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Daphnia Pulex: The Mixed Messages of Mutations

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DAPHNIA PULEX: THE MIXED MESSAGES OF MUTATIONS

by

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DEDICATION

For my grandparents, most of whom I didn’t get to know as well as I would have liked, but all of whom taught me lessons that I continue to learn from and be inspired by.

ACKNOWLEDGEMENTS

I would like to thank my fellow lab members, Trenton Agrelius, Rachel Schomaker, and Jake Swanson as well as our Lab Technician, Krista Harmon, and several other graduate students and undergraduate students, Sydney Mastalerz, Heather Bruck, and Cina Patel, who assisted me with questions and listened to my complaints over the course of my research.

ABSTRACT

While mutations are almost universally considered to be more often deleterious than beneficial, their precise interactions between different populations and individual lines have been largely overlooked. Using mutation accumulation lines of four clones of obligately asexual *Daphnia pulex*, this research is intended to investigate the degree to which spontaneous mutation would affect fitness-related traits after roughly 100 generations. The expectation was that there would be a visible decrease in juvenile specific growth rate, the surrogate measurement used for fitness, across all four clones due to the deleterious nature of mutation in a selection free environment. Through measuring birth mass in one food environment and juvenile specific growth rate in two food environments, this research shows that while on average, mutations do show a largely deleterious trend, there are variations in intensity of this effect as well as even some instances of beneficial effects when looking at individual traits. Two clones showed the expected result of a significant ($p<0.05$) decline in juvenile specific growth rate, while the other two clones did not. The birth mass data itself may explain some of this as it is a significant variable in the juvenile specific growth rate equation. In the birth mass data, two clones started small, and became larger due to mutation, whereas the other two clones started larger and became small, effectively switching positions with one another instead of merging on a median mass. Overall, the nature of mutations remains mildly misunderstood, in that different populations may respond differently to the same conditions.

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CHAPTER 1

CHANGES IN BIRTH MASS IN *DAPHNIA PULEX*

1.1 INTRODUCTION

Size at birth predicts life history traits in a number of vertebrate and invertebrate species (Simcic and Brancelj 1997, Ridgway et al. 2011, Souza et al. 2021). The general concept is that if an organism is born smaller, an organism is only able to acquire so many resources to grow, and more resources will be required until an organism reaches reproductive maturity (Ocampo et al. 2012, Bukovinsky et al. 2012) whereas if it is born larger, then it already has a head start in growth compared to the smaller births of the same species. While smaller individuals can recover some of this mass through "catch up growth," the rapid accumulation of mass can have other detrimental effects on fitness in the long term (Singhal 2017). While not all species respond the same to these conditions, and there are exceptions when it can be beneficial for offspring to be born small, largely in times of resource scarcity when a delayed maturation may be beneficial in allowing environmental factors to change prior to reproduction would occur, or in predator avoidance when a smaller size makes an organism more difficult to detect visually (Alkimin et al. 2020), a larger birth mass is usually indicative of a higher base fitness level.

The genus *Daphnia* includes over 100 species (Ebert 2005) which can vary significantly in all life history traits, including in birth and mature dry mass, from *D. hyalina* with juveniles weighing roughly 2.4µg each to *D. magna* juveniles with a mass of over five times that at 12.6µg and mature masses of roughly 22µg and 179µg respectively (Simcic and Brancelj 1997). The typical measurement of *Daphnia* used in research is body length, as this allows continued use of the organism throughout its life cycle, though it is not always strongly correlated to dry mass (Simcic and Brancelj 1997) so, when practical, dry mass is a preferable measurement. After birth, *Daphnia* take between five and ten days to reproduce with their first clutch, depending on the species and food availability (Ebert 2005), through a process called cyclic parthenogenesis which allows for the asexual reproduction of female "daughter clones" when conditions are favorable or for sexual reproduction when genetic recombination may be more beneficial in taking advantage of the pool of traits available to the population when resources may be limited (Ebert 2005). *Daphnia* are also capable of producing resting eggs, called ephippia, which settle on the pond or lakebed and will hatch when conditions are favorable. These are of particular importance to *Daphnia* spp. that inhabit ephemeral ponds because they rely on these ephippia to renew their population with regular seasonality.

Size at birth can be an indicator for what conditions were present in the environment of the mother in *Daphnia*. Variations in size can be representative of food quality variation, predation events, pathogens, or shifts in chemical content of the water (Bownik 2017, Bukovinsky et al. 2012, Garbutt and Little 2014, Heugens et al. 2006). When *Daphnia* are heavily predated by fish, they give birth sooner to smaller offspring

(Alkimin et al. 2020), which allows them to avoid visual detection by the fish they share lake space with. However, when nutrition sources are scarce, *Daphnia* mothers will give birth to fewer, but larger offspring which provides them with a greater amount of initial resources (Garbutt and Little 2014, Bukovinsky et al. 2012).

Due to its importance in establishing an individual's fitness, my first objective is to determine how spontaneous mutations influence size at birth, using *Daphnia pulex* as a model system. I will evaluate the effect of spontaneous mutations on size at birth with an experimental mutation accumulation experiment that includes multiple ancestors. This will allow me to test the null hypothesis that the effects of spontaneous mutation on size at birth are independent of genetic background.

1.2 METHODS

1.2.1 Source Clones

In the four clones of *Daphnia pulex* used for this experiment, SED, MORG, TRO, and LIS, it is expected that there would be some initial variation as they were sourced from different ponds from around the Northern Midwest of the United States and Southern Canada. These clones in particular are obligate asexual reproducers that can still produce males, but the males do not provide anything to the populations genetically.

The ancestral ephippia that are hatched are reared for several acclimatization generations in order to remove any maternal effects that may linger from the collection site, thus putting all clones on relatively equal ground in regard to extra-organismal effects, though some natural variation is still expected between the different clones (Chopelet et al. 2008).

Measuring size at birth for multiple clones of *D. pulex* and being able to compare these to their ancestral controls directly will allow us to see how the spontaneous accumulation of mutations has affected, if at all, the birth mass and, by extension, the expectation of fitness for the mutation accumulation lines positively or negatively. With the majority of mutations being deleterious and a smaller size at birth representing a decrease in fitness, the mutation accumulation lines should show a smaller size at birth than their ancestral controls.

1.2.2 Mutation Accumulation (MA) Lines

MA lines of 4 clones, MORG, SED, TRO, and LIS have been maintained for over 3 years. A single mother of each clone was used to produce 30 neonates that were then separated, and each placed into beakers with ~100mL of filtered lake water, water was obtained from the Saluda River then filtered through a fiberglass paper based filtration system, and fed daily ad libitum algae, specifically *Ankistrodesmus falcatus*. The lines that were initially set up were defined as generation 1 for each clone. Every 2 weeks transfers occur where one individual offspring for each line was selected at random and moved from the existing beaker to a new beaker and marked as the successive generation. Two additional individuals were placed as backups in 50-mL centrifuge tubes with ~40 mL filtered lakewater for use in case of death or accidental selection of a male as the primary individual. In the event of complete die-off, due to the recurrent production of only male offspring, no ephippia production, or the death of the generational mother, attempts to revive the line from previous generations were made, however if this still resulted in failure due to lack of available organisms from repeated difficulties with a particular line, then the line was marked dead.

