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Filtering efficiency and feeding mechanisms of Daphnia pulex on Microcystis aeruginosa and Nannochloropsis

Elizabeth Orlowicz
University of New Hampshire - Main Campus, ecs52@wildcats.unh.edu

Title: Filtering efficiency and feeding mechanisms of *Daphnia pulex* on *Microcystis aeruginosa* and *Nannochloropsis*

Author: Elizabeth Orlowicz[1]

[1] Department of Biological Sciences, University of New Hampshire, Durham, NH 03824

Abstract

The filtering and feeding rate of *Daphnia pulex* from the Old Durham Reservoir, Durham, NH, were measured to determine the feeding efficiency on different concentrations of non-toxic *Nannochloropsis* (Class Eustigmatophyceae) and toxigenic cyanobacteria *Microcystis aeruginosa*. Direct observations of thoracic appendage beats and post-abdominal rejections were also measured at $5*10^5$ and 10^6 cells mL^{-1} concentrations of *Nannochloropsis* and *M. aeruginosa* to examine the feeding mechanisms of the *D. pulex*. In the presence of increasing *Nannochloropsis* concentrations, the filtering rates decreased and the feeding rates increased. When exposed to *M. aeruginosa*, both the filtering and feeding rates decreased. The thoracic beats decreased and the post-abdominal rejections increased from $5*10^5$ and 10^6 cells mL^{-1} of both food types. Similarly, the thoracic beats decreased and post-abdominal rejections increased from food suspensions of *Nannochloropsis* to *M. aeruginosa*. These results indicate that when compared to *Nannochloropsis*, *M. aeruginosa* had a negative effect on the feeding rates over a wide range of food concentrations of the *Daphnia*. The lowered feeding rates are in part due to a reduction in collection rates (i.e. lower thoracic appendage beats) and an increase in the food rejection as evidenced by the higher post abdominal rejection rate.

Introduction

A bloom of toxic cyanobacteria in a lake can create a public health problem as well as pose a problem for the health of the lake by altering the zooplankton composition, since cladocerans are less adapted to feed on some cyanobacteria, while calanoids seem to be best adapted (Haney 1987, Fulton III *et al*. 1988). Some zooplankton, such as *Daphnia*, can consume cyanobacteria even in the presence of toxins. As a result, phytoplankton and cyanobacteria, such as *Nannochloropsis* and *M. aeruginosa*, form the basis of many trophic pathways in lakes. *Daphnia*, therefore, provide a critical role in connecting the phytoplankton to upper trophic levels and in controlling of the phytoplankton composition and concentration in a lake (Lampert 1987 Feeding, Burns 1968, DeMott 1991). The blooms, therefore, can affect the whole lake ecosystem as well as higher trophic level organisms outside of the lake, such as humans. The toxins produced by cyanobacteria can cause liver damage, liver cancer, as well as neurotoxicity, which pose a great risk to humans, who could potentially consume these toxins through drinking lake water or by consuming fish that have lived in a toxic lake (Hitzfeld *et al.* 2000).

Herbivorous cladocerans create a filtering current by rhythmic pumping of their thoracic legs that draws water and any suspended food towards the mandibles and mouth where it is digested (Downing *et al.* 1984). The post-abdomen cleans out the food groove and filtering appendages with a quick thrust up and outwards, removing excess or undesirable material (Downing *et al.* 1984). Feeding can be disrupted by poor food source, algal cell toxicity, or an array of other physical factors such as temperature and light intensity (Forsyth *et al.* 1992, Haney *et al.* 1985, de Senerpont Domis *et al.* 2012). There is not much information available on the

effect of cell concentration on filtering and feeding rate, and the feeding mechanisms of cladocerans in suspensions of edible cyanobacteria.

Daphnia readily ingest phytoplankton that can be collected with their thoracic legs without impeding the feeding, and that are nutritious (Haney *et al.* 1985, DeMott *et al.* 1991). Within the size range of roughly 1 to 50 μm *Daphnia* are capable of ingesting different varieties of phytoplankton, which includes *Nannochloropsis* and *Microcystis aeruginosa*.

This study was designed to examine the feeding efficiency of *Daphnia pulex* from a local New Hampshire reservoir on *Nannochloropsis* and cultured *M. aeruginosa*. The phytoplankton were of similar size and shape, but with different taxonomic, nutritional, and chemical characteristics (*Microcystis* was toxigenic). The filtering and feeding rate of the *D. pulex* will give insight into their ability to feed on different bloom concentrations and compositions and further the understanding of the mechanism by which cyanobacteria, such as *M. aeruginosa*, alter *D. pulex* responses to different concentrations of phytoplankton.

