**Title**

Simulation of rapidly evolved grazing adaptations in Cladocera zooplankton*: Daphnia magna* and *Daphnia pulex*

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**Abstract**

In the summer of 2021, two species of Cladocera zooplankton*: Daphnia magna* and *Daphnia pulex*, were isolated from experimental mesocosms and subjected to a 24-hour grazing experiment to determine rapid evolution in grazing rates had occurred in response to varying competitive environments in the source mesocosms. The populations spent one year prior to the grazing experiment exposed to either intra- or interspecific competition, with either oligotrophic or eutrophic nutrient levels. The aim of my research was to design and analyze a simulation of the grazing experiment, in order to develop a plausible model for the mechanism of any observed evolved variation in population grazing rates sourced from low-nutrient environments. The simulation involved a Holling Type II functional response with genetic variation in some of the model parameters. I also conducted an analysis of variance on the resulting simulated data, which confirmed that grazing rates (based on final algal concentrations after 24 hours) depended on the focal clone’s previous competition environment, the competitive environment in the grazing experiment, and the interaction of these two factors. If these results are indicative of the results of the experiment, it would suggest that rapid evolution is a significant factor to be considered when evaluating how an organism responds to changes in their environment.



**Introduction**

Natural systems are increasingly under threat from anthropogenic climate change (Maclean & Wilson 2011). In freshwater aquatic systems, anthropogenic stressors include warming temperatures and eutrophication (Martin & Jeppesen 2007). Aquatic organisms have demonstrated in some instances that they can shift ranges (Comte et al. 2013) or evolve adaptations (Catullo et al. 2019) in response to these stressors, but these studies tend to focus on adaptive ecological or evolutionary responses of individual species. Few studies consider both ecological and evolutionary responses at the same time (referred to as eco-evolutionary dynamics; Fussmann et al. 2007), in the context of how interactions with other species (such as mutualisms, predation, or competition) might mediate these eco-evolutionary responses. There is research that eco-evolutionary contributions affect community shifts (Hattich et al. 2021) and when evolutionary dynamics are ignored, a significant amount of variation in community composition may go unexplained (Pantel et al. 2015). In order to better understand ecosystem functioning, feedback loops between ecological and evolutionary change need be considered. Also, as anthropogenic effects grow in relation to the growth of human population and its ecological footprint, it becomes increasingly important to understand community interactions, such as competition, and the role eco-evolutionary responses play in the changing environment.

Some existing studies have demonstrated that freshwater invertebrates can evolve in their ability to compete with one another (Hart et al. 2019). Another set of studies have shown that freshwater invertebrates can evolve in response to eutrophication (Hairston et al. 1999; Chislock et al. 2019;). The goal of the research described here is to determine how freshwater crustaceans of the genus *Daphnia* respond to eutrophication, and more specifically, whether they evolve rapidly, whether this rapid evolution in individual species can alter community dynamics, and whether evolutionary trajectories differ when *Daphnia* grazers evolve alone or in competition with a second grazer species.

To observe whether competition drives trait evolution and if this trait evolution feeds back to impact community ecological dynamics, two species of Cladocera zooplankton, *Daphnia* *magna* and *D.* *pulex*, were placed in an outdoor mesocosm experiment at two nutrient levels (oligotrophic and eutrophic). For an additional treatment, *Daphnia* were placed in either mixed tanks (with both species present) or in isolated tanks (with one grazer species present). After one year under these conditions, we isolated clones from these mesocosms, placed them in a common garden environment to purge maternal effects, and conducted an experiment to determine evolution of grazing ability on low- and high-quality food resources. Grazing ability was quantified as the difference in algae consumption after 24 hours, comparing across populations that had been exposed to intra- or interspecific competition in the mesocosm experimental mesocosms. The grazing experiment thus measures the effects of short-term evolution on *Daphnia* while subjecting them to no, intra-, or interspecific competition. In order to develop an appropriate statistical model for the data resulting from this grazing experiment, I conducted a simulation model and analyzed the resulting simulated data. I hypothesized that *Daphnia* would experience Hollings Type II feeding rates (Holling, 1959). In addition, I hypothesized *Daphnia* would have genetic variation in their handling time, and that an analysis of variance in the final concentration of algae after 24 hours would be a sufficient model to observe among-population variation in grazing ability due to evolved changes in handling time.



**Methods**

*Experimental and Laboratory Work*

In the summer of 2020, an experiment to study evolution and coevolution in freshwater invertebrates was set up at the University of Konstanz Limnological Institute. To set up the experiment, 30 different 300 L tanks were filled with 270 L of filtered water from Lake Konstanz. The lake water was filtered with a 25-micrometer filter in order to exclude everything except bacterioplankton and phytoplankton and it was added to the tanks on August 19th. On August 25th, two levels of nutrient treatments were added to the tanks. The tanks assigned to a low-nutrient level were given 25 micrograms/liter of nitrogen and 150 micrograms/liter of phosphorus (a high nutrient treatment was included as well but is not used in the subsequent grazing experiment and simulation). This level corresponds to that of an oligotrophic lake where the high-level nutrient treatment’s hypereutrophic level can cause toxic phytoplankton blooms (Sawyer 1966).

Two species of *Daphnia*, *D. pulex* and *D. magna*, were added into the 30 experimental mesocosms to evaluate the effect competition may have on community dynamics, and more specifically, on intraspecific genetic variation and rapid evolution during the course of the experiment (the experiment will last until October 2025). On September 1st, *Daphnia* were added to the tanks. 10 of the 30 mesocosms received only *D*. *magna* (treatment Dm), 10 received only *D*. *pulex* (Treatment Dp), and the final 10 received the two species (which will thus experience competition with one another for algal and bacterial resources; treatment Dp+Dm).

A picture containing text

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Figure 1: Mesocosm experimental array at the University of Konstanz Limnological Institute.300 L tanks are numbered corresponding to their experimental treatment: all tanks receive either low or high nutrients, and either D. magna alone, D. pulex alone, or D. magna + D. pulex in competition. The tanks are held in a pool of water to maintain more consistent temperatures.

The *Daphnia* introduced into the mesocosms for the experiment were hatched from dormant eggs, which are produced as part of their natural life cycle. *Daphnia* have two different strategies for reproduction. During their growing season, i.e. warmer months, the species reproduce asexually. During this time, a female *Daphnia*, under the right conditions, will produce a clutch of eggs that will hatch and remain in their dorsal brood chamber for approximately 3 days. After 3 days in their mother’s brood pouch, the neonates will be released. These juvenile *Daphnia* reach sexual maturity in 6-10 days and given the right conditions, will continue reproducing female *Daphnia* asexually (I therefore refer to lineages of asexual mothers and daughters as clones). Sexual reproduction occurs under stressed conditions. These conditions are most commonly associated with freezing temperatures as winter approaches, decreasing oxygen levels as ponds may become dry, and with decreasing daylight length (Dodson et al. 2010). The sexual cycle of *Daphnia* has two stages. First, the female gives birth to males. Second, the female produces haploid eggs which requires a male *Daphnia* for fertilization. These haploid eggs are protected with a hard external shell (called an ephippia) and released into the water column. These ephippia are resistant to freezing, drying, and passage through the digestive systems of predators. To hatch, these dormant eggs must receive environmental cues that signal conditions have become more favorable (i.e., spring warming, rehydration of the pond, increased daylight length; Schwartz & Hebert 1987). This method of reproduction ensures the survival of the population during winter months and increases the population’s genetic diversity (due to the genetic mixing of sexual reproduction).