1.2.3 Ancestor Hatching

Ephippia from the ancestor that originated the MA lines that have been stored in 1.5mL microcentrifuge tubes in a -80°C freezer between uses. When creating a hatching tray, microcentrifuge tubes of ephippia are removed from the -80°C freezer and approximately 10 ephippia are placed into each well of 6-well plates with a small amount of filtered lake water. The trays are then placed in a -20°C freezer for 2 weeks, followed by a 4°C fridge for 1 week, and then the final hatching chamber, a 20°C incubator for hatching. The eggs within the ephippia will hatch between 4 and 14 days after being placed in the hatching chamber. The trays were checked daily for any individuals that hatched. Once hatched, individuals were placed into 200mL beakers with 150mL of filtered lake water and allowed to develop a significant enough population to establish the acclimatization populations.

1.2.4 Acclimatization Phases

At the start of the size-at-birth assays, five female individuals were taken from each line of each clone to establish an initial acclimatization generation. The MA lines were between an average of 50 and 100 generations, depending on clone, when initially taken for the beginning of acclimatization. Each individual was placed in a 150mL beaker with 100mL of filtered lake water and fed 20,000 cells of *A. falcatus* every day. They were transferred to clean filtered water every Monday, Wednesday, and Friday and were monitored for reproductive events. Once the third clutch was reached, the lines were then moved to the second acclimatization phase. For each line 10 individuals were taken, and each placed in their own 150mL beaker with 100mL of filtered lake water. Transfers to new water continued on Mondays, Wednesdays, and Fridays and feeding of

20,000 cells of *A. falcatus* occurred daily. When this phase had their second clutch, monitoring began for their developmental progress of their third clutches so that the *Daphnia* mothers could be placed in filtered lake water with no algae present immediately prior to giving birth in order for the neonates to be distributed into high and low food treatments without pre-existing algae in their systems.

1.2.5 Experimental Phase

The experimental phase began by placing 18 neonates from each line on a slide at birth. First, for each line, after the mother had birthed neonates in a beaker with no algae present, 18 neonates were placed directly onto microscope slides and placed into an incubator at 60°C for 48hrs and then into desiccation boxes to remove any moisture present.

1.2.6 Data Collection

The dry mass was then obtained for each *Daphnia* using a Mettler-Toledo UMX2 Ultra-Microbalance. Being that neonates are extremely small, they were measured 2 at a time and then divided by 2 in order to obtain dry masses, however the 4-day low and high food treatment daphnia were all measured individually. This was recorded on paper before being transferred to Excel.

1.2.7 Data Analysis

Analysis was primarily conducted in R using the lme4 package. ANOVA tests were conducted to determine significance of individual MA lines against the control

group for each clone. Final statistics were exported back to Excel in order to create the figures and tables.

1.3 RESULTS

The MA lines had a larger mass than the ancestral Control lines in the clones MORG and SED, though they had a smaller mass in the clones TRO and LIS, as shown in Figure 1.1 below. The increase in mass in MORG and SED and decrease in mass of TRO and LIS show different clones responding distinctly from one another. Both TRO and LIS ancestral clones had a higher absolute mass than MORG and SED, which then decreased in the MA lines, whereas MORG and SED increased from a low starting mass in the ancestral clone to a higher mass in the MA lines, similar to the ancestral mass of TRO and LIS. s

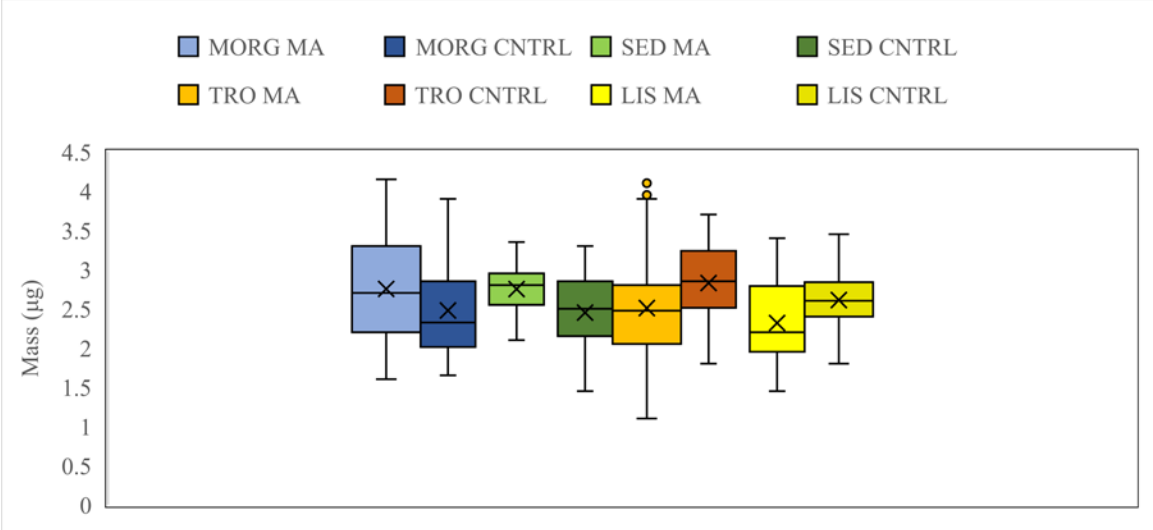

Figure 1.1 – Absolute mass at birth of individual *Daphnia pulex*

When comparing the individual MA lines to the ancestral control lines, it can be seen that at least half of their MA counterparts were significantly different (Figure 1.2, Tables 1.2, 1.3, 1.4, 1.5) from the ancestors, and largely in the same direction with SED and MORG having primarily larger MA neonates and TRO and LIS having primarily smaller MA neonates.