Methods

Daphnia pulex from the Old Durham Reservoir in Durham, NH were kept in culture at 20°C in glass mason jars covered by black lids to prevent the animals from getting caught in the surface tension. Every few days, the *D. pulex* were fed *Nannochloropsis*. To minimize the variation in size, adults were removed by pipette to a new jar filled with filtered-aerated well water when a cohort of newly born *Daphnia* were present. Once *D. pulex* reached the adult stage (10 to 15 days), they were used in the experiment outlined below.

The *Nannochloropsis*, class Eustigmatophyceae, which was used, is a unicellular spherical marine phytoplankton that ranges in size from 2 to 4 μm (Baker *et al.* 2012). The cells used in this experiment were an average size of 3.39 μm (Table 1). It is commonly used as a food source for microcrustaceans because it has a rich storage of fatty acids (Baker *et al.* 2012).

The cultured *M. aeruginosa* (UTEX 2385) that was used had an average size of 4.53 μm (Table 1) (Trubetskova *et al.* 2003). In the natural environment, this cyanobacteria is generally collected as large colonies, but individual cells can be found in lakes when broken off from larger, colonial *Microcystis* colonies. *Microcystis aeruginosa* produces the toxin microcystin (MC) which is known as a hepatotoxin for vertebrates and can also reduce the growth rate and sometimes cause death in *Daphnia* (DeMott *et al.* 1991, Trubetskova *et al.* 2003). Potential causes of reduced growth and death are toxicity, poor nutritional value due to its poor lipid content, bad taste, and morphology (DeMott *et al.* 1991).

To create a calibration curve of *Nannochloropsis* and *Microcystis aeruginosa*, 50 mL suspensions of 10^4, 10^5, $5*10^5$, 10^6, $5*10^6$, and 10^7 cells mL^{-1} were placed in small mason jars with 20 *D. pulex* each. There were three replicates with *D. pulex* and three controls run during each experimental test. In addition, 3-mL Nalgene bottles were prepared for water samples. Once the stock solution was created, a sample of the initial concentration of the solution was removed into one of the Nalgene bottles and placed aside to be counted on the hemacytometer later. A 50 mL sample of stock concentration solution was poured into each jar and 20 *D. pulex* of the approximate same age and size were transferred by pipette to three of the jars. The time when the last *D. pulex* was placed into the jar was recorded. One hour after the *D. pulex* were place in the jar, the suspension in the jar was gently mixed with a pipette to re-suspend any settled food. Then a small sample of each jar was placed in a 3-mL Nalgene bottle to be later counted in a hemacytometer.

After the termination of the first experiment, six additional jars were prepared with 50 mL of a new concentration for another run of the procedure outlined above. The same *D. pulex* were used in the following experiment and were pipette from the previous suspension to the new suspension. The procedural steps were repeated until all concentrations had been tested.

Immediately after the experiment was terminated, five *D. pulex* individuals from each replicate were measured using an Olympus SZX16 scope with a mounted Sony HD CM05 Handycam. An image of 15 individuals and the stage micrometer was taken under the same magnification and ImageJ was used to determine the size of each individual. These sizes were averaged together and used to correct the filtering rate and feeding rate measurements to a 2 mm long *Daphnia* using the linear regression of body length versus filtering rate, assuming log filtering rate equals the body length raised to the 2.3 power.

The filtering rate (F) was calculated using the cell-count method outlined by Downing and Rigler (1984). Two cell counts were completed using a hemacytometer for each sample for a total minimum count of 50 cells. The average cell count was found for each and used in Gauld's (1951) filtering rate equation which is:

$$F=v*((\log C_o- \log C_t)/(0.4343*t))$$

where F is the filtering rate in mL per individual per hour, v is the volume of water per animal (mL), C_o is the concentration of the control vessel (cells per mL), C_t is the concentration of the corresponding *Daphnia* vessel (cells per mL), and t is the time of feeding, which in this case was one hour. Feeding rate (f), cells eaten per individual per hour, was measured using the equation below:

$$f=F*\text{average concentration during trial run}$$

Using this method, it is assumed that any cells passed through the gut undigested were not re-suspended. This method was used rather than a radioisotope method because the equipment required for this procedure was easily accessible, and it could easily be run over several days and multiple times.

A functional response curve was created by comparing food concentration with filtering rate and feeding rate of each different food type to visualize trends in satiation levels, plateauing levels, and incipient limiting concentration (ILC) as described by Downing and Rigler (1984). The ILC is the critical concentration that marks a turning point in a functional response curve, where grazing rate decreases with respect of food concentration (Downing 1984).

It should be noted that tests of each different concentration of each food suspension were run in series, but the *D. pulex* became very sluggish and most died after four or more runs of the experiment. The *Daphnia* did not seem to be responding to the amount of handling they were experiencing. To reduce this, only two to three concentrations were run in series and, rather than pouring the *Daphnia* from one concentration jar to the other, the individuals were transferred using a pipette to the newest jar. This method also helped to reduce the amount of suspension transferred from one concentration to the next.