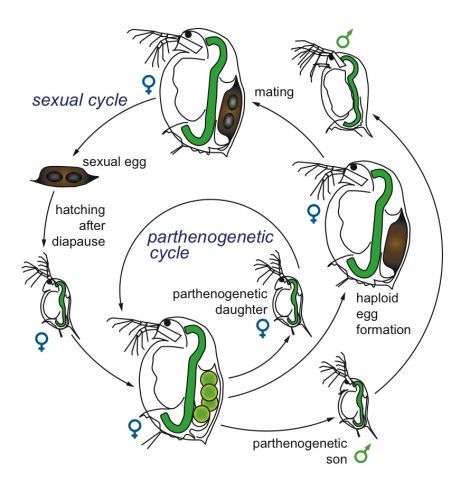


Figure 2: Daphnia life cycle, spanning an adult female Daphnia’s (top) parthenogenetic (asexual) and sexual cycle. Image from Ebert 2005.

The initial population of *D*. *pulex* and *D.* *magna* added to the mesocosms in 2020consisted of 22 unique clones which were hatched from dormant eggs collected from 5 different artificial ponds (Ismainger Teiche, near Munich, Germany; location: 48°13'18.3"N, 11°45'17.9"E). From there, all mesocosms received an initial density of 0.326 individuals / L. In the solo Dp and Dm treatments, 4 individuals per clone were added to each 270 L mesocosm and in the Dp+Dm mixed treatments, 2 individuals per clone of both species were added. The *Daphnia* in the mesocosms grew in these conditions (receiving weekly additions of nitrogen and phosphorus to maintain the target levels per low and high nutrient treatment) until November 5, 2020, when they were drained of water and covered. The tanks remained in this condition until March 15, 2021, when the tanks were refilled with filtered lake water to a volume of 270 L. Bacterio-, phyto-, and zooplankton re-established populations naturally from their over-winter dormant stages, and tanks were supplemented with target nutrient levels weekly.



In the summer of 2021, we collected *Daphnia* directly from the water column of each tank in order to determine whether populations had evolved in their grazing ability in response to nutrient and competition treatments. Between July 29 and August 6, we collected *Daphnia* from tanks and placed individuals in laboratory cultures. Clones collected from each tank were placed in common garden laboratory conditions for 3 generations, to eliminate the effects of maternal environment for phenotypic traits. A maternal effect occurs when an offspring’s phenotype is determined not only by its environment and experiences, but also the genotype and environment of its mother (LaMontagne & McCauley 2001). Maternal effects can increase individual fitness during environmental fluctuations. In *D*. *magna*, research shows that there is a significant relationship between maternal size and size of neonates hatched from ephippial eggs. As a result, larger mothers produce larger offspring, which subsequently grow to become large adults that produce more eggs. (Boersma *et al.* 2000). For the experiment to measure evolution of grazing ability, it was important to control for maternal effects and thus measure phenotypic variation that is due only to genetic variation.



Clones were kept in a controlled laboratory environment (20 deg C, on a 16:8-hour light: dark cycle), in 100ml of filtered Lake Konstanz water. Cultures were fed 1ml of the green algae *Scenedesmus* every Monday, Wednesday, and Friday. Before isolating each clone into culture, we verified the species and sex of the clone. Once a single female *Daphnia* was identified, we isolated it into 90 ml of filtered lake water in a 100 ml jar. We labeled all jars with the species and clone number. Once a parental (generation P) clone reproduced, we confirmed all neonates were female and placed 3 of these neonates in their own new 100 ml jar, marking them as the F1 generation. The first 2 times a F1 clones reproduced we isolated the F1 mother into a new jar and discarded the clutch. On the third clutch, we confirmed all neonates were females, and placed 3 individuals into their own jar, marking them as the F2 generation. The first two clutches of the F2 mothers were similarly discarded by isolating the mother into her own jar. Once the F2 parent clone has produced her third clutch we confirmed that they were all female and isolated three replicate neonates into their own jars. These three individuals mark the F3 generation and can be used in the grazing experiment without the interference of maternal effects.

These F3 *Daphnia* were maintained as clonal lineages in the constant laboratory conditions described above. In October-November 2021, researchers at the Limnological Institute performed an experiment to measure grazing rates of *Daphnia* on 5 distinct phyto- and bacterioplankton species. Because the results of this experiment are not currently available, I conducted a simulation to better understand how to model the expected results, and I developed a simple statistical model to analyze the data that will be produced by this experiment.

*Simulation of evolution of grazing experiment*

The goal of our simulation experiment was to simulate a 24h grazing experiment done at the University of Konstanz to compare differences in algae consumptions between *D.* *magna* and *D.* *pulex* from the Dp, Dm, and Dp+Dm treatments (in low nutrients)*.* I simulated the change in algae concentrations over a 24h period, using the same initial algae concentrations that were used in the laboratory common garden experiment. To simulate *Daphnia* grazing algae, I used a version of Gauld’s equation (after Allen et al. 1995):



Where the feeding rate ( is determined by the volume in ml () located in the test vessels multiplied by the difference in resource concentration at hour 0 () and the final resource concentration at hour 24 (, divided by the duration of the experiment (24 hours).



In order to determine the feed rate ( in the equation above I used the Holling Type II Functional Response model (Holling, 1959; after Schenone et al. 2021):



A type II functional response models a situation where the consumer’s rate of intake decelerates as it continues to consume more prey due to the assumption that the consumer’s capacity to process more food is limited.

Diagram

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Figure 3: Functional response represents the intake rate of a given consumer (y axis) as a function of food density (x axis). I focused on Type II where the predator’s intake eventually decelerates as the density of the prey population grows. Figure from: <https://commons.wikimedia.org> /w/index.php?curid=7870276

*Daphnia* encounters food items per unit of food density (in hours, h-1) and is multiplied by resource density (*R*, cells / ml*)* over the attack rate multiplied with handling time (*h*, hours). I chose this functional response model to consider that *Daphnia’s* capacity to consume is limited by their handling time. I used the Holling type II model above and calculated the resulting feeding rate that is used in the algae concentration model, in order to estimate the expected concentration after 24 hours (c24).

The results from the simulation were obtained using the statistical programming language R (version 3.6.2, 2019). The simulation assumed that all *Daphnia* share a common, invariable attack rate (*a* = 1.7 h-1). We thus focused on interspecific and intraspecific variation in handling time (*h*). For clones not exposed to competition (treatments Dm and Dp), *D. magna* handling times were drawn from a random normal distribution with mean 0.1 and standard deviation 0.001 (*hdm* ~ N(0.1, 0.001) )and *D. pulex* handling time for each clone was *hdp* ~ N(0.05, 0.001). For the clones from mixed tanks (treatment Dp+Dm), and therefore exposed to competition, *hdm* ~ N(0.09, 0.001) and *hdp* ~ N(0.049, 0.001). Values for these and for other parameters in the model are given in Table 1 and 2.



|  |  |
| --- | --- |
| **Species and competition history** | **Handling time (h)** |
| *D.* *magna* from isolated tank | .1 |
| *D.* *pulex* from isolated tank | .05 |
| *D.* *magna* from mixed tank | .09 |
| *D.* *pulex* from mixed tank | .049 |

Table 1: Chosen handling rates for Daphnia based on evolutionary history

|  |  |  |  |
| --- | --- | --- | --- |
| **Volume** | **Time** | **Attack Rate** | **Resource Density** |
| 40 mL | 24 hours | 0.8 (h-1) | 9.72 mg / L |

Table 2: Various parameters used to simulate the feeding patterns based on Gauld’s Equation and the Holling Type II Functional Response model

As in the laboratory experiment, we simulated grazing of each focal experimental clone in each of 8 treatments (Figure 4).

