC-medium). The species Is a common phytoplankton in freshwater ecosystems and a high quality food source for zooplankton (Buchberger, Stibor, Neusius, Nickelsen, & Stockenreiter, 2020). The *Nannochloropsis* was from a RotiGrow mix containing dead algae, and was held in a 5 °C room. This algae mix is a good quality food source for rotifers, containing a high amount of fatty acids (Lubzens, Gibson, Zmora, & Sukenik, 1995).

The optical density of *C. reinhardtii* was measured with a PV4 spectrophotometer (at 800nm) each day to calculate the ration needed to have the right amount of carbon for the food. To be precise the density was measured three times and the mean density of the algae was used for the further calculations that day. As the carbon amount was calculated, *Nannochloropsis* was added, resulting in a carbon amount where 30% was from *Nannochloropsis* and 70% was from *C. reinhardtii*. The amount for each food level was then calculated from the combined mix, with carbon being the determining factor. The three food rations used were 0.3 mg C (high food), 0.175 mg C (mean food) and 0.05 mg carbon (low food).

2.4 Preparation

Prior to the experiment the clones were held in stock cultures in 3 separate jars per clones with 4-7 individuals in each jar. They were fed an intermediate amount (0.175 mg C) of food each other day. Two weeks before starting my experiment I took out 20 individuals, 10 of each clone, from the stock cultures at age 0. These individuals were supposed to be the mothers all the *Daphnia* I used in my experiment. As they did not produce enough offspring, I took individuals from both the mother *Daphnia* and the stock cultures when I started my experiments. Due to the lack of offspring, I was not able to start my experiment on the two clones at the same day. The 13th of November I started my experiment on Aicha and the 14th of November I started my experiment on Pippi. For the Aicha-clone I took some offspring from two different jars of the stock cultures (clutch 9 and 10), and some offspring from one of the “mothers” (clutch 4). The individuals of the Pippi-clone were all taken from the same jar of the stock cultures (clutch 8).

2.5 Statistical analysis

The data were collected in tables using Microsoft Excel, and exported as text files (.txt). The files were read in RStudio (version 1.4.1103), which was the program used for producing plots and performing statistical tests and analysis. The plots were made using the package “ggplot2”.

An ANOVA-analysis was used to see if the treatments were significantly different. Diagnostic plots from the ANOVA was looked at to indicate if there were any highly influential data points. A post-hoc analysis was performed to indicate which treatments were significantly different from each other. A Tukey HSD test (Tukey`s Honestly Significant Difference) with a 95% confidence level was done, where confidence intervals and estimates illustrated differences in the treatments. To indicate differences the test compares the mean of one group to another. Plots were produced to visually present the results.

2.6 Complications

The experiment was supposed to only include female *Daphnia (*figure 1*)*. As the *Daphnia* grew bigger 26 of the individuals from the Pippi clone turned out to be males. Males are very hard to recognize in offspring, and in general without the use of a microscope. Due to that they were not identified as males until their size was measured. Male *Daphnia*, as seen in figure 2, are distinguished from females by their smaller body size, body shape, the hook on their first legs (Ebert, 2005). The males were also more red colored than the females.

Due to the dominating abundance of males in the Pippi-clone looking at eggs and offspring from that clone was not an option. On the other hand, it gave me an opportunity to look at the growth pattern of females from one clone and males from another. With enough data of both females and males, this study shows how both genders of *D. magna* may respond to unpredictability in food variation. There was no point in comparing the response of the two different genotypes like planned as males and females have different life histories, and growth patterns (Ebert, 2005). Still, the responses in the three treatments was still just as relevant for both clones.

Some complications were posterior to the experiment suspected to be results of a bacterial infection in the *Daphnia* cultures. This infection could explain the lack of eggs and even bigger lack of offspring, as well as the high abundance of males in the Pippi-clone (Ebert, 2005). The *Daphnia* that had eggs usually abandoned them after a few days. Due to this I had a narrower variety of data than expected. What I did have enough data on was somatic growth and molting.

3. Results

A factor that strongly affected my results was the fact that only 4 of the Pippi-individuals were females, the remaining 26 were males. This gave me one clone with 30 females (Aicha) and one with 26 males (Pippi). The 4 females from the Pippi clone are excluded from the plots and statistical analysis, but are shown in table 2 (marked "*"). 3 of the females were in the predictable variation treatment and 1 female in the unpredictable variation treatment. This left me with data on 10 individuals from constant, 7 from predictable variation and 9 from the unpredictable variation treatment for the Pippi-clone.

One Aicha-individual from the unpredictable treatment (individual 5) was a lot smaller than the others. It was 2.5 mm in length at age 11, and very pale from age 6. It had not received a particularly lower amount of food than the other *Daphnia* in the unpredictable treatment either. As this individual had a strong effect on my data (as my sample size was small for each treatment) it was excluded from the analysis as well as the plots on mean size. It is present but marked with a star in the plots of the individual sizes, marked with red in the plot on molting, marked with a "*" in table 1.

The days from 0 to 11 I will refer to as the juvenile stage, although *Daphnia* from various treatments did not grow in the same pace. The production of the first clutch is used to determine maturation, and the start of the adult stage. Due to the lack of reproduction, I will use age 11 as a representation of the end of the juvenile stage, as it was at this age I measured the individuals. The analysis will shed light on the difference in growth in terms of age, rather than difference of growth at the exact life stages.

Table 1: Data on all the Aicha individuals. The individual marked with "*" is the one excluded from the statistical analysis. Size is in millimeters.