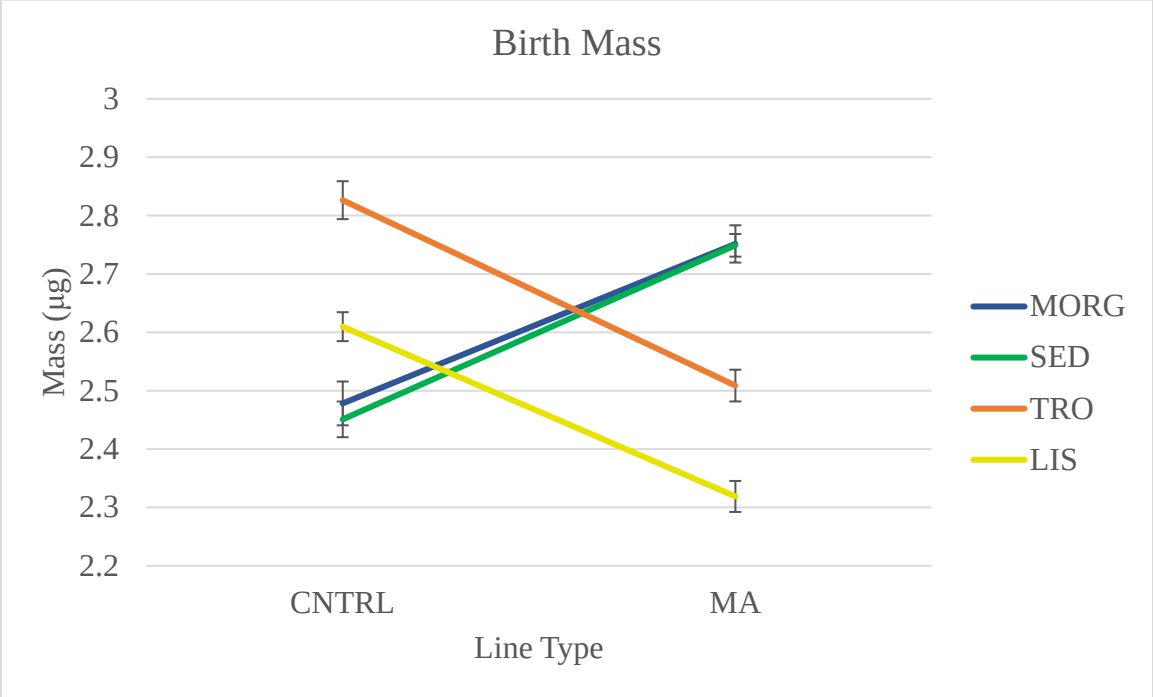

Figure 1.2 – Change in absolute mass at birth in four sets of mutation accumulation lines. The mean number of generations for the MA lines were MORG=93.44, SED = 72.25, TRO = 86.54, and LIS = 52.2.

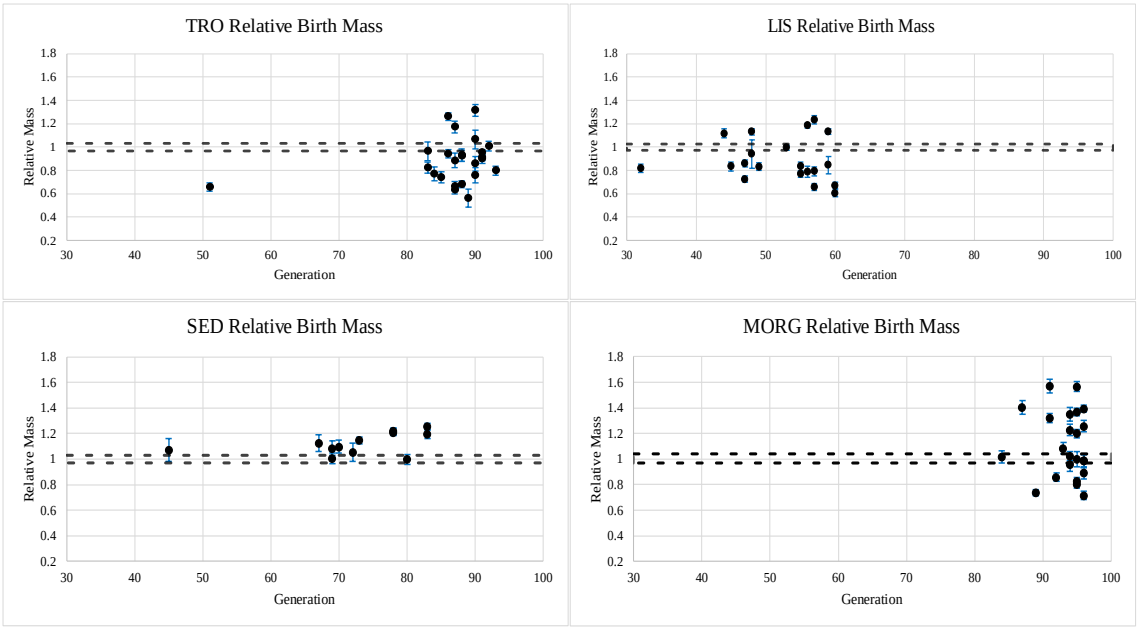

Figure 1.3 – Relative mass of MA lines compared to Control lines. MA lines for SED and MORG are primarily larger than their control lines whereas TRO and LIS are primarily smaller.

Table 1.1 –Analysis of the birth mass for the four clones of *D. pulex.*

	MORG	SED	TRO	LIS
Control Lines				
Mean (μg)	2.478	2.451	2.826	2.610
Variance (μg)	0.304	0.202	0.228	0.133
CV	0.222	0.183	0.169	0.140
MA Lines (Avg Generation)	(93.43)	(72.25)	(86.54)	(52.2)
Mean (μg)	2.751	2.749	2.509	2.319
Percent Change	11.02%	12.16%	-11.22%	-11.15%
Variance (μg)	0.422	0.081	0.320	0.254
CV	0.236	0.104	0.225	0.217
Per Generational Change				
Mean (ΔM)	0.0029	0.0041	0.0037	0.0056
Variance (ΔV)	0.0013	0.0017	-0.0011	0.0023
ANOVA Tests (MA v Control)				
p-value	1.9×10^{-7}	2.2×10^{-15}	$3.6\times10^{-12*}$	$4.9\times10^{-13*}$
F-statistic	27.743	67.74	50.205	54.754
df (Groups / Lines)	1 / 628	1 / 430	1 / 646	1 / 574

Table 1.2 - MORG MA lines compared to control lines ANOVA test. Degrees of freedom for all lines were 115. For MORG, 14 of the total 23 lines were significantly different from the ancestors. Of these, 4 were smaller (red) than the ancestor and 10 were larger (blue).

Line	1	2	3	4	5	6	7
p-Value	0.833011	**0.008858**	**3.94E-05**	0.153775	0.744436	**3.03E-06**	0.550344
F-statistic	0.044656	7.091016	18.28866	2.061507	0.106778	24.11063	0.358814
Line	9	10	11	14	15	16	17
p-Value	**8.87E-06**	**0.000829**	**1.02E-11**	**0.003156**	**0.010301**	0.061496	0.259721
F-statistic	21.63791	11.78889	57.29415	9.094495	6.804377	3.56595	1.282875
Line	18	19	20	22	23	24	26
p-Value	0.836704	**0.000641**	0.974305	**3.7E-07**	**6.44E-07**	**0.021113**	**0.000228**
F-statistic	0.042675	12.31946	0.001042	29.11753	27.77989	5.466441	14.48503
Line	29						
p-Value	**1.01E-11**						
F-statistic	57.32345						

Table 1.3 - SED MA lines compared to control lines ANOVA tests. Degrees of freedom for all lines were 115. For SED, 6 of the total 12 lines were significantly different from the ancestors. Of these, none were smaller than the ancestor and all 6 were larger (blue).