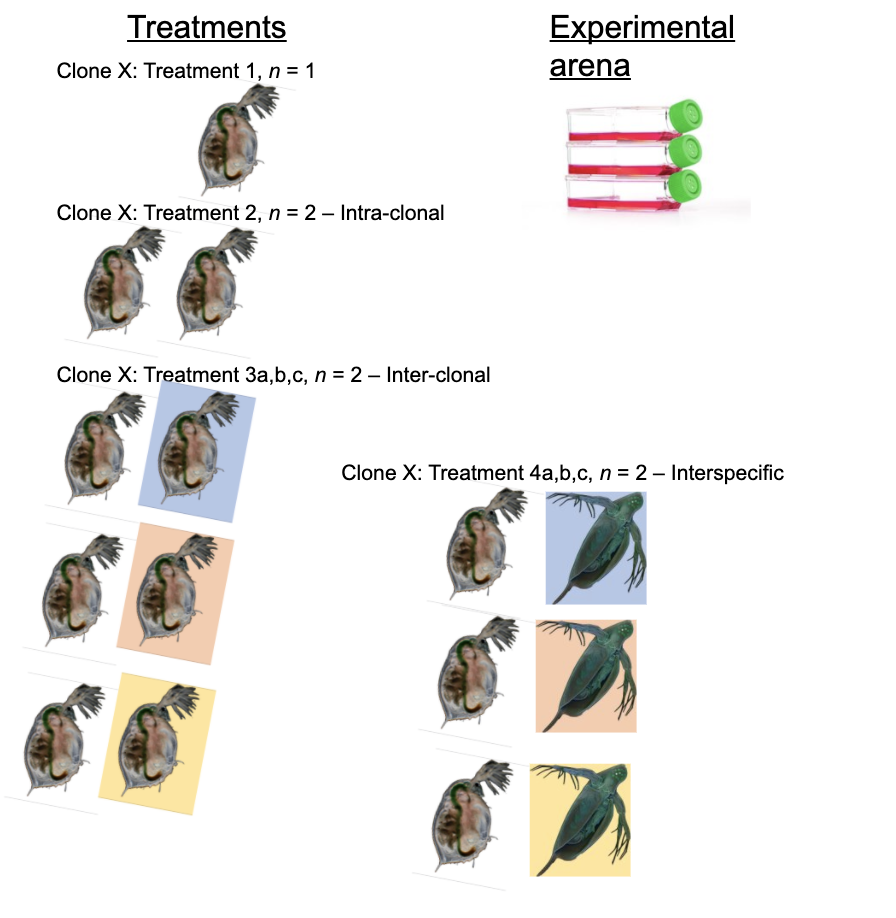


Figure 4: Experimental design of the 24-hour grazing experiment

Our first treatment (treatment 1) represents the focal clone (*D. magna* or *D. pulex*) alone. Our second treatment (2) simulated intra-clonal competition between the focal clone and a genetically identical sister of the same clone. To consider inter-clonal competition, we chose 3 reference clones (3a, 3b, 3c) to simulate 3 instances where a focal clone and a reference clone of the same species competed in the grazing experiment (in the actual experiment, these reference clones were drawn from clones used to stock the initial tank populations in 2020). And lastly, to simulate interspecific competition we choose 3 reference clones again (4a, 4b, 4c) to represent our focal clone in competition with the other species (in the true experiment, these were again drawn from initial 2020 stock clones). All 8 of these treatments (1, 2, 3a, 3b, 3c, 4a, 4b, and 4c) were replicated 3 times for each of our mesocosm tank clones (i.e. repeated in 3 experimental containers). Altogether, we simulated the feeding rates (F) of *D.* *pulex* from 10 dp\_only tanks, *D.* *magna* from 10 dm\_only tanks, and both *D.* *pulex* and *D. magna* from 10 mixed (Dp+Dm) tanks.

The reference clones were also given distinct handling times that were drawn from the same random normal distribution as the clones in the monospecific (dm and dp) treatments: *h*dm-focal ~ N(0.1, 0.001) and *h*dp-focal ~ N(0.15, 0.001). These were chosen at the beginning of the simulation and held as constant throughout the simulated replicates. We simulated the 3 replicates of the experiment using this procedure, and we introduced among-replicate variance by adding a small amount of random noise to all final c24 values, as c24 ~ N(c24, 0.001). The simulation thus produced 4,800 unique values of c24, the algal concentration in a simulated experimental container after 24 hours: one value per focal clone (of 100 focal clones of *D*. *pulex* and 100 focal clones of *D.* *magna*) for each of 8 grazing treatments, each replicated 3 times.

*Analysis of simulated data*

The goal of our simulation was to analyze the effects previous tank conditions have on *Daphnia’*sgrazing rate, to determine whether populations differ due to rapid evolution. I performed a 2-way ANOVA to determine if previous tank history (*comp-evol*: single species or 2 species together), the current grazing environment (*comp-env*: alone, intra-clonal, inter-clonal, or inter-specific), and their interaction are significant predictors of final c24 values (and therefore whether genetic difference in handling time are enough to significantly impact algal concentrations in the simulation). The resulting linear model included two main effects: previous tank environment (with clone nested within tank nested within *comp-evol*) and current competition environment. I ran two separate ANOVA tests for each focal species: *D.* *magna* and *D.* *pulex.* In addition, I created boxplots to visualize the distribution of c24 values across competition environments.

**Results**

The final mean algal concentrations (c24) of *D.* *magna* originating from the single-species tank treatment was 4.516 ± 0.0423, while the average c24 of *D.* *pulex* originating from the single-species tank treatment was 3.689 ± 0.0436. For *D.* *magna* originating from a mixed-species tank the average c24 value was 4.38 ± 0.0423 for *D*. *pulex* originating from a mixed-species tank the average c24 value was 3.667 ± 0.0436.

I performed a 2-way ANOVA to analyze the effect the clones previous tank environment (*comp-evol*) and the competition environment in the grazing treatment (*comp-env*) had on the final algal concentration after 24 hours of the experiment. I conducted an ANOVA for both focal species (*D. magna*, Figure 7a;and *D. pulex*, Figure 7b). The two-way ANOVA for *D. magna* (Table 3) revealed that the previous tank environments (comp-evol) affected the final c24 value (F1 = 1.66×104, p < 0.001), as well as the current grazing environment (comp-env; F3 = 1.46×106, p < 0.001), The interaction of these two factors affected the magnitude of the result of the c24 values (F3 = 4.78×102, p < 0.001). The nested factor of tank (comp-evol x tank; F2 = 3.44 x 101, p < 0.001) significantly impacted c24, as well as clone nested within tank (*comp-evol* × *tank* × *clone*; F2 = 8.85, p < 0.001).

The two-way ANOVA for *D*. *pulex* (Table 4) revealed that the previous tank environments (comp-evol) affected the final c24 value (F1 = 1.42x102, p < 0.001), as well as the current grazing environment (comp-env; F3 = 1.01x106, p < 0.001) The interaction of these two factors affected the magnitude of the result of the c24 values (F3 = 4.09, p = .007). The nested factor of tank (comp-evol x tank; F2 = 5.71, p = .003) significantly impacted c24 values as well as clone nested within tank (*comp-evol* × *tank* × *clone*; F2 = 2.94x101, p < 0.001).