Aicha - Constant food level

Individual	Sex	Molts 11 days	Molts 18 days	Size 11 days	Size 18 days	Eggs	Age first eggs	Offspring
1	Female	6	8	3.25	3.65	0		
2	Female	6	8	3.05	3.5	0		
3	Female	6	8	3.05	3.5	0		
4	Female	6	8	3	3.5	2	7	0
5	Female	6	8	3.15	3.6	3	7	0
6	Female	6	8	3.2	3.6	0		
7	Female	6	8	3.2	3.55	0		
8	Female	6	8	3.2	3.6	0		
9	Female	6	8	3.15	3.6	0		
10	Female	6	8	3.25	3.75	0		

Aicha Predictable variation in food

Individual	Sex	Molts 11 days	Molts 18 days	Size 11 days	Size 18 days	Eggs	Age first eggs	Offspring
1	Female	6	8	2.8	3.1	2	11	0
2	Female	6	8	3.2	3.6	0		
3	Female	6	8	3	3.35	0		
4	Female	6	8	3.2	3.55	0		
5	Female	6	8	3.05	3.5	0		
6	Female	6	8	2.8	3.3	0		
7	Female	6	8	3	3.4	0		
8	Female	6	8	2.9	3.4	0		
9	Female	6	8	3	3.6	0		
10	Female	6	8	3.1	3.55	0		

Aicha - Unpredictable variation in food

Individual	Sex	Molts 11 days	Molts 18 days	Size 11 days	Size 18 days	Eggs	Age first eggs	Offspring
1	Female	6	8	3.05	3.5	0		
2	Female	6	8	2.9	3.5	4	13	
3	Female	6	8	2.95	3.4	4	13	
4	Female	6	8	2.95	3.35	0		
5*	Female	6	8	2.5	3.1	2	9	
6	Female	7	9	3	3.6	0		
7	Female	6	8	2.9	3.45	2	13	
8	Female	5	7	3	3.5	2	9	
9	Female	6	8	2.9	3.3	0		
10	Female	6	8	3.05	3.4	0		

Table 2: Data on all the Pippi-individuals (females included). The individual marked with "*" are the ones excluded from the plots and statistical analysis. Size is in millimeters.

Pippi Constant food level

Individual	Sex	Molts 11 days	Molts 18 days	Size 11 days	Size 18 days				
1	Male	5	7	2.35	2.65				
2	Male	5	7	2.25	2.6				
3	Male	5	7	2.35	2.65				
4	Male	6	7	2.35	2.5				
5	Male	5	7	2.4	2.4				
6	Male	5	7	2.35	2.6				
7	Male	5	7	2.4	2.5				
8	Male	5	7	2.4	2.55				
9	Male	5	7	2.25	2.45				
10	Male	5	7	2.35	2.55				

Pippi Predictable variation in food

Individual	Sex	Molts 11 days	Molts 18 days	Size 11 days	Size 18 days	Eggs	Age first eggs	Offspring	Age of reproduction
1	Male	5	7	2.3	2.5				
2	Male	5	7	2.2	2.4				
3*	Female	6	8	3.05	3.5		0		
4	Male	5	7	2.15	2.35				
5	Male	5	7	2.3	2.5				
6	Male	6	8	2.2	2.35				
7	Male	5	7	2.25	2.45				
8*	Female	5	7	2.4	3	3	12	3	15
9	Male	5	7	2.25	2.45				
10*	Female	5	7	2.9	3.4	7	12	6	15

Pippi unpredictable variation in food

Individual	Sex	Molts 11 days	Molts 18 days	Size 11 days	Size 18 days	Eggs			
1	Male	5	7	2.2	2.4				
2	Male	5	7	2.15	2.4				
3	Male	5	DEAD	2.2	DEAD				
4	Male	5	7	2.15	2.45				
5	Male	5	7	2.2	2.4				
6	Male	5	7	2.4	2.5				
7	Male	5	7	2.25	2.4				
8	Male	5	7	2.2	2.4				
9*	Female	5	7	3.05	3.4	0			
10	Male	6	8	2.3	2.55				

Table 1 and 2 shows data from all the individuals present in this experiment, including data on the few individuals that had eggs and offspring, and the 4 female individuals of the Pippi-clone. Two of these individuals reproduced, both were in the predictable treatment. Pippi-individual 3 in the predictable treatment died at age 12. This was the only *Daphnia* that died during the experiment. The cause of death is not known, although it looked like it had been hurt when transferred with the pipette, and died from the wound.

3.1 Mean size and individual size

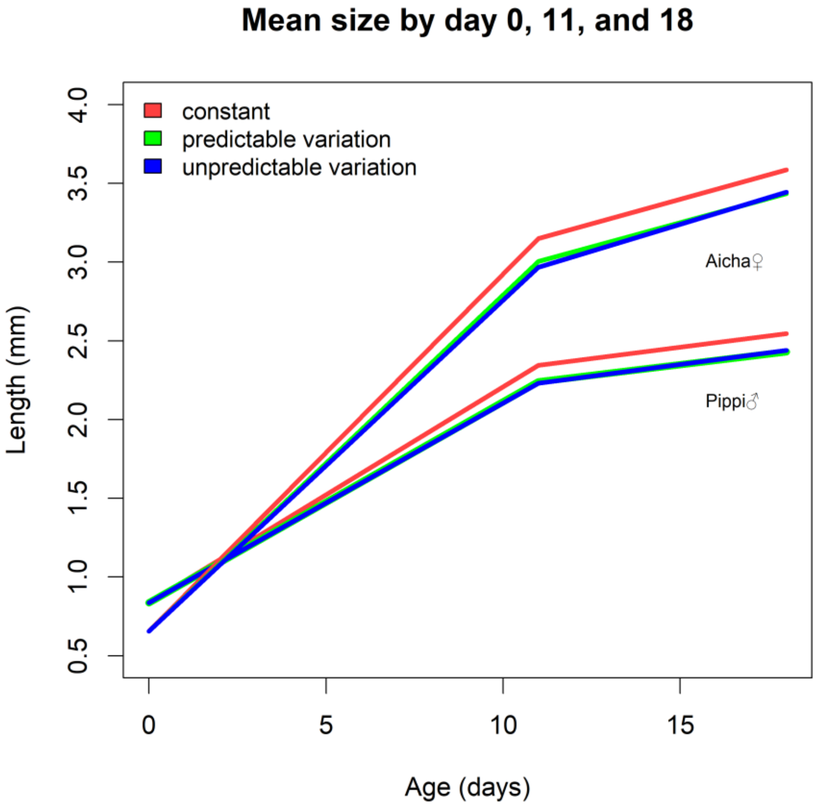

Figure 5: Mean size of individuals in each treatment for Aicha (females) and Pippi (males) at age 0, 11 and 18.