During the test run with *M. aeruginosa*, there was a significant mortality of *Daphnia* in concentrations of $5*10^5$ and 10^6 cells mL^{-1}. Thus, thoracic beats (TB) and post-abdominal rejections (PAR) of the *Daphnia* were collected to examine the *Daphnia* individual's physical reactions. Three *D. pulex* individuals were secured by their dorsal side to three different thin lines of Vaseline in a Petri dish. A $5*10^5$ cell mL^{-1} *Nannochloropsis* suspension was gently transferred by pipette around the *Daphnia*, as to not remove the *Daphnia* from the Vaseline. The individuals were left in the suspension for about 1 minute to acclimate to the solution. Then, each individual's thoracic beats were counted three times for 10 seconds each, then post-

abdominal rejections were counted three times for 1 minute. This procedure was repeated for a 10^6 cell mL^{-1} *Nannochloropsis* solution, a sample of filtered-aerated well water, a $5*10^5$ cell mL^{-1} *M. aeruginosa* solution, and a 10^6 cell mL^{-1} *M. aeruginosa* solution, respectively. The thoracic beats per minute and post-abdominal rejections per minute were calculated and compared to the filtering rate and feeding rate.

Statistics were calculated using SigmaPlot version 12.3. The statistical significance ($p<0.05$) of the filtering and feeding rates for both *Nannochloropsis* and *M. aeruginosa* as well as thoracic beats and post-abdominal rejections between *Daphnia* individuals, between concentrations, and between different food types were tested using a one-way ANOVA with a Tukey test, a one-way repeat measure ANOVA with a Tukey test, and a student's t-test.

Results

The filtering rate of a 2 mm long *Daphnia pulex* steadily decreased as concentration of *Nannochloropsis* increased (Figure 1). The incipient limiting concentration, based on the tested concentrations, was approximately $5*10^5$ cells mL^{-1}. The filtering rate at this point was 2.39 mL ind^{-1} h^{-1}. Filtering rates differed between $5*10^5$ cell mL^{-1} and $5*10^6$ cell mL^{-1} (Tukey p=0.008) as well as $5*10^5$ cell mL^{-1} and $5*10^7$ cell mL^{-1} (Tukey p=0.005). The filtering rates at 10^4 cells mL^{-1} were highly variable, probably because the cell counts were near the lower limit of resolution for a hemacytometer. The feeding rate still increased after $5*10^5$ cells mL^{-1} and did not seem to plateau (Figure 1). The maximum feeding rate of $2.13*10^6$ cells ind^{-1} h^{-1} was reached at $1*10^7$ cell mL^{-1}, but there was substantial variability at this level (Figure 1). Feeding rates did not significantly differ between food concentrations or food type (one-way ANOVA p=0.120)

The filtering and feeding rate of a 2 mm long *D. pulex* consuming *Microcystis aeruginosa* had the same general response. Peak filtering rate of 0.24 mL ind^{-1} h^{-1} and feeding rate of $1.41*10^5$ cell ind^{-1} h^{-1} occurred at $5*10^5$ cells mL^{-1} (Figure 2). These levels are much lower than those found at peak levels for *Nannochloropsis*. Before the peak, there was a dip in the filtering and feeding rate at $1*10^5$ cell mL^{-1}. This is probably not part of a response pattern, but simply shows that the effects of the *M. aeruginosa* on filtering and feeding rates are variable at low concentrations. There was no plateau as found with the *Nannochloropsis*, but a steady decline in feeding rate after the peak. Minimum filtering and feeding rates occurred at $5*10^6$ cells mL^{-1} (Figure 2). During experiments run at this concentration, up to 13 *Daphnia* in each jar were observed on the bottom of the jar, either lying motionless or spinning. Those that were motionless were found to be dead at the end of the experiment. There was statistical significance in the filtering rate and feeding rate data from $1*10^5$ cell mL^{-1} and $5*10^5$ cell mL^{-1} (T-test p=0.007 and p=0.004).

The adverse effects of the *M. aeruginosa* at this concentration were very apparent, so the thoracic beats and post-abdominal rejections of *D. pulex* were tested to show the difference in feeding behavior between filtered aerated well water (control), *Nannochloropsis*, and *M. aeruginosa*. At a concentration of $5*10^5$ cell mL^{-1} *Nannochloropsis*, the individual *Daphnia*'s thoracic beats had statistical difference from one another (one-way repeat measure ANOVA p=0.003). The same occurred for individuals at $1*10^6$ cell mL^{-1} *Nannochloropsis* (one-way repeat measure ANOVA p=0.024) and at $1*10^6$ cell mL^{-1} *M. aeruginosa* (one-way repeat measure ANOVA p=0.031). The variation within the individual *Daphnia* was expected and may have contributed to the error in the results.