Line	1	2	7	9	10	11	12
p-Value	**0.048785**	0.255758	**0.000545**	**0.002089**	0.946584	0.410233	0.170894
F-statistic	3.966465	1.304543	12.65481	9.915021	0.004508	0.683099	1.898744
Line	17	18	22	25			
p-Value	**0.018871**	0.120457	0.965972	**0.000898**			
F-statistic	5.672865	2.447542	0.001828	11.625			

Table 1.4 - TRO MA lines compared to control lines ANOVA tests. Degrees of freedom for all lines were 115. For TRO, 15 of the total 24 lines were significantly different from the ancestors. Of these, 12 were smaller than the ancestor and 3 were larger.

Line	1	2	3	4	5	7	8
p-Value	**1.52E-07**	**0.002783**	**1.96E-08**	0.184361	**9.24E-06**	0.124262	**0.033145**
F-statistic	31.29354	9.343641	36.45685	1.783474	21.54421	2.397689	4.650488
Line	9	10	11	12	13	15	16
p-Value	**1.6E-05**	0.306161	0.082423	0.856518	0.43264	**0.00056**	**0.046282**
F-statistic	20.30427	1.056549	3.069829	0.032839	0.620075	12.5989	4.058526
Line	17	18	20	21	22	23	24
p-Value	0.248027	**0.002937**	0.259577	0.547129	**2.18E-07**	**3.23E-09**	**4.45E-08**
F-statistic	1.348029	9.236799	1.283654	0.364642	30.40815	41.16601	34.36878
Line	29	30					
p-Value	**1.05E-11**	**0.015368**					
F-statistic	57.18804	6.053225					

Table 1.5 - LIS MA lines compared to control lines ANOVA tests. Degrees of freedom for all lines were 115. For LIS, 18 of the total 20 lines were significantly different from the ancestors. Of these, 13 were smaller than the ancestor and 5 were larger.

Line	2	3	4	5	6	7	9
p-Value	**0.00164**	**1.3E-13**	**1.7E-10**	**3.6E-11**	**0.00492**	**0.00065**	**0.00567**
F-statistic	10.3941	70.6387	49.13246	53.57026	8.219902	12.29506	7.949115
Line	10	12	13	14	17	18	19
p-Value	**2.2E-06**	**1.7E-05**	0.24132	**0.01412**	**0.00013**	**0.00354**	0.90096
F-statistic	24.8077	20.0965	1.3871	6.21099	15.6596	8.86504	0.01556
Line	20	21	22	23	24	25	
p-Value	**0.000643**	**2.82E-06**	**2.79E-05**	**0.000539**	**0.000181**	**5.09E-08**	
F-statistic	12.31204	24.27732	19.05817	12.67895	14.98181	34.02803	

1.5 DISCUSSION

The hypothesis that the birth mass would decrease in successive generations was only partially supported. The four clones of *D. pulex* operated in two distinct patterns, with mutation operating in different directions between the four clones. The first pattern, MORG and SED, had a low mass in the ancestral lines and then increased to a greater mass in the MA lines, which is what was proposed would occur to all the clones due to how *Daphnia* generally respond to negative conditions (Ocampo et al. 2012), in this case represented by mutation accumulation. For the second pattern, however, TRO and LIS had an ancestral mass that was high and then the birth mass of the MA lines became low, in direct opposition to the initial hypothesis. The four clones did not converge on a central mass either, but rather almost switched positions regarding mass obtained. The four clones were measured at different average number of generations since their establishment, averages of 52.2, 72.25, 86.54, 93.43, however this too did not have any correlation with the degree of change seen in the birth mass of any of the four clones from either the two that increased in birth mass nor the two that decreased in birth mass.

One common factor seen in three of the four clones was an increase in variation of birth mass for the MA lines, with the exception of SED though this may have to do with SED only having 12 lines of the initial 30 lines remaining at the time of the experiment. For the three clones that showed this increase in variation, there were also individual lines that did show both an increase and a decrease in birth mass from their ancestral controls. When all four clones were viewed as a whole, the distribution of significant positive and negative changes in birth mass, 24 and 29 respectively, are roughly equivalent in probability of occurring.

The potential causes MORG and SED increasing in birth mass while TRO and LIS decreased in birth mass may partially be found in the current state of the MA lines. SED and LIS are two lines that tend to struggle to reproduce, which can at times cause a decrease in number of offspring and increase in size at birth (Zhou et al. 2019, Gerber et al. 2018), which would be in line with what was observed for SED but not for LIS. Similarly, MORG and TRO are generally more stable reproductively, producing a significant number of female offspring each generation which would allow for a more normal size (Jeong and Simpson 2019), which would be in accordance with the observation for TRO but not for MORG. As all the daphnia clones were collected from ephemeral ponds, they were not subject to fish predation that would influence a quickened reproduction cycle with smaller offspring (Alkimin et al 2020), and as stated in the methods, they were reared for three stabilization generations to remove any such effects (Lampert and Trubetskova 1996). To further explore these results, measurements could be taken at regular generational intervals to examine if the MA lines birth mass

represents a linear deviation from the ancestral controls or if there is positive and negative variability in birth mass across numerous generations of the same clone.

CHAPTER 2

JUVENILE SPECIFIC GROWTH RATE AS A MEASURE OF FITNESS

2.1 INTRODUCTION

The rate at which an organism reaches reproductive maturity is a parameter that influences the fitness of an organism. The average time to maturity in *D. pulex* is approximately 7 days (Schwartz et al. 2016). Instead of waiting until the *Daphnia* were fully mature and recording reproductive events, juvenile specific growth rate (g_j) has been found to be a robust surrogate for *r*, the exponential population growth rate and a common measure of fitness (Lampert and Trubetskova 1996). The juvenile specific growth rate involves measuring resource allocation for growth of a neonate prior to reaching reproductive maturity through dry mass measurements. Through examining this rate, an estimation for overall fitness can be made.

Understanding the effects of mutation on fitness are of particular importance in understanding a species ability to either sustain itself or grow. Mutation accumulation experiments allow us to examine any changes in a population in the absence of selection. Examining the mutation accumulation lines in order to observe how mutation has affected the fitness of *D. pulex* allows for a more focused view on what compounding mutations could have over multiple generations. While multiple small mutations may be observed, any large deleterious mutation could lead directly to decline in the mutation accumulation line, removing it from the pool of available test lines (Toline and Lynch 1994). While mutations with such a negative impact on fitness that it caused the line to die off can't be accounted for directly, assumptions can be made regarding the averages

of results in that the findings would be skewed to being more fit as the most unfit of the mutation accumulation lines did not survive to be tested.

While it is important to understand how mutations can impact an organism in general, it is also important to understand how mutations may impact organisms under different conditions. To this effect, investigating the impact of mutations in multiple environments can be used to see if any mutations are occurring that impact that environment directly. When it comes to fitness, resource acquisition remains a strong predictor (Lampurt and Trubetskova 1996) so manipulating food levels would allow for the examination of how resource limitation in addition to mutation accumulation impacts the fitness of *Daphnia*.