As shown in figure 5, the Pippi individuals were bigger than the Aicha individuals at birth, with a size of 0.837mm and 0.656mm respectively. In the constant treatment the mean size was significantly higher than in predictable and unpredictable variation in both males and females after 11 and 18 days. Even though male and female *Daphnia* grew differently, they had similar patterns across the treatments.

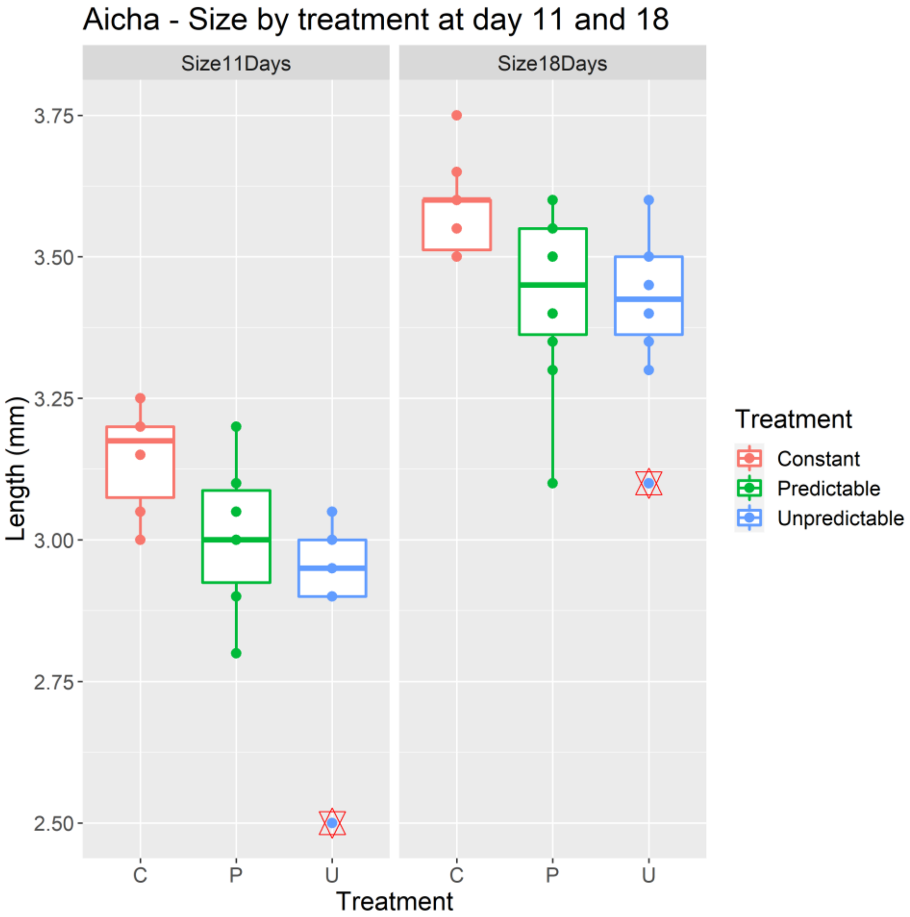

Figure 6: Size of individuals in each treatment for Aicha (females) at age 11 (left) and 18 (right). Individual 5 from unpredictable variation is the outlier marked with a star. This individual was excluded from the analysis.

As shown in figure 6 individuals in the constant treatment grew bigger than individuals in the two other treatments. At 11 days the Aicha-individuals had a mean size of 3.15mm in the constant treatment with a standard deviation of 0.09, 3.01 mm in predictable variation with a standard deviation of 0.14, and 2.97 mm in unpredictable variation with a standard deviation of 0.061. At 18 days the mean size was 3.59mm in the constant treatment with a standard deviation of 0.08, 3.44mm for predictable variation with a standard deviation of 0.16, and 3.44mm for unpredictable variation with a standard deviation of 0.09.

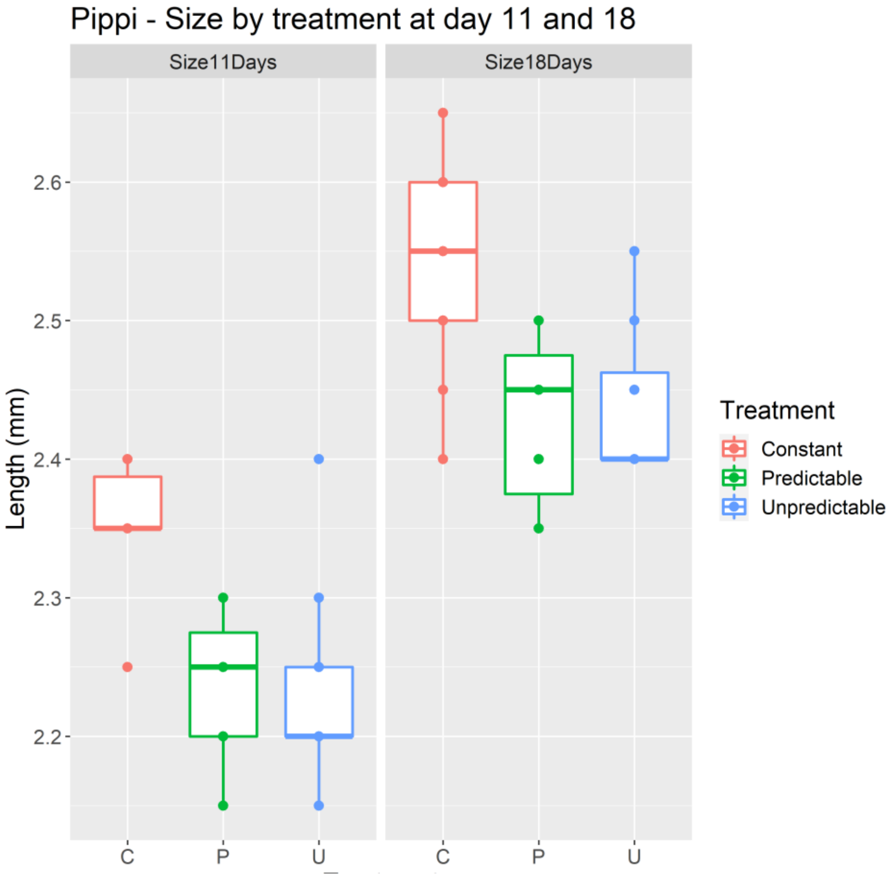

Figure 7: Size of individuals in each treatment for Pippi (males) at age 11 and 18.