The average thoracic beats per minute decreased from *Nannochloropsis* to *M. aeruginosa* at each concentration (Figure 3). The thoracic beat rate also decreased from $5*10^5$ cell mL^{-1} to $1*10^6$ cell mL^{-1} (Figure 3). The maximum rate of beats occurred at $5*10^5$ cell mL^{-1} *Nannochloropsis*, which correlates with maximum filtering rate (Figure 1 and 3). However, thoracic beats did not differ at $p<0.05$ (one-way ANOVA $p=0.225$).

Variation between individual *Daphnia*'s post-abdominal rejections when fed *M. aeruginosa* was only found in *Daphnia* at $5*10^5$ cell mL^{-1} (one-way repeat measure ANOVA $p=0.035$). The average rate of post-abdominal rejections increased dramatically when *M. aeruginosa* was introduced at either concentration (Figure 4). It only slightly increased (3.44 to 3.67 PAR min^{-1}) from $5*10^5$ cell mL^{-1} to $1*10^6$ cell mL^{-1}. The rate of post-abdominal rejections increased when any food type was introduced to the solution compared to the rate with filtered-aerated well water (Figure 4). Average post-abdominal rejection rates did not differ with food type (*Nannochloropsis* or *M. aeruginosa*) or food concentration ($5*10^5$ cell mL^{-1} or $1*10^6$ cell mL^{-1}). (one-way ANOVA $p=0.549$).

Discussion

The filtering rate of *Daphnia pulex* on *Nannochloropsis* reached its incipient limiting concentration at $5*10^5$ cell mL^{-1} and decreased steadily from there (Figure 1). As filtering rate decreased, feeding rate increased and reached a small plateau (Figure 1). The highest feeding rate occurred at the highest concentration, which also had the lowest filtering rate. This follows the idea that, as the concentration increases and more food is available, less water has to be filtered to collect a greater amount of food. This curve acted as a standard for healthy *Daphnia* feeding because *Nannochloropsis* is a nutritious phytoplankton that was used to culture the *Daphnia* used in these experiments.

If the response to *Microcystis aeruginosa* was similar to *Nannochloropsis*, then the curve for that should be similar to that of *Nannochloropsis*, but, as was discussed above, the response of the *Daphnia* was different, suggesting that something about the *M. aeruginosa* caused this change.

The filtering and feeding rate of *M. aeruginosa* reached incipient limiting concentration at $5*10^5$ cell mL^{-1} (Figure 2). The filtering and feeding rate sharply declined, with the lowest levels at $5*10^6$ cell mL^{-1} (Figure 2). There was an unexplained drop in filtering and feeding rate at $1*10^5$ cell mL^{-1}. This could be due to the fact that different cohorts of *Daphnia* were used to create the portions of the curve from $1*10^4$ to $1*10^6$ to $5*10^6$ cells mL^{-1} and from $1*10^5$ to $5*10^5$ cell mL^{-1}. The variability between cohorts may have resulted in this since the condition of the cohorts prior to the experiment may have been different, which could have positively or negatively affected the filtering and feeding rate.

The *Daphnia* showed negative effects at concentrations of $1*10^6$ cell mL^{-1} and $5*10^6$ cell mL^{-1} *M. aeruginosa*. After one hour, some jars had as many as 13 unresponsive *Daphnia* on the bottom of the jar. The extreme reaction after only one hour was curious, so thoracic beats and post-abdominal rejections of the *Daphnia* were determined to understand the mechanisms behind the death and significant decrease in filtering and feeding rate of the *Daphnia* compared to that of *Daphnia* consuming *Nannochloropsis*.

The size variation that occurred within the individual *Daphnia* used in the thoracic beat experiment should be noted, but other studies have found that *Daphnia carinata* of different sizes beat consistently at about 326 ± 15 beats min^{-1} with little variation; therefore, limb beat rates were not dependent upon body length (Forsyth *et al.* 1992). The average beats of the

Daphnia pulex in the control for this experiment was about 181 beats min^{-1}. Thoracic beat rates decreased from lower to higher food concentrations and from *Nannochloropsis* to *M. aeruginosa* suspensions. The decrease from low to high concentration may occur because fewer beats are required to take in the required amount of food at higher concentrations since there is more food per milliliter. The decrease in beat rates from *Nannochloropsis* to *M. aeruginosa* is possibly a result of the negative effects of microcystin produced by the *M. aeruginosa* on the *Daphnia* or the poor quality of the food. In a similar experiment by Forsyth *et al.* (1992), *D. carinata* were exposed to varying percentages of *Anabaena* filtrate. They found thoracic limb beat frequency was depressed over the range of concentrations with little variation in the control from low concentration to high (Forsyth 1992). It was determined by direct observation that the *Daphnia* were inhibited by the toxic component of the *Anabaena* (Forsyth *et al.* 1992), not mechanically, as found by Chow-Fraser and Sprules (1986). The *M. aeruginosa* used in this experiment were of such similar size and shape to the nutritional *Nannochloropsis* that were readily consumed, that ideas of mechanical inhibition of thoracic beat rates by *M. aeruginosa* are not readily supported. Haney (1985) actually found that *Daphnia pulex* feed more readily on fractions less than 10 μm. Therefore, some other factor, such as the presence of microcystin produced by *M. aeruginosa*, could be causing the decrease in thoracic beats.