Df Sum Sq Mean Sq F value Pr(>F)

comp\_evol 1 4.8 4.8 1.661e+04 < 2e-16 \*\*\*

comp\_env 3 1275.5 425.2 1.459e+06 < 2e-16 \*\*\*

comp\_evol:tank 2 0.0 0.0 3.437e+01 3.12e-15 \*\*\*

comp\_evol:comp\_env 3 0.4 0.1 4.784e+02 < 2e-16 \*\*\*

comp\_evol:tank:clone 2 0.0 0.0 8.850e+00 0.000153 \*\*\*

comp\_evol:tank:comp\_env 6 0.0 0.0 9.900e-01 0.430495

comp\_evol:tank:clone:comp\_env 6 0.0 0.0 2.550e-01 0.957395

Residuals 1176 0.3 0.0

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Signif. codes: 0 ‘\*\*\*’ 0.001 ‘\*\*’ 0.01 ‘\*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Table 3: 2 Way ANOVA D. magna where comp\_evol is the tank environment the focal clone was isolated from and comp\_env is the grazing treatment (alone, intra-clonal, inter-clonal, or inter-specific)

Df Sum Sq Mean Sq F value Pr(>F)

comp\_evol 1 0.1 0.1 1.419e+02 < 2e-16 \*\*\*

comp\_env 3 1356.9 452.3 1.006e+06 < 2e-16 \*\*\*

comp\_evol:tank 2 0.0 0.0 5.712e+00 0.0034 \*\*

comp\_evol:comp\_env 3 0.0 0.0 4.089e+00 0.0067 \*\*

comp\_evol:tank:clone 2 0.0 0.0 2.939e+01 3.51e-13 \*\*\*

comp\_evol:tank:comp\_env 6 0.0 0.0 1.650e-01 0.9860

comp\_evol:tank:clone:comp\_env 6 0.0 0.0 8.470e-01 0.5339

Residuals 1176 0.5 0.0

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Signif. codes: 0 ‘\*\*\*’ 0.001 ‘\*\*’ 0.01 ‘\*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Table 4: 2-way ANOVA D. pulex where comp\_evol is the tank environment the focal clone was isolated from and comp\_env is the grazing treatment (alone, intra-clonal, inter-clonal, or inter-specific)

Graphical user interface, chart

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Figure : Box plot displaying overall C24 value distributions for both species (left: D. pulex; right: D. magna). The center bold line denotes the median C24 value, the box edges denote the quartile ranges, and the top and bottom bars denote the min and max C24 Values.

**Conclusions**

*Daphnia* are considered to be organisms with genomic features that are particularly flexible for rapid evolution in response to changing environments (Figure 2; Colbourne et al. 2011). We expect *D. magna* and *D*. *pulex* to experience adaptive evolutionary responses to nutrient competition treatments, even over the rapid time scale of one year (Pantel et al. 2015). The goal of this research project was to observe the differences in grazing patterns between populations who spent one year’s time in either isolated single-species tanks in mixed-species tanks. To do this, we isolated clones from both environments and performed a common garden experiment to purge the clones of any maternal effects. From here we put them through a 24-hour grazing experiment to detect any differences in grazing rates. We evaluated grazing rates by measuring algae concentration after 24 hours in the grazing arena. In order to better understand how to predict final algae concentrations, and whether evolved genetic variation affects algal concentration, I simulated the grazing experiment using handling times that varied based on the *Daphnia*’s previous competitive history.

I was successfully able to model realistic levels of algal concentrations expected in the accompanying grazing experiment, using the Hollings Type II feeding rates (Holling 1959). Although this response model has been used for grazers in some theoretical studies (Schenone et al. 2021), some empirical studies have shown evidence for a Type III response (Sarnelle & Wilson 2008) while others have observed the Type II response (Porter et al. 1983). The simulation I have developed can be easily modified to model this functional response (Appendix). Another interesting approach would be to design the grazing experiment and statistical model that explicitly determines the type of functional response observed (Coblentz & DeLong 2021).

For the simulation I chose various handling times based on how we predict *Daphnia* will react as a function of their previous competitive environments. Because handling time represents an individual’s ability to find and process prey, we hypothesized that species sourced from heterospecific, mixed-species environments would evolve to have a lower handling time. Handling time includes the time it takes for the predator to find prey, consume the prey, and prepare to repeat this process. Under competition stress, predators may evolve to complete this process quicker, to ensure an adequate prey intake. However, it is not actually known whether it takes longer to find prey when there is competition, and it is important to consider how well the handling rates we chose for the simulation compare to nature. There’s a lack of clear consensus regarding *Daphnia*’s various handling times, and whether they evolve. Experimental research has been done regarding *Daphnia*’s feeding parameters. It has been hypothesized that there are 3 processes (feeding currents, food collection, and transportation to mouth) that contribute to *Daphnia*’s selective feeding, with the probability of ingestion relying on the likelihood of encountering, pursuing, capturing, and ingesting the prey (Hartmann & Kunkel 1991). A more holistic understanding of *Daphnia* handling times would be helpful to consider further how these rates can evolve under various competition environments.

Some of the model parameters were chosen to reflect actual values used in the grazing experiment (i.e. volume, c0), but the fixed values for attack rate and the genetic variation in the values for handling time represent hypotheses (that await subsequent comparison with experimental data). In this research project, I chose to model a known level of variation due to the handling rate of the focal clone and of the reference clone, and due to among-replicate variation. These all contribute to overall variation in the model-produced c24 values. The exercise was to then determine whether the true sources of variance I included in the simulation were subsequently captured by the ANOVA statistical model. The ANOVA results accurately indicated that final concentration values were driven by the environment clones evolved in (their treatments in the mesocosms), the environment they compete in (alone, intra-clonal, inter-clonal, interspecific), and the interaction of these two factors. The statistical model thus correctly captures that evolved changes in handling time, of the magnitude included in the simulation, can be detected among populations. Of course, it would be critical to better understand the effects of different levels of variance due to this factor that could still be detected by the ANOVA (a power analysis: Green & MacLeod 2016). By including variation among the 8 competition treatments, we also consider phenotypic plasticity. Phenotypic plasticity is an organism’s ability to change based on environmental stimuli. This often causes variation in the interactions between the organism and its environment, which may affect ecological organization through interactions such as competition or predation (Miner et al. 2005). There is evidence that *Daphnia* have shown plasticity in their grazing ability in response to low concentrations of food (Lampert 1994), but it is not known how intra and interspecific competition may impact this.

The laboratory grazing experiment considered 5 different algal species, including the low-nutrition toxic blue-green algae, Cyanobacteria. This simulation only considers grazing habits in terms of the concentration of algae consumed in 24 hours but does not vary in aspects such as whether either species of *Daphnia* preferred particular species of algae, and whether these preferences would lead to a greater likelihood for coexistence. The laboratory grazing experiment, and thus my simulation, also focused on tanks with low nutrient levels. In the mesocosm environments with eutrophic (high) nutrient levels, lower algal diversity (and thus fewer types of food resources) and blooms of toxic cyanobacteria are more likely to occur (Anderson et al. 2002). Interestingly, *Daphnia* have been previously shown to adapt to the presence of toxic cyanobacteria (Hairston et al. 1999), and *D.* *pulex* and *D.* *magna* have been shown to have different tolerances for poor quality food resources (Bengtsson 1987). In the simulation, we could potentially account for these differences by tracking not only the overall concentration of algae, but also varying the attack rate or handling time of *D.* *magna* or *D*. *pulex*, depending on the algal species they differentially prefer or tolerate.

There is existing evidence for the evolution of *Daphnia* grazing rates (Park & Post 2017). After taking two populations of *Daphnia*, one from lakes with anadromous predatory fish (alewife) and the other from landlocked lakes (with genetically distinct alewife populations) and performing a 24-hour grazing experiment, they found that the two populations with divergent life histories had significantly different consumption patterns. Although the results of the grazing experiment conducted in the summer of 2021 are not yet available, we expect that *Daphnia* sourced from mixed-species tanks will have different grazing patterns than those sourced from single-species tanks. More specifically, we expect that *Daphnia* that come from a competitive environment will adapt to graze in a way that maximizes their survival. This may be observed either in more rapid handling times, or in divergent preferences of algal resources among competitors (i.e. niche partitioning, Barabàs et al. 2018). Our expected results, combined with Park & Post (2017), should suggest that rapid adaptation is likely to be one of the responses organisms show to variation in the selection pressures imposed by their environment.