The males (Pippi) were significantly smaller than the females (Aicha). The constant treatment caused bigger individuals than the two other treatments, as shown in figure 7. After 11 days the constant treatment had the biggest individuals with a mean size of 2.35mm, and a standard deviation of 0.06. Individuals from the predictable variation had a mean size of 2.24mm, and a standard deviation of 0.06. In the unpredictable variation there was a mean size of 2.23mm, with a standard deviation of 0.079. After 18 days the constant treatment still had the biggest individuals with a mean size of 2.55 and a standard deviation of 0.083. The predictable variation caused a mean size of 2.43 with a standard deviation of 0.064, while the unpredictable variation caused a mean size of 2.44, and a standard deviation of 0.058.

3.2 ANOVA-analysis and Tukey`s HSD test

The ANOVA-analysis tested significant differences in individual size across the treatments for Aicha and Pippi independently. However, it did not point out which treatments were significantly different, and which were not. According to the ANOVA-test the size after 11 and 18 days was significantly different in both the Pippi- and Aicha-clone with a P-value <0.05. For the Aicha individuals the size after 11 days had a P-value of 0.00158 and an F-value of 8.354, with 26 degrees of freedom. The size after 18 days had a P-value of 0.0126 and F-value of 5.203 for the same clone.

For the Pippi-clone the size after 11 days had a P-value of 0.000967 and an F-value of 9.531, with 23 degrees of freedom. The size after 18 days showed a P-value of 0.0033 and an F-value of 7.492 for this clone. Diagnostic plots from the analysis showed that the data were true to the assumptions made by using an ANOVA. No data points were outside cooks distance, meaning that no individual data point was highly influential on the data.

Table 3: Results from the TukeyHSD-test which detected differences in size between the treatments. Confidence intervals and the degree of difference pairwise between treatments.

Post hoc test - TukeyHSD 95% confidence level				
Differences in size				
Aicha ♀				
Age	**11**		**18**	
	difference	confidence interval	difference	confidence interval
Predictable-Constant	0.15	-0.26 to -0.03	0.15	-0.28 to -0.02
Unpredictable-Constant	0.18	-0.3 to -0.06	0.14	-0.27 to -0.01
Unpredictable-Predictable	0.04	*-0.16 to 0.08*	0.01	*-0.12 to 0.14*
Pippi ♂				
Age	**11**		**18**	
	difference	confidence interval	difference	confidence interval
Predictable-Constant	0.11	-0.19 to -0.03	0.12	-0.20 to -0.03
Unpredictable-Constant	0.12	-0.19 to -0.04	0.11	-0.19 to -0.02
Unpredictable-Predictable	0.01	*-0.09 to 0.7*	0.01	*-0.08 to 0.01*

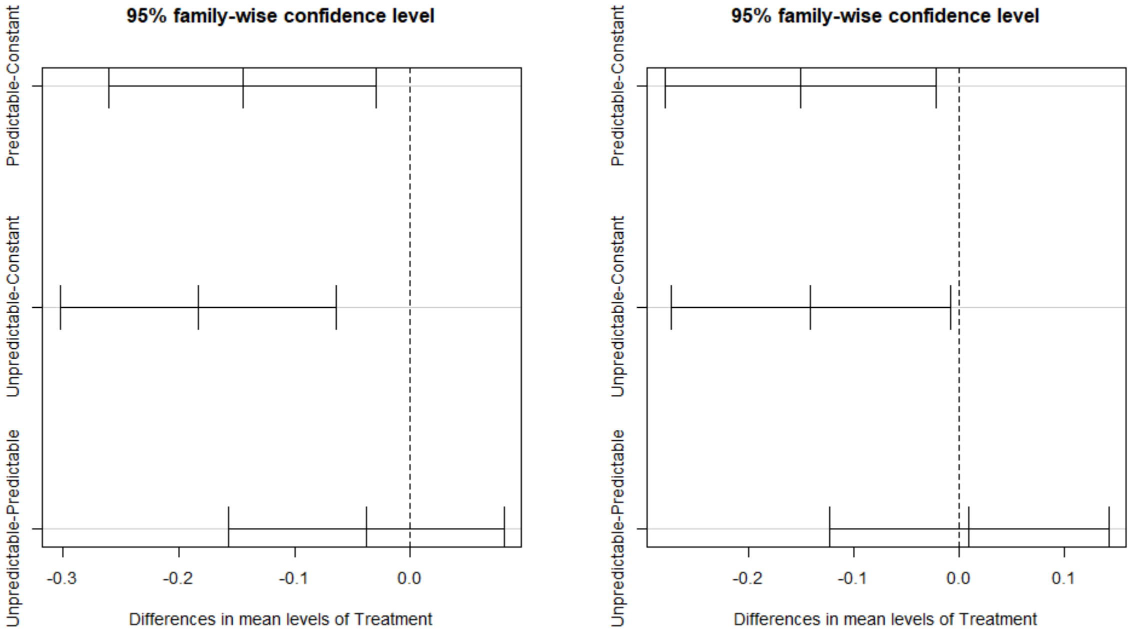

Figure 9: Tukey HSD-plots showing the significant differences between treatments in size at age 11 (left) and 18 (right) for Aicha.