After many generations, mutations should have enough time to accumulate to impact fitness (Toline and Lynch 1997), and mutations are generally expected to be deleterious (Halligan and Keightly 2009). With this, how significant an impact will mutation accumulation have on the fitness of *Daphnia*? While generally it is anticipated that all lines will show reduced fitness, will all lines follow this trend? The expectation is that the four clones will all show reduced fitness, though not necessarily at the same rate.

2.2 METHODS

To estimate g_j and its sensitivity to diet in each MA and ancestral line, measures of dry mass at birth and dry mass after four days of growth under two different food treatments were obtained. As a continuation of the methods covered in Chapter 1, specifically subsection 1.2.5 Experimental Phase, following the placement of neonates on microscope slides, 15 additional neonates from each line were placed into a high food treatment and a further 15 neonates from each line were placed into a low food treatment.

The high food treatment continued to receive 20k cells per mL of *A. falcatus* in filtered lake water per day, whereas the low food treatment received 5k cells per mL of *A. falcatus* in 100ml of filtered lake water per day. When initially placed into treatment groups, *Daphnia* were separated three neonates per 100ml of filtered lake water, they were then transferred after 24 hours and placed two *Daphnia* per 100ml of filtered lake water. After an additional 24 hours, they were again transferred, this time to only one individual per 100ml of filtered lake water. After a final 24 hour period, for a total of 96 hours or four days, 12 *Daphnia* from each line were placed on microscope slides and then placed in a drying oven for up to 48 hours prior to being placed in desiccation boxes to remove all water from the *Daphnia*, then their dry mass was taken on a Mettler Toledo UMX2 Ultra-Microbalance.

Data analysis was performed using the lme4 package in R, after first calculating the juvenile specific growth rates were calculated using the formula $g_j=\frac{\ln\left(\frac{w2}{w1}\right)}{t}$ where w_2 is the dry mass at day 4, w_1 is the dry mass at birth, and t is the time, 4 days in this case. These values were bootstrapped in R 1000 times to obtain a measure for error within the data.

Estimations of genomic mutation rate were performed using the Bateman-Mukai method adapted for asexually reproducing populations by Lynch (1997).

2.3 RESULTS

The findings of this experiment are that the primary influencer on mutations that affect fitness were the ancestral clone from which the *Daphnia* originated from. MORG and SED both showed significant difference between the MA and Ancestral lines in both food treatments (Table 2.1). LIS showed a moderately significant deleterious effect of

mutation for the high food treatment (Table 2.1). TRO did not show any significance by the ancestral lines at all (Table 2.1).~~When compared to one another, the four ancestral lines showed no significant differences in sensitivity of juvenile specific growth rate to food level.~~ In contrast, the MA lines did show a difference among the clones (Table 2.2), suggesting mutational separation over time. The relationship between the food treatments for MORG, SED, and TRO MA lines were all uniform, whereas LIS had an increase in juvenile specific growth rate as food level increases in the ancestor as compared to the MA line (Figure 2.1). In addition to the within clone comparisons (Table 2.1), if we ignore the genetic background of the juvenile specific growth rates and compare the total MA lines to the total Control lines, the resulting p-values are 0.0097 and 3.15×10^{-5} for the low food and high food treatments respectively.

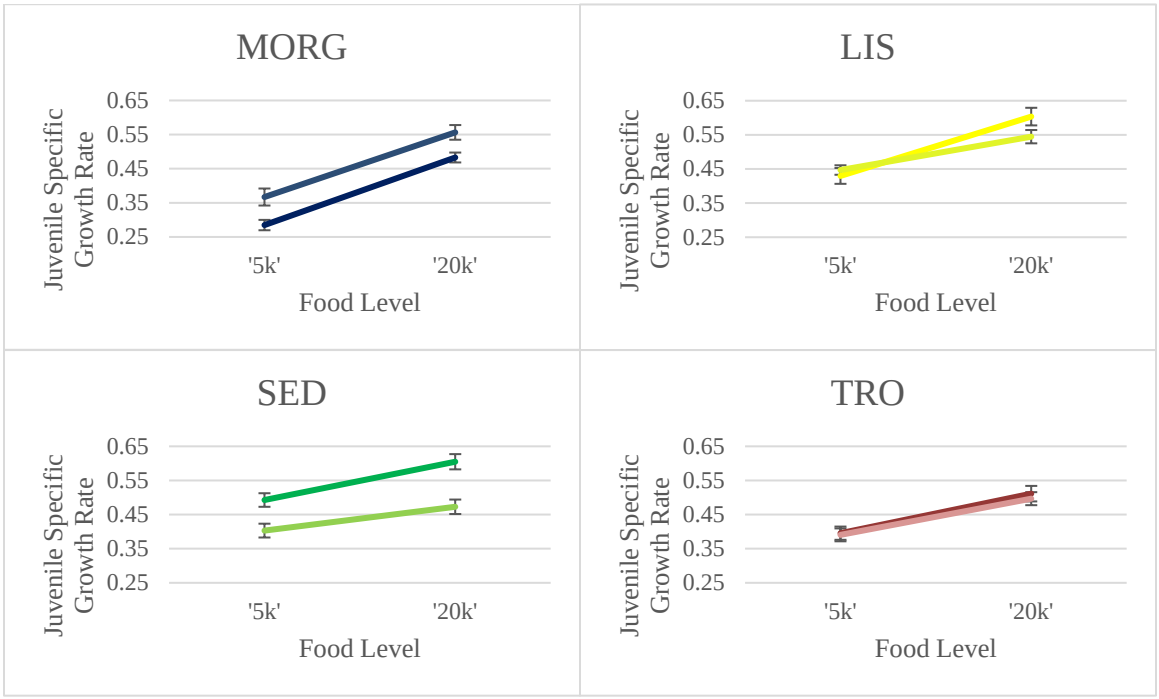

Figure 2.1 – Reaction Norms of the juvenile specific growth rate for the four clones of *D. pulex*. Food level is measured in cells of the algae *Ankistrodesmus falcatus* per mL of filtered lake water. For SED, MORG, and TRO the upper line is the ancestral control and

lower line is the MA line. For LIS, the line that has a higher juvenile specific growth rate at 20k is the ancestral control.