**Acknowledgments**

There are many people I want to thank for their contributions to this project. Firstly, I would like to extend a huge thank you to my supervisor, Jelena Pantel, who gave me this opportunity and provided both guidance and encouragement through the entirety of the project. And to who, in tandem with Lutz Beck at the University of Konstanz, designed the mesocosm and grazing experiment I was lucky enough to help on. I’d like to thank Alayna Amrein and Lynn Elhadjali for their work in setting up the mesocosm experiment. Next, I would like to thank my teammates Sara Zientek, Malia Elder, and Carolina Almaraz whose help in the common garden experiment was paramount. And finally, to everyone at the Limnological Institute who made this research possible, Pia, Angelica, etc. Thank you, I could not write this without all your help.

**Appendix**

## type II feeding rate equation variables. Variation per each clone.

#handling time for conspecific treatments and dp/dm alone DEPENDING on tank

h\_dm\_only <- .1

h\_dp\_only<- .05

#handling time for heterospecific treatments DEPENDING on tank

h\_dm <- 0.09

h\_dp <- 0.049

h\_dp\_4a <- .05 + rnorm(1, sd=.001)

h\_dp\_4b <- .05 + rnorm(1, sd=.001)

h\_dp\_4c <- .05 + rnorm(1, sd=.001)

h\_dm\_3a <- .1 + rnorm(1, sd=.001)

h\_dm\_3b <- .1 + rnorm(1, sd=.001)

h\_dm\_3c <- .1 + rnorm(1, sd=.001)

#consistent for all clones

d\_attack <- 0.8 ## JHP: edited

density <- 9.72 ## JHP: 9.72 micrograms / L

##algae concentration after 24 hours variables

t <-24

vol <- 40

#function to determine algae concentration after 24 hours

alg\_conc <- function(d\_handling,d\_attack,density,t,vol){

feed\_rate <- ((d\_attack\*density)/(1+(d\_handling\*d\_attack\*density)))

c24 <- ((-(feed\_rate\*t)/vol)+density)

conc<- list(c24=c24)

return(conc)

}

alg\_conc\_comp <- function(d\_handling,d\_attack,density,t,vol,d\_handling\_ref){

feed\_rate <- ((d\_attack\*density)/(1+(d\_handling\*d\_attack\*density)))

feed\_rate\_ref <- ((d\_attack\*density)/(1+(d\_handling\_ref\*d\_attack\*density)))

c24 <- ((-((feed\_rate+feed\_rate\_ref)\*t)/vol)+density)

conc<- list(c24=c24)

return(conc)

}

#Setting up the data frame

# dat <- array(NA,dim=c(1600,8),dimnames=list(NULL,c("tank","tank\_trt","clone","trt\_graze","focal\_sp","rep","handling\_time","co","c24"))) ## JHP: dimensions off

dat <- array(NA,dim=c(1600,9),dimnames=list(NULL,c("tank","tank\_trt","clone","trt\_graze","focal\_sp","rep","handling\_time","co","c24")))

dat <- as.data.frame(dat)

dat$tank\_trt<-c(rep('low\_dp',40),rep('high\_dp\_dm',160),rep('high\_dp',80),rep('high\_dp\_dm',80),rep('low\_dm',40),rep('high\_dm',40),rep('low\_dp\_dm',80),rep('low\_dm',40),rep('high\_dp\_dm',80),rep('high\_dm',40), rep('high\_dp',40),rep('low\_dm',40),rep('low\_dp\_dm',80),rep('low\_dm',40),rep('high\_dm',40),rep('low\_dp',80),rep('low\_dp\_dm',80),rep('low\_dm',40),rep('high\_dm',40),rep('high\_dp\_dm',80),rep('high\_dp',40),rep("low\_dp\_dm",80),rep('low\_dp',80),rep('high\_dp',40),rep('high\_dm',40),rep('low\_dp\_dm',80))

dat$tank<-c(rep(1,40),rep(2,80),rep(3,80),rep(4,40),rep(5,40),rep(6,80),rep(7,40),rep(8,40),rep(9,80),rep(10,40),rep(11,80),rep(12,40),rep(13,40),rep(14,40),rep(15,80),rep(16,40),rep(17,40),rep(18,40),rep(19,40),rep(20,80),rep(21,40),rep(22,40),rep(23,80),rep(24,40),rep(25,80),rep(26,40),rep(27,40),rep(28,40),rep(29,40),rep(30,80))

dat$trt\_graze <- rep(c('1','2','3a','3b','3c','4a','4b','4c'),200)

dat$handling\_time<- c(rep(h\_dp\_only + rnorm(1, sd=.001),8),rep(h\_dp\_only + rnorm(1, sd=.001),8),rep(h\_dp\_only + rnorm(1, sd=.001),8),rep(h\_dp\_only + rnorm(1, sd=.001),8),rep(h\_dp\_only + rnorm(1, sd=.001),8),

rep(h\_dp + rnorm(1, sd=.001),8),rep(h\_dp + rnorm(1, sd=.001),8),rep(h\_dp + rnorm(1, sd=.001),8),rep(h\_dp + rnorm(1, sd=.001),8),rep(h\_dp + rnorm(1, sd=.001),8),

rep(h\_dm + rnorm(1, sd=.001),8),rep(h\_dm + rnorm(1, sd=.001),8),rep(h\_dm + rnorm(1, sd=.001),8),rep(h\_dm + rnorm(1, sd=.001),8),rep(h\_dm + rnorm(1, sd=.001),8),

rep(h\_dp + rnorm(1, sd=.001),8),rep(h\_dp + rnorm(1, sd=.001),8),rep(h\_dp + rnorm(1, sd=.001),8),rep(h\_dp + rnorm(1, sd=.001),8),rep(h\_dp + rnorm(1, sd=.001),8),

rep(h\_dm + rnorm(1, sd=.001),8),rep(h\_dm + rnorm(1, sd=.001),8),rep(h\_dm + rnorm(1, sd=.001),8),rep(h\_dm + rnorm(1, sd=.001),8),rep(h\_dm + rnorm(1, sd=.001),8),

rep(h\_dp\_only + rnorm(1, sd=.001),8),rep(h\_dp\_only + rnorm(1, sd=.001),8),rep(h\_dp\_only + rnorm(1, sd=.001),8),rep(h\_dp\_only + rnorm(1, sd=.001),8),rep(h\_dp\_only + rnorm(1, sd=.001),8),

rep(h\_dp\_only + rnorm(1, sd=.001),8),rep(h\_dp\_only + rnorm(1, sd=.001),8),rep(h\_dp\_only + rnorm(1, sd=.001),8),rep(h\_dp\_only + rnorm(1, sd=.001),8),rep(h\_dp\_only + rnorm(1, sd=.001),8),

rep(h\_dp + rnorm(1, sd=.001),8),rep(h\_dp + rnorm(1, sd=.001),8),rep(h\_dp + rnorm(1, sd=.001),8),rep(h\_dp + rnorm(1, sd=.001),8),rep(h\_dp + rnorm(1, sd=.001),8),

rep(h\_dm + rnorm(1, sd=.001),8),rep(h\_dm + rnorm(1, sd=.001),8),rep(h\_dm + rnorm(1, sd=.001),8),rep(h\_dm + rnorm(1, sd=.001),8),rep(h\_dm + rnorm(1, sd=.001),8),

rep(h\_dm\_only + rnorm(1, sd=.001),8),rep(h\_dm\_only + rnorm(1, sd=.001),8),rep(h\_dm\_only + rnorm(1, sd=.001),8),rep(h\_dm\_only + rnorm(1, sd=.001),8),rep(h\_dm\_only + rnorm(1, sd=.001),8),

rep(h\_dm\_only + rnorm(1, sd=.001),8),rep(h\_dm\_only + rnorm(1, sd=.001),8),rep(h\_dm\_only + rnorm(1, sd=.001),8),rep(h\_dm\_only + rnorm(1, sd=.001),8),rep(h\_dm\_only + rnorm(1, sd=.001),8),