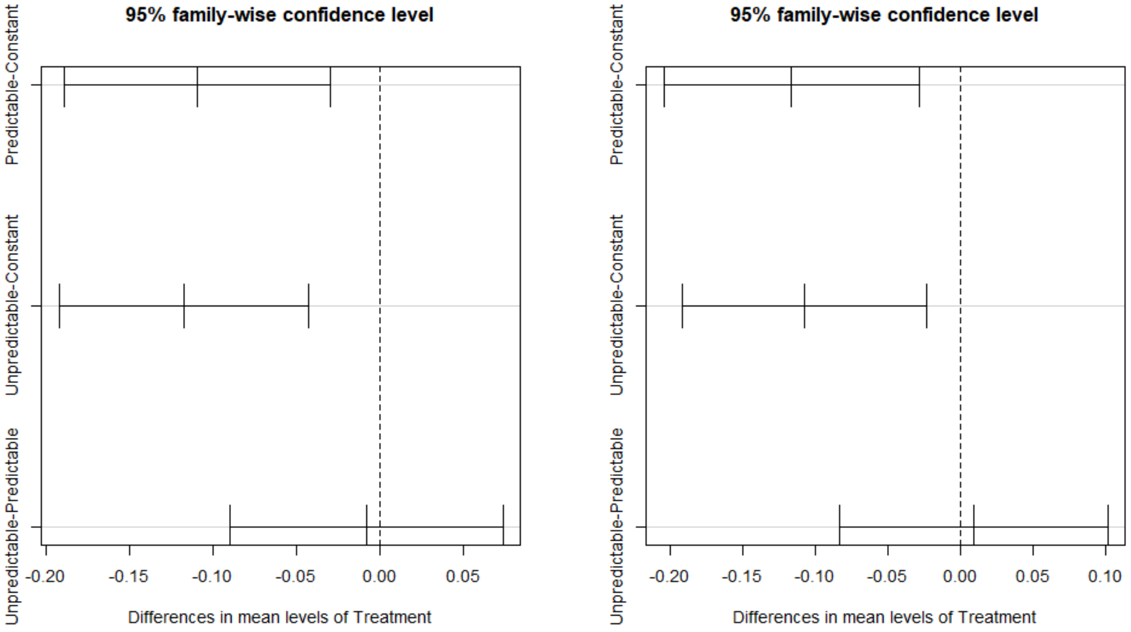

Figure 10: Tukey HSD-plots showing the significant differences between treatments in size at age 11 (left) and 18 (right) for Pippi.

The TukeyHSD-test indicated differences in size between the food treatments pairwise. This test compares the mean in one group to the mean in another group. The confidence intervals and differences are presented in table 3. Confidence intervals between two treatments determined if they were significantly different, where intervals not containing 0 indicated that there was a significant difference. By this I could conclude that the mean size in the constant food treatment was significantly different from both the mean size in the predictable variation treatment and the unpredictable variation treatment. This was the case at age 11 and 18, in both clones. Figure 9 and 10 visually presents the differences in mean size, and confidence intervals between the treatments in the Aicha- and Pippi-clones respectively.

At the final day of the experiment, at age 18, the mean size difference was 0.15 between constant and predictable, 0.14 between constant and unpredictable, and 0.01 between predictable and unpredictable, for Aicha-individuals. The Pippi-individuals had a mean size difference of 0.12 between constant and predictable, 0.11 between constant and unpredictable, and 0.01 between predictable and unpredictable at age 18 (table 3).