Lampert (1987 Laboratory) found that assimilation efficiencies of cyanobacteria are highly variable, but that *Daphnia magna* had an assimilation efficiency of about 21% for *M. aeruginosa*. Low assimilation levels indicate that *M. aeruginosa* is not a very good food source for *Daphnia* since more food must be consumed to acquire the nutrition to grow and reproduce, and therefore more energy must be expended to collect food. This coupled with the toxins that *M. aeruginosa* is known to produce, and can help explain the negative effects of feeding on pure *M. aeruginosa* at different concentrations seen in the present study.

Lürling (2003) found that the presence of *Microcystis* in food mixtures had a great effect on growth development and survival of *Daphnia magna*. The average time to kill 50% of the *Daphnia* used in his experiment was shorter when microcystin was present in the food than when it was not. Clearance rates on mixtures of food suspensions of *Microcystis aeruginosa* and *Scenedesmus obliquus* were higher in strains not containing microcystin than on mixtures with the microcystin. Trubetskova and Haney (2006) found that when *Daphnia magna* were exposed to pure *Microcystis* as a food source, only 35% survived. After six days of exposure to pure *Microcystis* there was a significant decrease in adult body length. Body weight was decreased after six days of exposure to 25% or more *Microcystis*, and egg production was decreased after six days of exposure to 12% or more *Microcystis*. The surviving *Daphnia* that were transferred back to a pure culture of "healthy" phytoplankton, such as *Chlorella*, from the two highest concentrations of *Microcystis* did not recover, but died within 3 to 5 days (Trubetskova and Haney 2006). This supports the idea that the presence of *M. aeruginosa* and, consequently microcystin, decreases thoracic beat rates and therefore clearance rates of the *Daphnia*, limiting the amount of nutritional food the *Daphnia* consume, which in turn inhibits the growth and survival of the *Daphnia.*

It should be noted that the *Daphnia pulex* used in this experiment may have adapted to living with cyanobacteria, so they may be capable of consuming and surviving higher levels than other populations of the same species (J. F. Haney, personal communication, December 14, 2012). However, even though the *Daphnia* from Old Durham Reservoir may be adapted to cyanobacteria, they still showed a negative reaction to the presence of *M. aeruginosa* compared to *Nannochloropsis*. The average rate of post-abdominal rejections (PAR) of a *D. pulex* in

filtered aerated well water was about 1.44 min^{-1}. In both $5*10^5$ and $1*10^6$ cell mL^{-1} *Nannochloropsis*, the rate PAR was 2.22 min^{-1} while in $5*10^5$ cell mL^{-1} *M. aeruginosa* it was 3.44 PAR min^{-1} and $1*10^6$ cell mL^{-1} *M. aeruginosa* was 3.67 PAR min^{-1}. The increase in rejections indicates that the presence of *M. aeruginosa* was irritating the *Daphnia*'s feeding mechanisms, causing them to try to clear out their thoracic limbs more often. Haney et al. (1995) found that post-abdominal rejection rates of *Daphnia carinata* increased with increasing saxitoxin (STX) concentrations associated with *Aphanizomenon flos-aquae*. The increase in both this study and Haney's (1995) supports the idea that *M. aeruginosa*'s presence decreases feeding of *Daphnia* by reducing their thoracic limb beats, increasing their PAR, leading to a decrease in filtering and feeding rate after a incipient limiting concentration was reached, as seen in Figures 1, 2, 3, and 4.

The lack of significance between treatments in this experiment could be a result of a type two error, i.e. the low number of replicates in the feeding study and the use of individual *Daphnia* for the direct observation of thoracic beats and post-abdominal rejections. However, the results showed expected patterns based on previous research.

Improvements to this experiment could be made in the number of trials and number of replicates used in the experiments to help eliminate type-two-error and improve data significance. It would be useful to develop a response curve using pico-cyanobacteria to determine how cyanobacteria of that size affect the filtering and feeding rate of *Daphnia*, and from there, what kind of effect they would have on the feeding mechanisms of the *Daphnia*. A similar suggestion could be made for colonial cyanobacteria, such as *Woronichinia*, since colonial forms are more readily found in the natural environment.