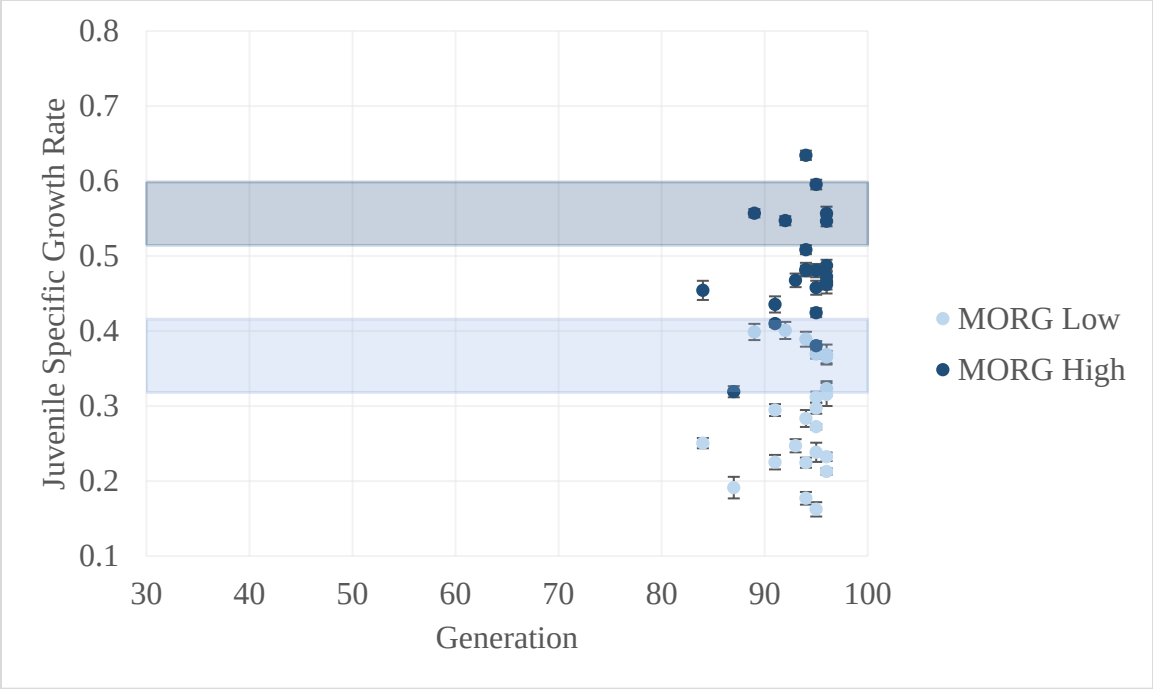

Figure 2.2 – 95% confidence interval of the mean for the High Food Treatment and Low Food Treatment shown as shaded areas with individual MA Lines shown as points with error bars.

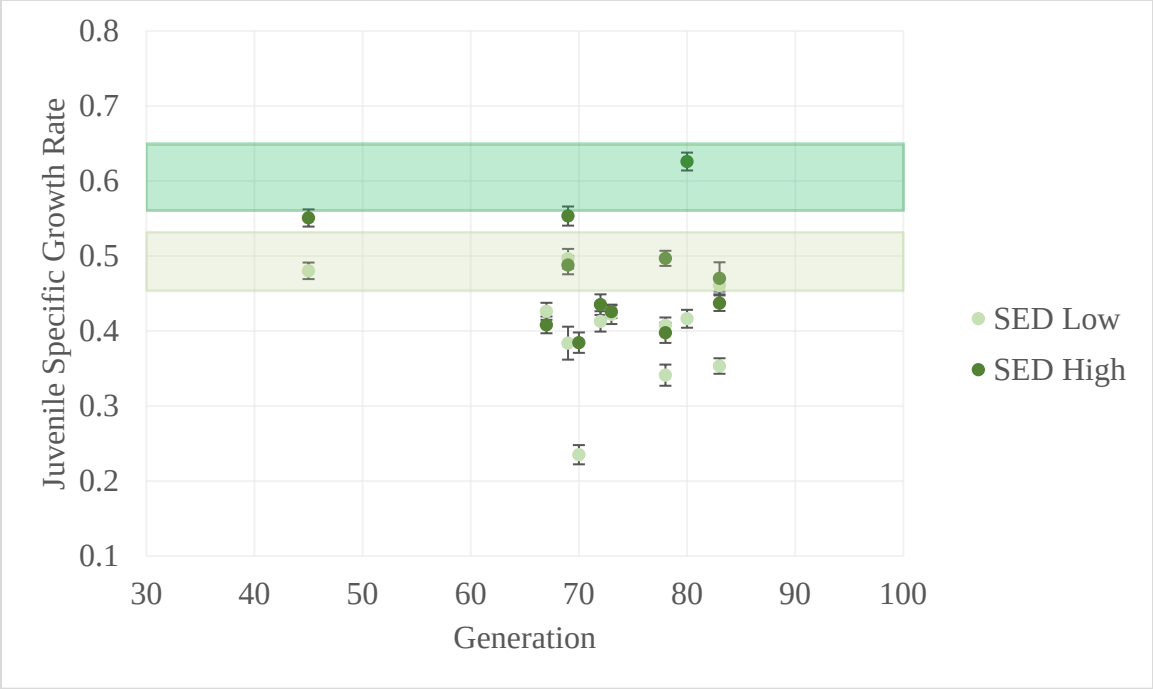

Figure 2.3 – 95% confidence interval of the mean for the High Food Treatment and Low Food Treatment shown as shaded areas with individual MA Lines shown as points with error bars.

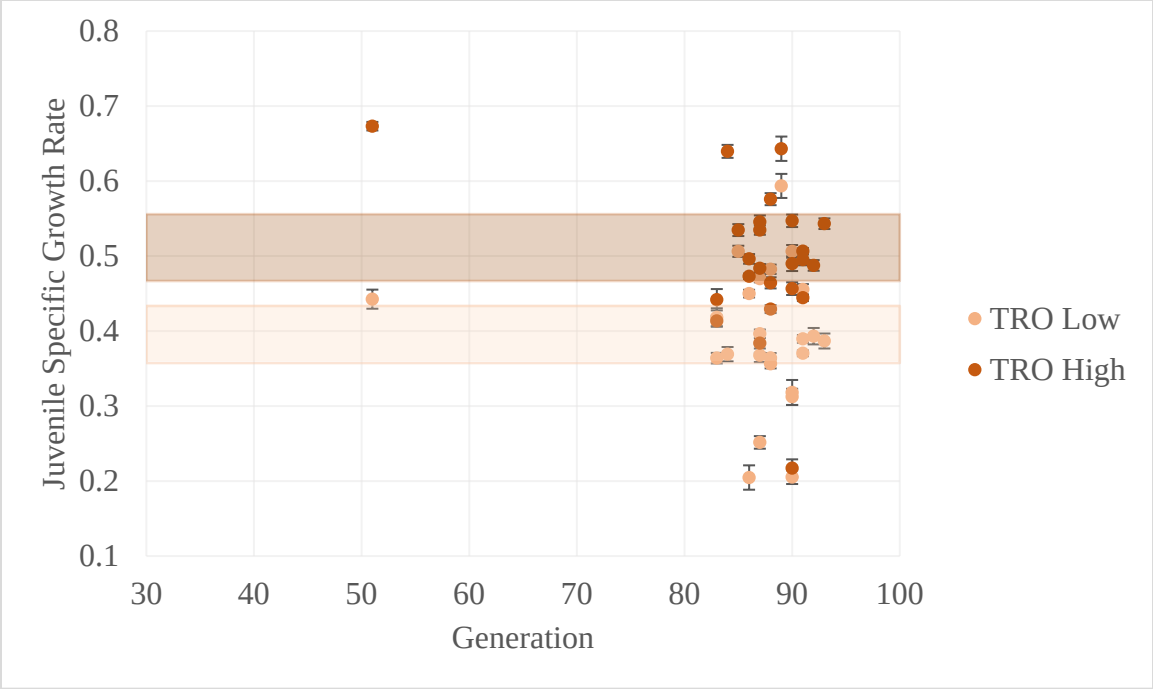

Figure 2.4 – 95% confidence interval of the mean for the High Food Treatment and Low Food Treatment shown as shaded areas with individual MA Lines shown as points with error bars.