3.3 Molting

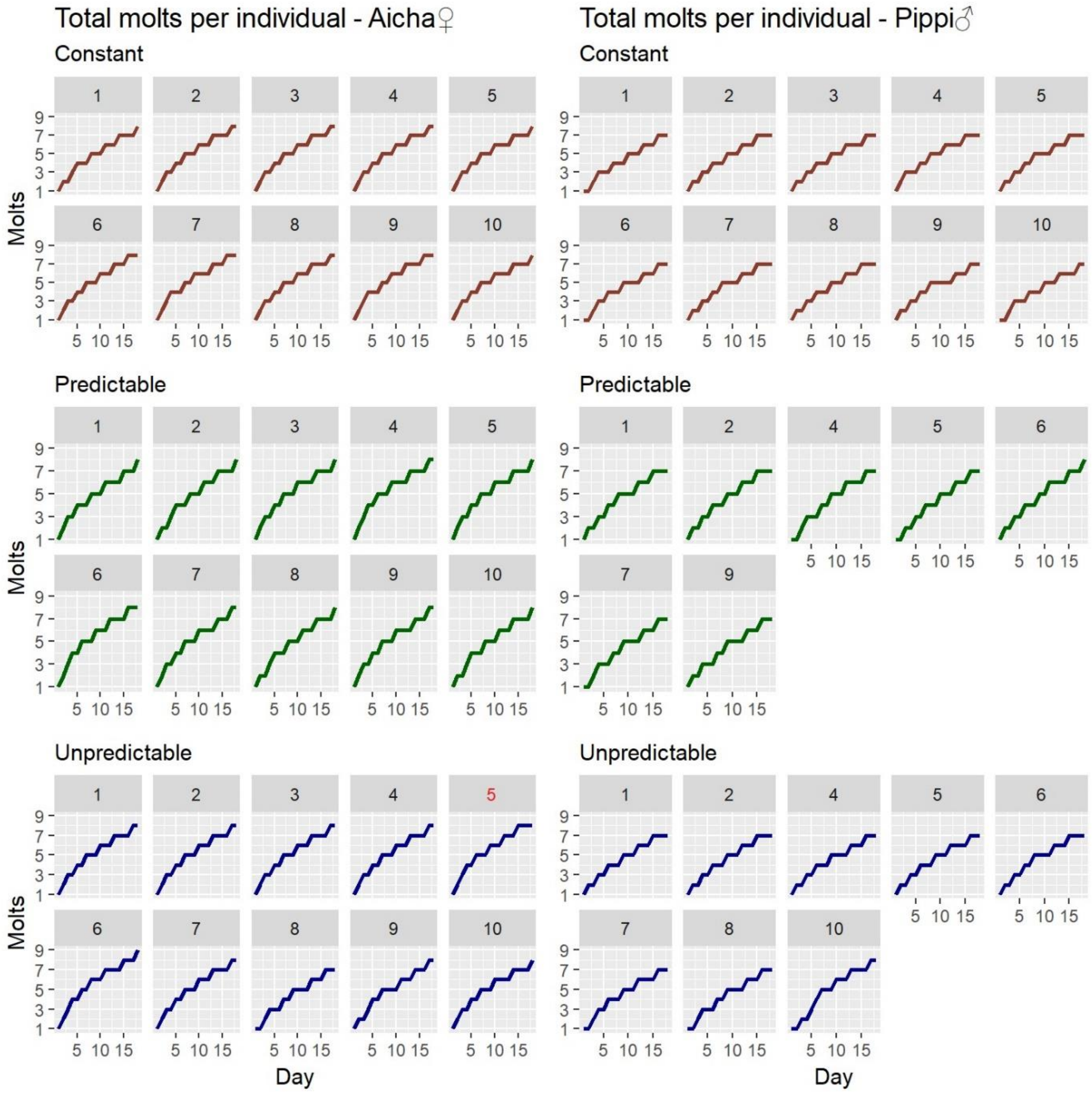

Figure 11: The molting pattern for each individual of Aicha (females) and Pippi (males) throughout the experiment. Aicha-individual 5 from unpredictable variation (marked red) was the one excluded from the analysis. Due to Pippi-individual 3 from the unpredictable treatment died at age 12 it was excluded from this plot.

The molting process for all female Aicha- and male Pippi-individuals are shown in figure 11. Pippi-individuals usually had molted 5 times at age 11 and 7 times at age 18, with a few exceptions (table 2). Most of the Aicha-individuals had molted 6 times at age 11 and 8 times at age 18, also with a few exceptions (table 1). There were no significant difference in either number of molts or molt rates between the treatments.

4. Discussion

My experiment showed no significant difference in growth for *Daphnia magna* exposed to predictable and unpredictable food variation. Individuals from these two food conditions were very similar in size at age 11 and 18. The same pattern between these treatments was found in both clones, meaning it applied to both females and males. These results deviates from my hypothesis that individuals exposed to a predictable food variation would perform better than those exposed to an unpredictable food variation. At the same time, my hypothesis predicting a higher growth rate for individuals given the constant food treatment was supported by the data. Constant food levels caused a significantly bigger mean size of *Daphnia* than the other two food treatments did. The constant treatment was used as a control group with favorable food conditions for *Daphnia*, by being fed the same intermediate ration of food every day.

4.1 The possible effect of low food rations

The daily fluctuations between high (0.3 mg C) and low (0.05 mg C) food ration in the predictable treatment after two days, gave the same total food amount as that of the constant treatment (0.175 mg C) after two days (0.35 mg C). At the end of the experiment *Daphnia* from these two treatments had been fed the same total amount of food (3.15 mg C). In the presence of such daily fluctuations in food, individuals may not be able to fully utilize the amount of food that is available (Ross, 1982). The amount of food an individual will be able to consume is determined by its body size (Ebert, 2005). Young individuals may not be able to benefit from the high food rations, but may still be affected by the low food rations. The total consumption of algae could therefore be lower for *Daphnia* exposed to the predictable variation in food, than the constant food on the course of the experiment, even though the total food ration was the same. As growth rate is determined by food consumption (Giebelhausen & Lampert, 2001), one would think this gave higher growth rates for *Daphnia* in the constant treatment.

The growth rate of individuals in the unpredictable food variation presumably diverged from that of the constant food level for the same reason as that of predictable food variation did. Fluctuations play a role in the total amount individuals are able to consume through a time period due to their daily limitation in food consumption, which is determined by their body size. In the unpredictable food treatment, there was no pattern to how often (in terms of days) the individuals were fed a high level or low level of food. The total amount received in the course of the experiment would also vary for individuals in this treatment. Final size did not seem to be dependent on total amount of food. Due to this, there might be a general life history response to fluctuating food availability, influencing the individual growth of the *Daphnia*.

4.2 Higher investment in somatic maintenance

Periods of food shortage often cause organisms to allocate more energy into somatic maintenance than growth and reproduction (Masoro & Austad, 1996). This is thought to be a strategy to cope with periods of low food availability. Reproduction is not beneficial if there is not enough food for offspring to survive. This response is said to be induced by calorie restriction, and causes extended life spans. A lot of animals is proven to use this strategy, from nematodes to humans (Pietrzak, Grzesiuk, & Bednarska, 2010). In a study on mice (*mus musculus*) (Shanley et al. 2000) this strategy, known as anti-aging, led to longer lifespans and delayed aging, and seems to be favored by natural selection. Young individuals of mice was shown to have a stronger response to calorie restriction than older individuals (Shanley & Kirkwood, 2000). A study of *Daphnia magna* by (Pietrzak et al. 2010) indicated that conditions of mild food stress can lead to extended lifespans in *Daphnia*. A negative correlation between early investment of first clutch and lifespan was also shown. High food availability led to early reproductive investment which had consequences for later life stages (Pietrzak et al., 2010). The days of low food availability present in two of the treatments in my experiment may have had an anti-aging effect on the *Daphnia.* This is supported by the observation that the molting rate was not to affected, but the growth rate was. Due to this there seemed to be a higher investment in somatic maintenance, than growth. The treatments seemed to have no influence on molting. As my experiment only lasted 18 days, I was not able to see how mortality and length of lifespans were affected. With days of full starvation, or higher variation in rations of food, the response could have been even stronger (Glazier & Calow, 1992).

4.3 Unpredictable food conditions

In nature food availabilities usually vary a lot (Barbosa et al., 2015; Betini et al., 2020). A condition where individuals receive a constant level of food each day is not realistic. In a more realistic ecosystem food sources are temporally unpredictable, dependent on biological processes involving other organisms. The degree of unpredictability depends on the ecosystem and environmental changes affecting the ecosystem. In general, unpredictable conditions are expected to cause environmental cues to be less effective for predicting future conditions. Environmental cues are used by organisms to modify investments to optimize individual fitness (Barbosa et al., 2015). This may explain why species usually are expected to perform worse with increased unpredictability in the environments.