The findings of this experiment indicate that as *M. aeruginosa* concentration increases, *D. pulex* filtering and feeding rates decrease after the incipient limiting concentration was reached. At concentrations after the drop, the thoracic beats of the *Daphnia* decrease and the post-abdominal rejections increase. When compared to *Nannochloropsis*, a healthier food for *Daphnia*, the filtering rates and feeding rates themselves are much higher, indicating that *M. aeruginosa* had a negative effect on the feeding mechanisms of the *Daphnia*, be it from the toxin content or other feeding inhibitors that may have been present.

Conclusion

The results and previous studies presented here show that phytoplankton concentration and type has a direct effect on *Daphnia pulex* filtering rate, feeding rate, thoracic beats, and post-abdominal rejections. As *Nannochloropsis* concentration increases, the filtering rate decreased and the feeding rate increased. As *Microcystis aeruginosa* concentration increased, the filtering rate and feeding rate decreased. At concentrations above $5*10^5$ and 10^6 cells mL^{-1} *M. aeruginosa*, some *D. pulex* died, possibly because there was too much toxic cyanobacteria present, creating some upper feeding limit which negatively affects the *Daphnia*. Thoracic beats of *D. pulex* decreased at higher concentrations and on food suspensions of *M. aeruginosa*. The reduction in beats means that there would be lower collection rates of food, which correlates to the lower filtering and feeding rates found at higher food concentrations and on food suspensions of *M. aeruginosa*. Post-abdominal rejections increased at higher concentrations and on food suspensions of *M. aeruginosa*. The higher rates of rejection at higher food concentrations and on food suspensions of *M. aeruginosa* indicate that more food needs to be removed from the food groove of the *Daphnia*, reducing filtering and feeding rate. The results of this study fit the trends established by previous studies and confirm that concentration and food type does influence

feeding efficiency of *D. pulex*, which can be linked to the effects on feeding mechanisms of *D. pulex*.

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Table 1: The average size of each phytoplankton was determined by averaging the sizes of 15 individual cells of each. *Microcystis aeruginosa* cells are slightly larger than *Nannochloropsis*, but not enough to be considered a variable in these experiments.