rep(h\_dp + rnorm(1, sd=.001),8),rep(h\_dp + rnorm(1, sd=.001),8),rep(h\_dp + rnorm(1, sd=.001),8),rep(h\_dp + rnorm(1, sd=.001),8),rep(h\_dp + rnorm(1, sd=.001),8),

rep(h\_dm + rnorm(1, sd=.001),8),rep(h\_dm + rnorm(1, sd=.001),8),rep(h\_dm + rnorm(1, sd=.001),8),rep(h\_dm + rnorm(1, sd=.001),8),rep(h\_dm + rnorm(1, sd=.001),8),

rep(h\_dm\_only + rnorm(1, sd=.001),8),rep(h\_dm\_only + rnorm(1, sd=.001),8),rep(h\_dm\_only + rnorm(1, sd=.001),8),rep(h\_dm\_only + rnorm(1, sd=.001),8),rep(h\_dm\_only + rnorm(1, sd=.001),8),

rep(h\_dp + rnorm(1, sd=.001),8),rep(h\_dp + rnorm(1, sd=.001),8),rep(h\_dp + rnorm(1, sd=.001),8),rep(h\_dp + rnorm(1, sd=.001),8),rep(h\_dp + rnorm(1, sd=.001),8),

rep(h\_dm + rnorm(1, sd=.001),8),rep(h\_dm + rnorm(1, sd=.001),8),rep(h\_dm + rnorm(1, sd=.001),8),rep(h\_dm + rnorm(1, sd=.001),8),rep(h\_dm + rnorm(1, sd=.001),8),

rep(h\_dm\_only + rnorm(1, sd=.001),8),rep(h\_dm\_only + rnorm(1, sd=.001),8),rep(h\_dm\_only + rnorm(1, sd=.001),8),rep(h\_dm\_only + rnorm(1, sd=.001),8),rep(h\_dm\_only + rnorm(1, sd=.001),8),

rep(h\_dp\_only + rnorm(1, sd=.001),8),rep(h\_dp\_only + rnorm(1, sd=.001),8),rep(h\_dp\_only + rnorm(1, sd=.001),8),rep(h\_dp\_only + rnorm(1, sd=.001),8),rep(h\_dp\_only + rnorm(1, sd=.001),8),

rep(h\_dm\_only + rnorm(1, sd=.001),8),rep(h\_dm\_only + rnorm(1, sd=.001),8),rep(h\_dm\_only + rnorm(1, sd=.001),8),rep(h\_dm\_only + rnorm(1, sd=.001),8),rep(h\_dm\_only + rnorm(1, sd=.001),8),

rep(h\_dp + rnorm(1, sd=.001),8),rep(h\_dp + rnorm(1, sd=.001),8),rep(h\_dp + rnorm(1, sd=.001),8),rep(h\_dp + rnorm(1, sd=.001),8),rep(h\_dp + rnorm(1, sd=.001),8),

rep(h\_dm + rnorm(1, sd=.001),8),rep(h\_dm + rnorm(1, sd=.001),8),rep(h\_dm + rnorm(1, sd=.001),8),rep(h\_dm + rnorm(1, sd=.001),8),rep(h\_dm + rnorm(1, sd=.001),8),

rep(h\_dm\_only + rnorm(1, sd=.001),8),rep(h\_dm\_only + rnorm(1, sd=.001),8),rep(h\_dm\_only + rnorm(1, sd=.001),8),rep(h\_dm\_only + rnorm(1, sd=.001),8),rep(h\_dm\_only + rnorm(1, sd=.001),8),

rep(h\_dm\_only + rnorm(1, sd=.001),8),rep(h\_dm\_only + rnorm(1, sd=.001),8),rep(h\_dm\_only + rnorm(1, sd=.001),8),rep(h\_dm\_only + rnorm(1, sd=.001),8),rep(h\_dm\_only + rnorm(1, sd=.001),8),

rep(h\_dp\_only + rnorm(1, sd=.001),8),rep(h\_dp\_only + rnorm(1, sd=.001),8),rep(h\_dp\_only + rnorm(1, sd=.001),8),rep(h\_dp\_only + rnorm(1, sd=.001),8),rep(h\_dp\_only + rnorm(1, sd=.001),8),

rep(h\_dp\_only + rnorm(1, sd=.001),8),rep(h\_dp\_only + rnorm(1, sd=.001),8),rep(h\_dp\_only + rnorm(1, sd=.001),8),rep(h\_dp\_only + rnorm(1, sd=.001),8),rep(h\_dp\_only + rnorm(1, sd=.001),8),

rep(h\_dp + rnorm(1, sd=.001),8),rep(h\_dp + rnorm(1, sd=.001),8),rep(h\_dp + rnorm(1, sd=.001),8),rep(h\_dp + rnorm(1, sd=.001),8),rep(h\_dp + rnorm(1, sd=.001),8),

rep(h\_dm + rnorm(1, sd=.001),8),rep(h\_dm + rnorm(1, sd=.001),8),rep(h\_dm + rnorm(1, sd=.001),8),rep(h\_dm + rnorm(1, sd=.001),8),rep(h\_dm + rnorm(1, sd=.001),8),

rep(h\_dm\_only + rnorm(1, sd=.001),8),rep(h\_dm\_only + rnorm(1, sd=.001),8),rep(h\_dm\_only + rnorm(1, sd=.001),8),rep(h\_dm\_only + rnorm(1, sd=.001),8),rep(h\_dm\_only + rnorm(1, sd=.001),8),

rep(h\_dm\_only + rnorm(1, sd=.001),8),rep(h\_dm\_only + rnorm(1, sd=.001),8),rep(h\_dm\_only + rnorm(1, sd=.001),8),rep(h\_dm\_only + rnorm(1, sd=.001),8),rep(h\_dm\_only + rnorm(1, sd=.001),8),

rep(h\_dp + rnorm(1, sd=.001),8),rep(h\_dp + rnorm(1, sd=.001),8),rep(h\_dp + rnorm(1, sd=.001),8),rep(h\_dp + rnorm(1, sd=.001),8),rep(h\_dp + rnorm(1, sd=.001),8),

rep(h\_dm + rnorm(1, sd=.001),8),rep(h\_dm + rnorm(1, sd=.001),8),rep(h\_dm + rnorm(1, sd=.001),8),rep(h\_dm + rnorm(1, sd=.001),8),rep(h\_dm + rnorm(1, sd=.001),8),

rep(h\_dp\_only + rnorm(1, sd=.001),8),rep(h\_dp\_only + rnorm(1, sd=.001),8),rep(h\_dp\_only + rnorm(1, sd=.001),8),rep(h\_dp\_only + rnorm(1, sd=.001),8),rep(h\_dp\_only + rnorm(1, sd=.001),8),

rep(h\_dp + rnorm(1, sd=.001),8),rep(h\_dp + rnorm(1, sd=.001),8),rep(h\_dp + rnorm(1, sd=.001),8),rep(h\_dp + rnorm(1, sd=.001),8),rep(h\_dp + rnorm(1, sd=.001),8),

rep(h\_dm + rnorm(1, sd=.001),8),rep(h\_dm + rnorm(1, sd=.001),8),rep(h\_dm + rnorm(1, sd=.001),8),rep(h\_dm + rnorm(1, sd=.001),8),rep(h\_dm + rnorm(1, sd=.001),8),

rep(h\_dp\_only + rnorm(1, sd=.001),8),rep(h\_dp\_only + rnorm(1, sd=.001),8),rep(h\_dp\_only + rnorm(1, sd=.001),8),rep(h\_dp\_only + rnorm(1, sd=.001),8),rep(h\_dp\_only + rnorm(1, sd=.001),8),