Through my experiment *Daphnia magna* did not seem to perform worse by unpredictable food conditions than by predictable ones. This may be a result of their evolutionary adaptation to cope with variable and unpredictable environments. *D. magna* live in environments with frequent fluctuations both in temperature and food resources. They are adapted to variable environments. In highly variable habitats such as rock pool systems, *D. magna* has to be adapted to fluctuations in both food and temperature (Wulff, 1980). In rock pools there are large seasonal, and even daily, variations where effects of weather conditions and biological activity plays a major role. *Daphnia* are proven to be highly adapted in relation to temperatures. In a previous study (Barbosa et al. 2015) found that *Daphnia magna* exposed to unpredictability in temperature showed the same fitness-related response as *D. magna* exposed constant temperatures. The individuals had about as long and produced about as many offspring during their lifetime in both temperature conditions (Barbosa et al., 2015). In my study, unpredictable food variation led to significantly different responses from constant food level in terms of growth. This indicates that the response to unpredictability in food and unpredictability in temperature may have different effects on *D. magna,* and both are crucial to look at to understand how adapted the species is to predictability.

5. Conclusion

It is not completely clear why *Daphnia magna* had the same growth-response to predictable food variation and unpredictable food variation. It might be a result of possible flaws in the experiment. A bigger sample size or the experiment lasting over a longer time period could have strengthened the results.

Still, if my result is a good representation of what we would observe in an even more robust experiment, Daphnia magna may be considered an evolutionary adapted species to unpredictable food conditions. More research on both unpredictability in food but also other environmental conditions, such as temperature, could provide a greater understanding on how adapted these small crustaceans are to possible future conditions emerging, as a result of rapid environmental changes.

6. References

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Appendix

Table A1: Recipe for the ADAM-medium (Klüttgen, Dülmer, Engels, & Ratte, 1994)

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

At UiO different concentrations are used for stock solution A and B:

A: $CaCl_2$ $2H_2O$ Calciumchloride dihydrate

108.2 g/L

B: $NaHCO_3$ Sodium bicarbonate ($CHNaO_3$)

22.2 g/L

C: SeO_2 Selenium dioxide

0.07 g/L

5L recipe for the WC-medium

1. Weigh out **0.575 grams** (575 mg) of the **TES** buffer (in cabinet) and add it to the 5-liter Erlenmeyer flask.
2. Fill the flask with **4.96 litres** of distilled water and place it on a stirrer plate with a magnet inside (rince magnet with ethanol then distilled water).
3. Add **5 ml** of each of the stock solutions* 1-6.
4. Add **10 ml** of stock solution 7 (trace elements).
5. Add **5 mL** of stock solution 8 (vitamins).
6. Let the medium stir for some minutes, then fill it into assigned clean flasks.
7. Label the flasks MWC + date + your initials.
8. Store the medium cold and dark.

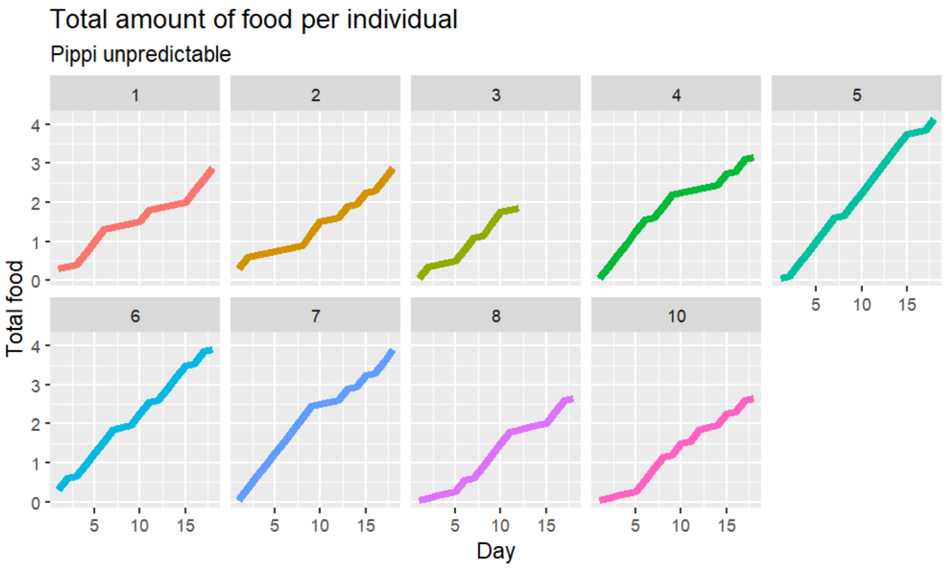

Figure A1: The total amount of food each male Pippi-individual received throughout the unpredictable variation treatment.

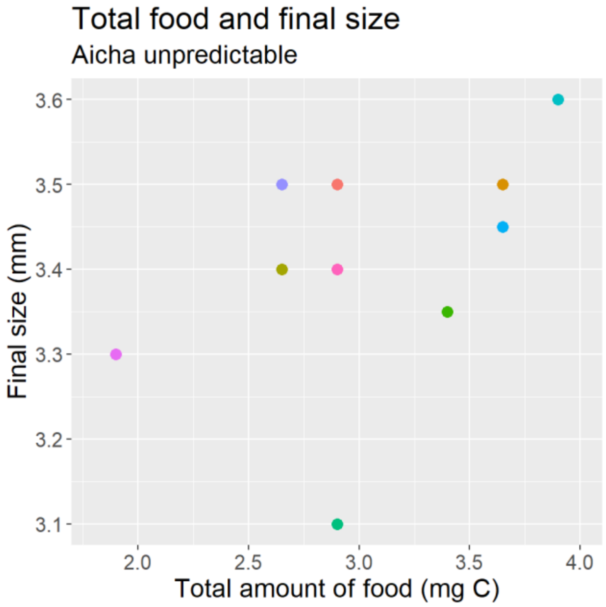

Figure A2: The total food amount and final size for Aicha-individuals.