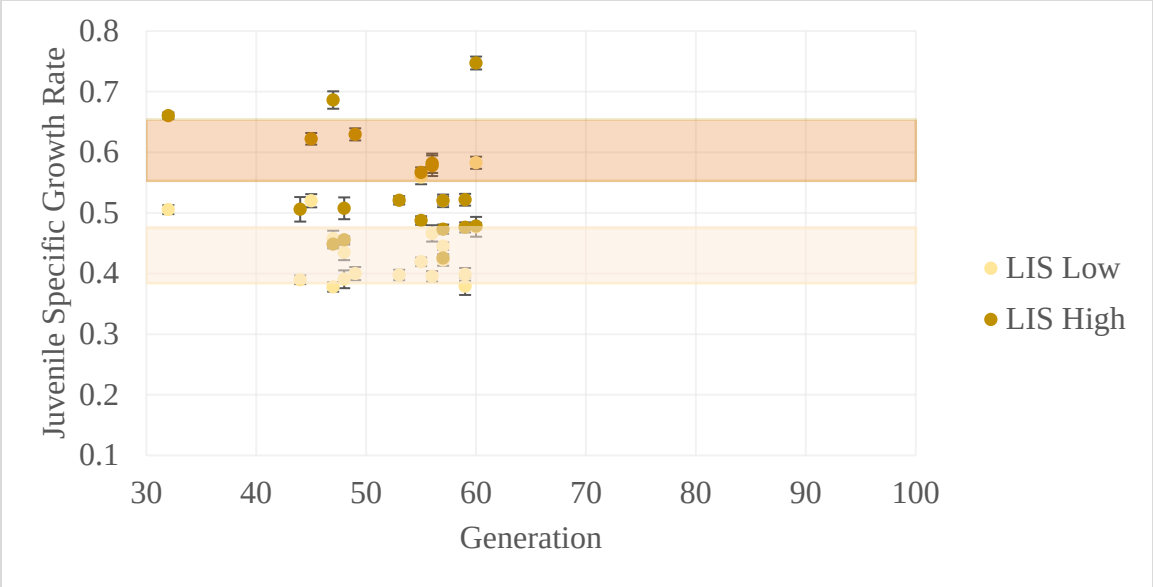

Figure 2.5 – 95% confidence interval of the mean for the High Food Treatment and Low Food Treatment shown as shaded areas with individual MA Lines shown as points with error bars.

Table 2.1 – Analysis of MA lines vs Control lines for both food treatments.

	MORG	SED	LIS	TRO
Low Food				
p-Value	0.005	0.004	0.501	0.872
F-Statistic	8.839	9.973	0.465	0.026
High Food				
p-Value	0.006	3×10^{-4}	0.077	0.644
F statistic	8.417	18.358	3.36	0.218

Table 2.2 – Statistical analysis of the juvenile specific growth rate for the four clones of *D. pulex*

Clone	U_{MIN}	$E[a]_{MAX}$	Control to MA p-value	Treatment p-value	Plasticity p-value
MORG	-0.16036	0.00491	< 0.001	< 0.001	0.819
SED	-0.80539	0.002268	< 0.001	< 0.001	0.317
LIS	-0.33128787	0.003404	0.313	< 0.001	0.68
TRO	0.001961*	-0.08653*	0.655	< 0.001	0.823

2.4 DISCUSSION

While the majority of MA lines showed a decrease in juvenile specific growth rate, one line in particular, TRO, not only was not statistically significantly different between ancestral controls and mutation accumulation lines, but also showed a roughly even degree of variation between positive and negative effects from the ancestor lines. The results showed that the greatest effect on juvenile specific growth rate was which clone they came from, followed by food treatment group. This suggests that their genetic background played a large role in determining their resiliency against spontaneous mutations when in ideal situations, however while resource limitations are in place, all four clones fell back to a slower growth rate which may have disguised underlying mutations or be more strongly conserved in order to protect the organism against these mutations having a significant negative effect when environmental conditions are already poor (Chainy et al 2016).

The importance of observing all four clones can also not be discounted. The results and overall impressions would have been much different had only a pair of clones

been tested depending on which two clones were selected. When looked at without regard to clone, some of the skew may have been mediated, but increased sample size in the populations from which samples are drawn, and not just the number of individuals helps us to provide a more complete picture of what is occurring (Ho et al. 2020).

When continuing this study, it would be good to look at the juvenile specific growth rate again in 50 to 100 generations to attempt to establish a trend for any surviving individual lines, as well as the clones as a whole. This would enable the observation of mutation rate and if it remains somewhat constant or if as mutations continue to accumulate, the rate itself begins to compound as they have greater and greater effect on fitness.

REFERENCES

Alkimin GD de, Nunes B, Soares AMVM, Bellot M, Gómez-Canela C, Barata C. 2020. Daphnia magna responses to fish kairomone and chlorpromazine exposures. Chemico-Biological Interactions. 325:109123. doi:10.1016/j.cbi.2020.109123.

Betini GS, Wang X, Avgar T, Guzzo MM, Fryxell JM. 2019. Food availability modulates temperature-dependent effects on growth, reproduction, and survival in Daphnia magna. Ecology and Evolution. 10(2):756–762. doi:10.1002/ece3.5925.

Bownik A. 2017. Daphnia swimming behaviour as a biomarker in toxicity assessment: A review. Science of The Total Environment. 601-602:194–205. doi:10.1016/j.scitotenv.2017.05.199.

Bukovinszky T, Verschoor AM, Helmsing NR, Bezemer TM, Bakker ES, Vos M, de Senerpont Domis LN. 2012. The good, the bad and the plenty: interactive effects of food quality and quantity on the growth of different Daphnia species. Getz WM, editor. PLoS ONE. 7(9):e42966. doi:10.1371/journal.pone.0042966.

Chainy, G. B., Paital, B., & Dandapat, J. (2016). An Overview of Seasonal Changes in Oxidative Stress and Antioxidant Defence Parameters in Some Invertebrate and Vertebrate Species. *Scientifica*, *2016*, 6126570. https://doi.org/10.1155/2016/6126570

Chopelet J, Blier PU, Dufresne F. 2008. Plasticity of growth rate and metabolism in Daphnia magna populations from different thermal habitats. Journal of Experimental

Zoology Part A, Ecological Genetics and Physiology. 309(9):553–562. doi:10.1002/jez.488. [accessed 2021 Mar 12]. https://pubmed.ncbi.nlm.nih.gov/18668644/.