Species	**Average Size (μm)**
Nannochloropsis	3.39
Microcystis aeruginosa	4.53

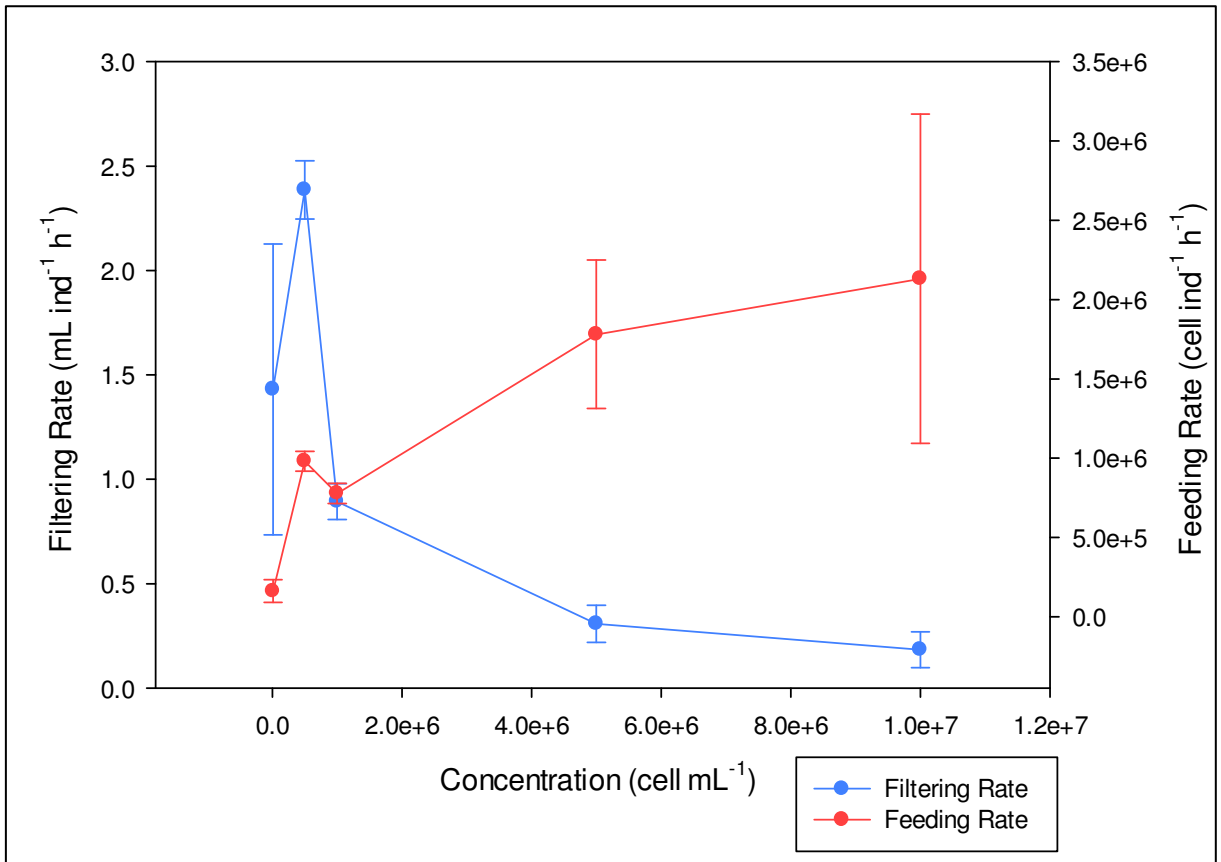

Figure 1: The filtering and feeding rate of a 2mm long *Daphnia pulex* on different concentrations of *Nannochloropsis*. The filtering rate reaches its incipitent limiting concentration at $5*10^5$ cell mL^{-1}. As filtering rate decreases, feeding rate increases, with the maximum feeding rate occuring at $1*10^7$ cell mL^{-1}.

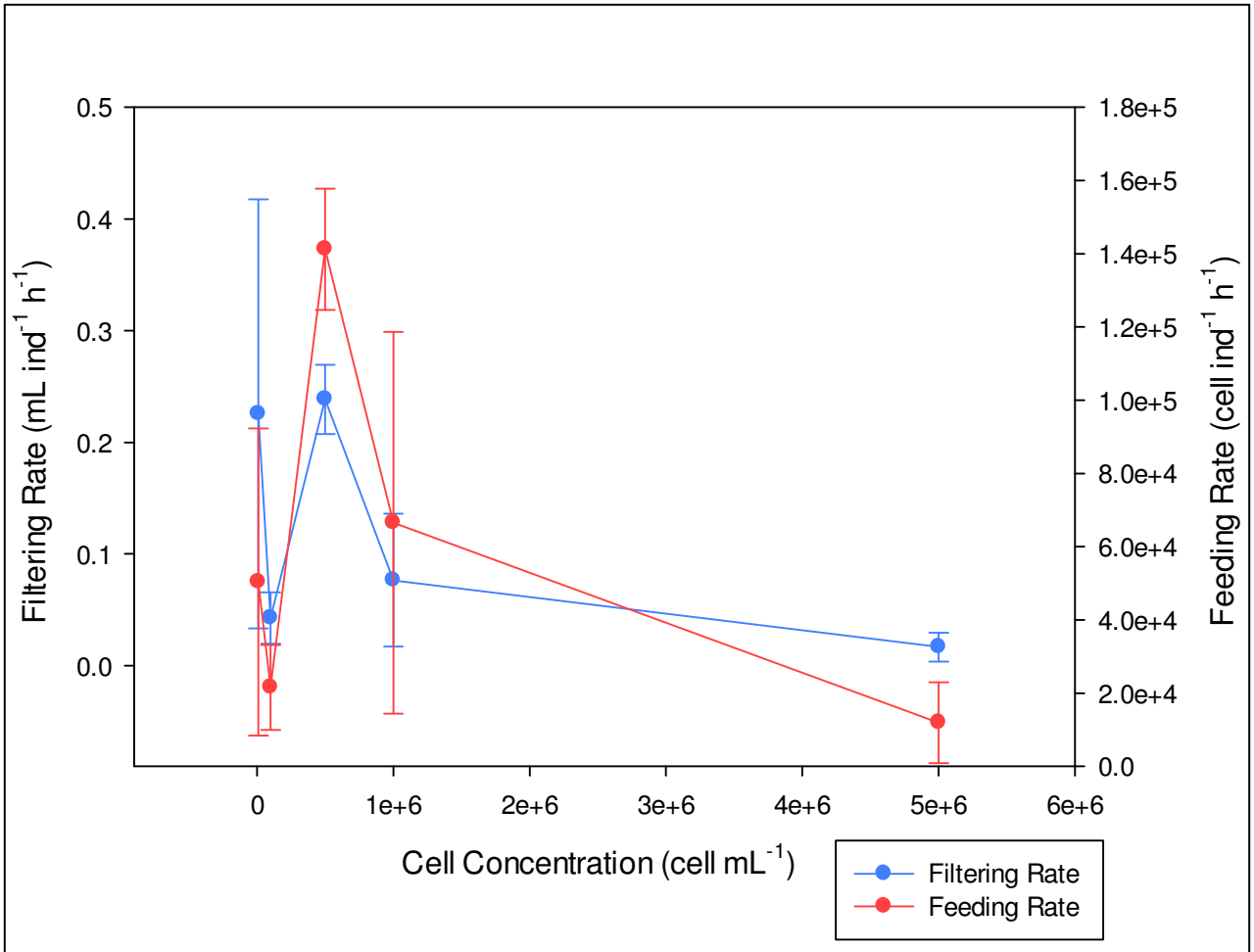

Figure 2: The filtering and feeding rate of a 2mm long *Daphnia pulex* on different concentrations of *Microcystis aeruginosa*. The filtering and feeding rate peak at $5*10^5$ cell mL^{-1}. After this, the filtering and feeding rate decrease substantially.