rep(h\_dp\_only + rnorm(1, sd=.001),8),rep(h\_dp\_only + rnorm(1, sd=.001),8),rep(h\_dp\_only + rnorm(1, sd=.001),8),rep(h\_dp\_only + rnorm(1, sd=.001),8),rep(h\_dp\_only + rnorm(1, sd=.001),8),

rep(h\_dp\_only + rnorm(1, sd=.001),8),rep(h\_dp\_only + rnorm(1, sd=.001),8),rep(h\_dp\_only + rnorm(1, sd=.001),8),rep(h\_dp\_only + rnorm(1, sd=.001),8),rep(h\_dp\_only + rnorm(1, sd=.001),8),

rep(h\_dm\_only + rnorm(1, sd=.001),8),rep(h\_dm\_only + rnorm(1, sd=.001),8),rep(h\_dm\_only + rnorm(1, sd=.001),8),rep(h\_dm\_only + rnorm(1, sd=.001),8),rep(h\_dm\_only + rnorm(1, sd=.001),8),

rep(h\_dp + rnorm(1, sd=.001),8),rep(h\_dp + rnorm(1, sd=.001),8),rep(h\_dp + rnorm(1, sd=.001),8),rep(h\_dp + rnorm(1, sd=.001),8),rep(h\_dp + rnorm(1, sd=.001),8),

rep(h\_dm + rnorm(1, sd=.001),8),rep(h\_dm + rnorm(1, sd=.001),8),rep(h\_dm + rnorm(1, sd=.001),8),rep(h\_dm + rnorm(1, sd=.001),8),rep(h\_dm + rnorm(1, sd=.001),8))

dat$clone<- rep(1:5, each=8)

dat$rep<-rep(1,1600)

dat$focal\_sp[dat$tank\_trt == 'low\_dp'] <- 'dp'

dat$focal\_sp[dat$tank\_trt == 'high\_dp'] <- 'dp'

dat$focal\_sp[dat$tank\_trt == 'low\_dm'] <- 'dm'

dat$focal\_sp[dat$tank\_trt == 'high\_dm'] <- 'dm'

dat$focal\_sp[dat$tank\_trt == 'low\_dp\_dm'] <- rep(c('dp','dm'),each=40)

dat$focal\_sp[dat$tank\_trt == 'high\_dp\_dm'] <- rep(c('dp','dm'),each=40)

dat$co <- rep(density,1600)

##C24 values will depend on which focal clone is present, what species that focal clone is (Dm or Dp), and thus what clone reference set 3a-c vs. 4a-c consists of

#\*\*\*\*In this version of the code Daphnia from low and high treamtents are identical\*\*\*\*#

for(i in 1:nrow(dat)){

if(dat$trt\_graze[i] == "1"){

xx <- alg\_conc(dat$handling\_time[i],d\_attack,density,t,vol)

dat$c24[i] <- xx$c24

} else if (dat$trt\_graze[i] == "2"){

xx <- alg\_conc\_comp(dat$handling\_time[i],d\_attack,density,t,vol,dat$handling\_time[i])

dat$c24[i] <- xx$c24

} else if (dat$trt\_graze[i] == "3a"){

xx <- alg\_conc\_comp(dat$handling\_time[i],d\_attack,density,t,vol,h\_dm\_3a)

dat$c24[i] <- xx$c24

} else if (dat$trt\_graze[i] == "3b"){

xx <- alg\_conc\_comp(dat$handling\_time[i],d\_attack,density,t,vol,h\_dm\_3b)

dat$c24[i] <- xx$c24

} else if (dat$trt\_graze[i] == "3c"){

xx <- alg\_conc\_comp(dat$handling\_time[i],d\_attack,density,t,vol,h\_dm\_3c)

dat$c24[i] <- xx$c24

} else if (dat$trt\_graze[i] == "4a"){

xx <- alg\_conc\_comp(dat$handling\_time[i],d\_attack,density,t,vol,h\_dp\_4a)

dat$c24[i] <- xx$c24

} else if (dat$trt\_graze[i] == "4b"){

xx <- alg\_conc\_comp(dat$handling\_time[i],d\_attack,density,t,vol,h\_dp\_4b)

dat$c24[i] <- xx$c24

} else{

xx <- alg\_conc\_comp(dat$handling\_time[i],d\_attack,density,t,vol,h\_dp\_4c)

dat$c24[i] <- xx$c24

}

}

#Setting up the data frame with all 3 reps

#dat2 <- array(NA,dim=c(1600,8),dimnames=list(NULL,c("tank","tank\_trt","clone","trt\_graze","focal\_sp","rep","co","c24")))

dat2 <- array(NA,dim=c(1600,9),dimnames=list(NULL,c("tank","tank\_trt","clone","trt\_graze","focal\_sp","rep","handling\_time","co","c24")))

dat2 <- as.data.frame(dat2) ## JHP: Is this supposed to be dat2?

dat2$tank\_trt<-c(rep('low\_dp',40),rep('high\_dp\_dm',160),rep('high\_dp',80),rep('high\_dp\_dm',80),rep('low\_dm',40),rep('high\_dm',40),rep('low\_dp\_dm',80),rep('low\_dm',40),rep('high\_dp\_dm',80),rep('high\_dm',40), rep('high\_dp',40),rep('low\_dm',40),rep('low\_dp\_dm',80),rep('low\_dm',40),rep('high\_dm',40),rep('low\_dp',80),rep('low\_dp\_dm',80),rep('low\_dm',40),rep('high\_dm',40),rep('high\_dp\_dm',80),rep('high\_dp',40),rep("low\_dp\_dm",80),rep('low\_dp',80),rep('high\_dp',40),rep('high\_dm',40),rep('low\_dp\_dm',80))

dat2$tank<-c(rep(1,40),rep(2,80),rep(3,80),rep(4,40),rep(5,40),rep(6,80),rep(7,40),rep(8,40),rep(9,80),rep(10,40),rep(11,80),rep(12,40),rep(13,40),rep(14,40),rep(15,80),rep(16,40),rep(17,40),rep(18,40),rep(19,40),rep(20,80),rep(21,40),rep(22,40),rep(23,80),rep(24,40),rep(25,80),rep(26,40),rep(27,40),rep(28,40),rep(29,40),rep(30,80))

dat2$trt\_graze <- rep(c('1','2','3a','3b','3c','4a','4b','4c'),200)

dat2$clone<- rep(1:5, each=8)

dat2$rep<-rep(2,1600)

dat2$focal\_sp[dat$tank\_trt == 'low\_dp'] <- 'dp'

dat2$focal\_sp[dat$tank\_trt == 'high\_dp'] <- 'dp'

dat2$focal\_sp[dat$tank\_trt == 'low\_dm'] <- 'dm'

dat2$focal\_sp[dat$tank\_trt == 'high\_dm'] <- 'dm'

dat2$focal\_sp[dat$tank\_trt == 'low\_dp\_dm'] <- rep(c('dp','dm'),each=40)

dat2$focal\_sp[dat$tank\_trt == 'high\_dp\_dm'] <- rep(c('dp','dm'),each=40)

#dat2$co <- rep(24000,1600)

dat2$co <- rep(density,1600)

#I added random noise based on the the final column of rep1

## So its dat, then dat1, dat2, dat3 as the 3 reps.

dat2$c24 <- dat$c24 + rnorm(1, sd=.01)