Ebert D. 2005. Epidemiology, and evolution of parasitism in Daphnia. Bethesda (MD): National Center for Biotechnology Information (US). [accessed 2021 Sep 29]. https://www.ncbi.nlm.nih.gov/books/NBK2042/.

Eyre-Walker A, Keightley PD. 2007. The distribution of fitness effects of new mutations. Nature Reviews Genetics. 8(8):610–618. doi:10.1038/nrg2146.

Flynn JM, Chain FJJ, Schoen DJ, Cristescu ME. 2016. Spontaneous mutation accumulation in Daphnia pulex in selection-free vs. competitive environments. Molecular Biology and Evolution. 34(1):160–173. doi:10.1093/molbev/msw234.

Garbutt JS, Little TJ. 2014. Maternal food quantity affects offspring feeding rate in Daphnia magna. Biology Letters. 10(7). doi:10.1098/rsbl.2014.0356.

Gerber N, Kokko H, Ebert D, Booksmythe I. 2018. Daphnia invest in sexual reproduction when its relative costs are reduced. Proceedings of the Royal Society B: Biological Sciences. 285(1871). doi:10.1098/rspb.2017.2176.

Halligan DL, Keightley PD. 2009. Spontaneous Mutation Accumulation Studies in Evolutionary Genetics. Annual Review of Ecology, Evolution, and Systematics. 40(1):151–172. doi:10.1146/annurev.ecolsys.39.110707.173437.

Heugens EHW, Tokkie LTB, Kraak MHS, Hendriks AJ, van Straalen NM, Admiraal W. 2006. Population growth of Daphnia magna under multiple stress conditions: joint effects of temperature, food, and cadmium. Environmental Toxicology and Chemistry. 25(5):1399. doi:10.1897/05-294r.1.

Ho, E., Macrae, F., Latta, L. C., McIlroy, P., Ebert, D., Fields, P. D., Benner, M. J., & Schaack, S. (2020). High and Highly Variable Spontaneous Mutation Rates in Daphnia. *Molecular biology and evolution*, *37*(11), 3258–3266. https://doi.org/10.1093/molbev/msaa142

Jeong T-Y, Simpson MJ. 2019. Reproduction stage differentiates the time-course regulation of metabolites in Daphnia magna. Environmental Science & Technology. 53(21):12764–12773. doi:10.1021/acs.est.9b03762.

Katju V, Bergthorsson U. 2018. Old trade, new tricks: insights into the spontaneous mutation process from the partnering of classical mutation accumulation experiments with high-throughput genomic approaches. Makova K, editor. Genome Biology and Evolution. 11(1):136–165. doi:10.1093/gbe/evy252.

Keightley PD, Halligan DL. 2008. Analysis and implications of mutational variation. Genetica. 136(2):359–369. doi:10.1007/s10709-008-9304-4.

Keightley PD, Lynch M. 2003. Toward a realistic model of mutations affecting fitness. Evolution. 57(3):683. doi:10.1554/0014-3820(2003)057[0683:tarmom]2.0.co;2.

Keith N, Tucker AE, Jackson CE, Sung W, Lucas Lledó JI, Schrider DR, Schaack S, Dudycha JL, Ackerman M, Younge AJ, et al. 2015. High mutational rates of large-scale duplication and deletion in Daphnia pulex. Genome Research. 26(1):60–69. doi:10.1101/gr.191338.115.

Lampert W, Trubetskova I. 1996. Juvenile Growth Rate as a Measure of Fitness in Daphnia. Functional Ecology. 10(5):631. doi:10.2307/2390173.

Latta LC, Peacock M, Civitello DJ, Dudycha JL, Meik JM, Schaack S. 2015. The Phenotypic Effects of Spontaneous Mutations in Different Environments. The American Naturalist. 185(2):243–252. doi:10.1086/679501.

Lynch M, Gutenkunst R, Ackerman M, Spitze K, Ye Z, Maruki T, Jia Z. 2017. Population genomics of Daphnia pulex. Genetics. 206(1):315–332. doi:10.1534/genetics.116.190611.

Ocampo Q. LE, Botero M, Fernando L. 2012 Jan 27. Measurements Population Growth and Fecundity of Daphnia Magna to Different Levels of Nutrients Under Stress Conditions. Aquaculture. doi:10.5772/29136.

Ridgway CL, Brage S, Anderssen S, Sardinha LB, Andersen LB, Ekelund U. 2011. Fat-free mass mediates the association between birth weight and aerobic fitness in youth. International Journal of Pediatric Obesity. 6(2-2):e590–e596. doi:10.3109/17477166.2010.526225.

Schwartz TS, Pearson P, Dawson J, Allison DB, Gohlke JM. 2016. Effects of fluctuating temperature and food availability on reproduction and lifespan. *Exp Gerontol*. 86:62-72. doi:10.1016/j.exger.2016.06.010

Simčič T, Brancelj A. 1997. Electron transport system (ETS) activity and respiration rate in five Daphnia species at different temperatures. Hydrobiologia. 360(1):117–125. doi:10.1023/a:1003117221455.

Singhal A. 2017. Long-Term Adverse Effects of Early Growth Acceleration or Catch-Up Growth. Annals of Nutrition and Metabolism. 70(3):236–240. doi:10.1159/000464302.

Souza LV, Meneck F, Parizotto GP, Franco M. 2021 Mar 12. Low birth weight and its relation to physical fitness parameters in children: Its negative effect on muscle strength and cardiorespiratory endurance. American Journal of Human Biology. doi:10.1002/ajhb.23595.

Spanier KI, Jansen M, Decaestecker E, Hulselmans G, Becker D, Colbourne JK, Orsini L, De Meester L, Aerts S. 2017. Conserved Transcription Factors Steer Growth-Related Genomic Programs in Daphnia. Genome Biology and Evolution. 9(6):1821–1842. doi:10.1093/gbe/evx127.

Suzuki A, Kato Y, Matsuura T, Watanabe H. 2014. Growth evaluation method by live imaging of Daphnia magna and its application to the estimation of an insect growth regulator. Journal of Applied Toxicology. 35(1):68–74. doi:10.1002/jat.2988.

Toline CA, Lynch M. 1994. Mutational divergence of life-history traits in an obligate parthenogen. Genome. 37(1):33–35. doi:10.1139/g94-005.

Zhou Q, Lu N, Gu L, Sun Y, Zhang L, Huang Y, Chen Y, Yang Z. 2020. Daphnia enhances relative reproductive allocation in response to toxic microcystis: Changes in the performance of parthenogenetic and sexual reproduction. Environmental Pollution. 259. doi:10.1016/j.envpol.2019.113890.