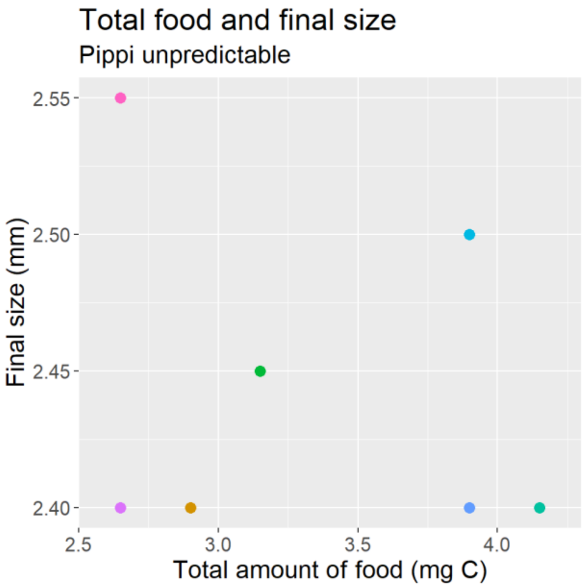

Figure A3: The total food amount and final size for Pippi-individuals.

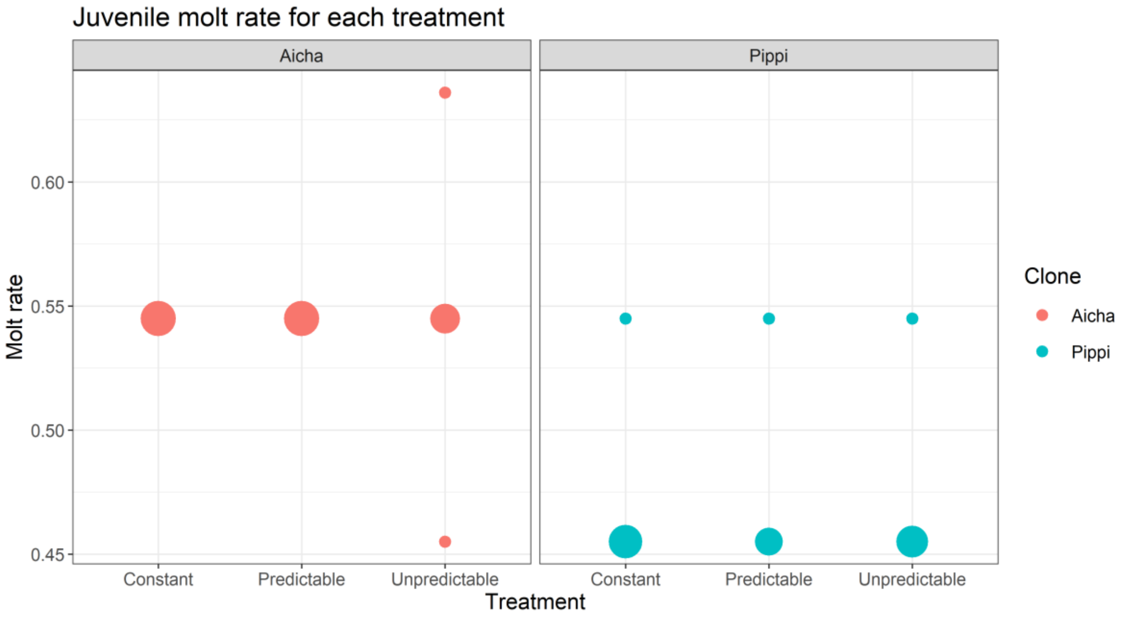

Figure A4: Molt rate from age 0-11 for Aicha (females) and Pippi (males).

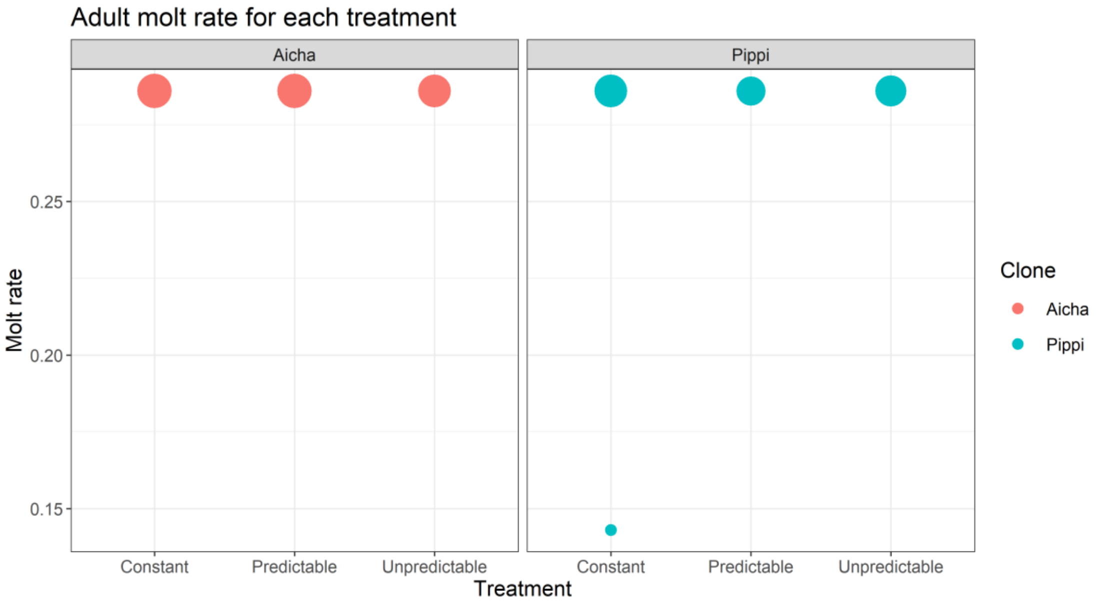

Figure A5: Molt rate from age 0-18 for Aicha (females) and Pippi (males).