#Setting up the data frame with all 3 reps

dat3 <- array(NA,dim=c(1600,8),dimnames=list(NULL,c("tank","tank\_trt","clone","trt\_graze","focal\_sp","rep","co","c24")))

dat3 <- as.data.frame(dat)

dat3$tank\_trt<-c(rep('low\_dp',40),rep('high\_dp\_dm',160),rep('high\_dp',80),rep('high\_dp\_dm',80),rep('low\_dm',40),rep('high\_dm',40),rep('low\_dp\_dm',80),rep('low\_dm',40),rep('high\_dp\_dm',80),rep('high\_dm',40), rep('high\_dp',40),rep('low\_dm',40),rep('low\_dp\_dm',80),rep('low\_dm',40),rep('high\_dm',40),rep('low\_dp',80),rep('low\_dp\_dm',80),rep('low\_dm',40),rep('high\_dm',40),rep('high\_dp\_dm',80),rep('high\_dp',40),rep("low\_dp\_dm",80),rep('low\_dp',80),rep('high\_dp',40),rep('high\_dm',40),rep('low\_dp\_dm',80))

dat3$tank<-c(rep(1,40),rep(2,80),rep(3,80),rep(4,40),rep(5,40),rep(6,80),rep(7,40),rep(8,40),rep(9,80),rep(10,40),rep(11,80),rep(12,40),rep(13,40),rep(14,40),rep(15,80),rep(16,40),rep(17,40),rep(18,40),rep(19,40),rep(20,80),rep(21,40),rep(22,40),rep(23,80),rep(24,40),rep(25,80),rep(26,40),rep(27,40),rep(28,40),rep(29,40),rep(30,80))

dat3$trt\_graze <- rep(c('1','2','3a','3b','3c','4a','4b','4c'),200)

dat3$clone<- rep(1:5, each=8)

dat3$rep<-rep(3,1600)

dat3$focal\_sp[dat$tank\_trt == 'low\_dp'] <- 'dp'

dat3$focal\_sp[dat$tank\_trt == 'high\_dp'] <- 'dp'

dat3$focal\_sp[dat$tank\_trt == 'low\_dm'] <- 'dm'

dat3$focal\_sp[dat$tank\_trt == 'high\_dm'] <- 'dm'

dat3$focal\_sp[dat$tank\_trt == 'low\_dp\_dm'] <- rep(c('dp','dm'),each=40)

dat3$focal\_sp[dat$tank\_trt == 'high\_dp\_dm'] <- rep(c('dp','dm'),each=40)

dat3$co <- rep(24000,1600)

#I added random noise based on the the final column of rep1

dat3$c24 <- dat$c24 + rnorm(1, sd=.01)

final<-cbind(dat,dat2,dat3)

## First, use only the low nutrient tanks

sub <- dat[dat$tank\_trt == "low\_dp" | dat$tank\_trt == "low\_dm" | dat$tank\_trt == "low\_dp\_dm",]

sub <- rbind(sub,dat2[dat2$tank\_trt == "low\_dp" | dat2$tank\_trt == "low\_dm" | dat2$tank\_trt == "low\_dp\_dm",])

sub <- rbind(sub,dat3[dat3$tank\_trt == "low\_dp" | dat3$tank\_trt == "low\_dm" | dat3$tank\_trt == "low\_dp\_dm",])

## A boxplot showing the overall distributions of the c24 values across grazing treatments.

boxplot(c24 ~ trt\_graze,data=sub)

## Divide analysis by species

sub\_dp <- sub[sub$focal\_sp == "dp",]

sub\_dm <- sub[sub$focal\_sp == "dm",]

par(mfrow=c(1,2))

colors = c(rep("#88CCEE",1),rep("#117733",1),rep("#44AA99",3),rep("#332288",3))

boxplot(c24 ~ trt\_graze, data=sub\_dp, ylim=c(3,7), main="Final Concentration of Algae After 24 Hours",names=c("1","2","3a","3b","3c","4a","4b","4c")

,xlab="Grazing Treatment Focal Species D. Pulex",ylab="Algea Concentration at 24h",las=2, cex.lab=.80, cex.axis=.70, border=colors)

legend("topright", legend = c("No competition", "Intra-clonal Competition", "Inter-clonal Competition", "Interspecific Competition"),

col = c("#88CCEE", "#117733", "#44AA99", "#332288"), pch=20, cex=.75)

boxplot(c24 ~ trt\_graze, data=sub\_dm, ylim=c(3,7), xlab="Grazing Treatment Focal Species D. Magna", main="Final Concentration of Algae After 24 Hours",

names=c("1","2","3a","3b","3c","4a","4b","4c"), ylab="Algea Concentration at 24h",par(las=3), cex.lab=.80, cex.axis=.70, border=colors)

legend("topright", legend = c("No competition", "Intra-clonal Competition", "Inter-clonal Competition", "Interspecific Competition"),

col = c("#88CCEE", "#117733", "#44AA99", "#332288"), pch=20, cex=.75)

## Analysis of variance - what factors contribute to c24 values?

## Recoded the data to have factors

## First, we rename the environments that focal clones evolved in 'comp\_evol'. Focal clones either evolved in intraspecific or interspecific competitiom environments.

sub\_dp$comp\_evol <- NA

sub\_dp$comp\_evol[sub\_dp$tank\_trt == "low\_dp"] <- "intra"

sub\_dp$comp\_evol[sub\_dp$tank\_trt == "low\_dp\_dm"] <- "inter"

## Second, we rename the grazing experiment treatments as comp\_env - this is the competition environment they were assayed in - either alone, with the same clone, with other clones of the same species, or with the other species.

sub\_dp$comp\_env <- NA

sub\_dp$comp\_env[sub\_dp$trt\_graze == "1"] <- "none"

sub\_dp$comp\_env[sub\_dp$trt\_graze == "2"] <- "intra\_clone"

sub\_dp$comp\_env[sub\_dp$trt\_graze == "4a" | sub\_dp$trt\_graze == "4b" | sub\_dp$trt\_graze == "4c"] <- "inter\_clone" # Because references clones 4a, 4b, and 4c are D. pulex

sub\_dp$comp\_env[sub\_dp$trt\_graze == "3a" | sub\_dp$trt\_graze == "3b" | sub\_dp$trt\_graze == "3c"] <- "inter\_spec" # Because references clones 3a, 3b, and 3c are D. magna

## We construct our linear model. We have 2 main factors that can drive c24 values - the tank competition environment (the clones were either drawn from tanks with only members of the same species, or in the presence of the 2nd species) which I code as "comp\_evol", and the competition environment in the grazing experiment itself (grazing in a bottle alone / "none", grazing in a bottle with another individual of the same clone / "intra\_clone", grazing in a bottle with another individual of the same species from another clone / "inter\_clone", and grazing in a bottle with an individual from the other species / "inter\_spec"). We have knowledge that the clones aren't drawn randomly from the two comp\_evol treatments - instead, clones are nested within tanks, and tanks are nested within the two treatments. My lm (linear model) syntax reflects that.

mod <- aov(lm(c24 ~ comp\_evol/tank/clone \* comp\_env,data=sub\_dp))

summary(mod)

sub\_dm$comp\_evol <- NA

sub\_dm$comp\_evol[sub\_dm$tank\_trt == "low\_dm"] <- "intra"

sub\_dm$comp\_evol[sub\_dm$tank\_trt == "low\_dp\_dm"] <- "inter"

sub\_dm$comp\_env <- NA

sub\_dm$comp\_env[sub\_dm$trt\_graze == "1"] <- "none"

sub\_dm$comp\_env[sub\_dm$trt\_graze == "2"] <- "intra\_clone"

sub\_dm$comp\_env[sub\_dm$trt\_graze == "4a" | sub\_dm$trt\_graze == "4b" | sub\_dm$trt\_graze == "4c"] <- "inter\_clone" # Because references clones 4a, 4b, and 4c are D. pulex

sub\_dm$comp\_env[sub\_dm$trt\_graze == "3a" | sub\_dm$trt\_graze == "3b" | sub\_dm$trt\_graze == "3c"] <- "inter\_spec" # Because references clones 3a, 3b, and 3c are D. magna

mod <- aov(lm(c24 ~ comp\_evol/tank/clone \* comp\_env,data=sub\_dm))

summary(mod)

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