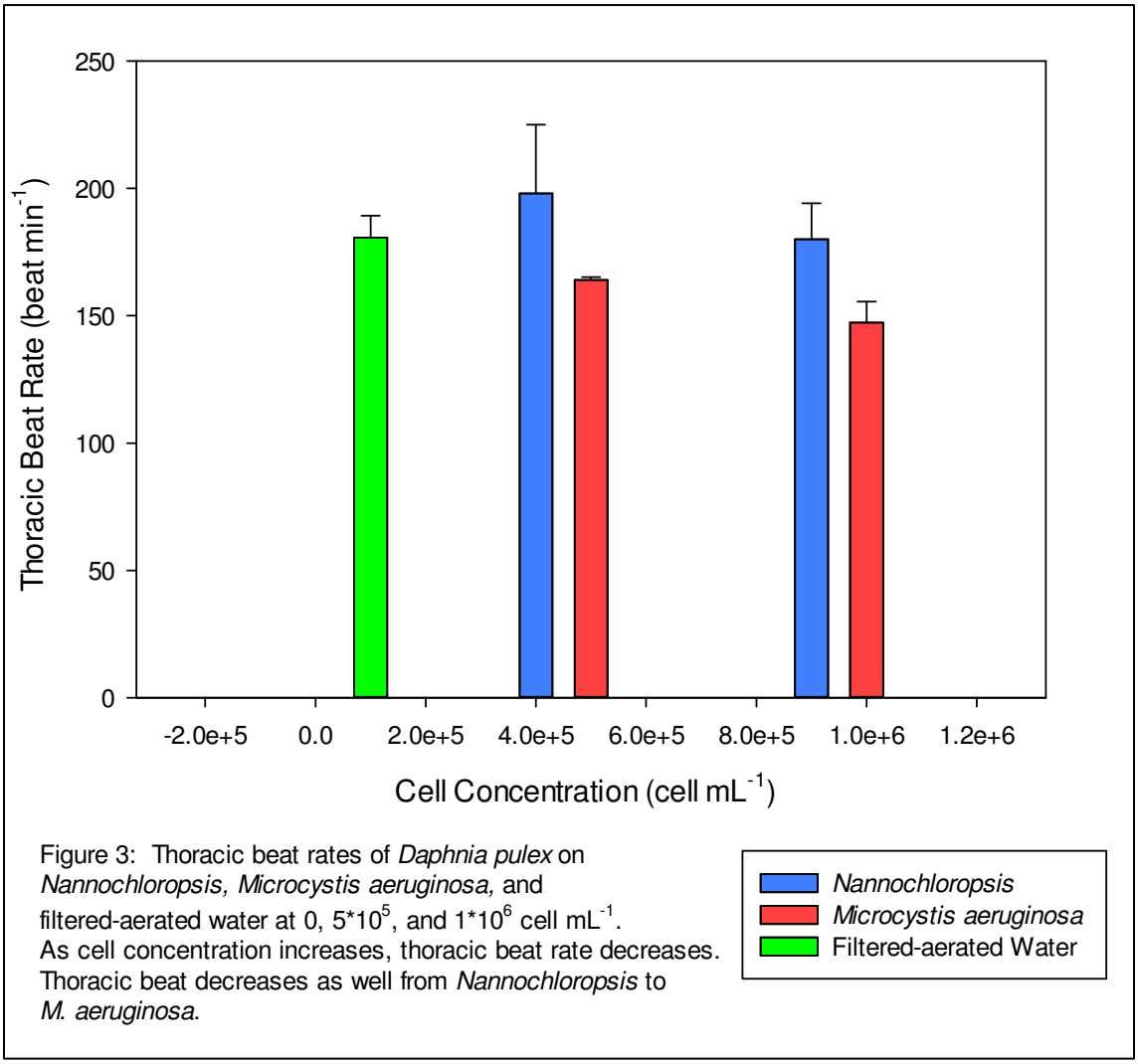

Figure 3: Thoracic beat rates of *Daphnia pulex* on *Nannochloropsis, Microcystis aeruginosa,* and filtered-aerated water at 0, $5*10^5$, and $1*10^6$ cell mL^{-1}. As cell concentration increases, thoracic beat rate decreases. Thoracic beat decreases as well from *Nannochloropsis* to *M. aeruginosa*.

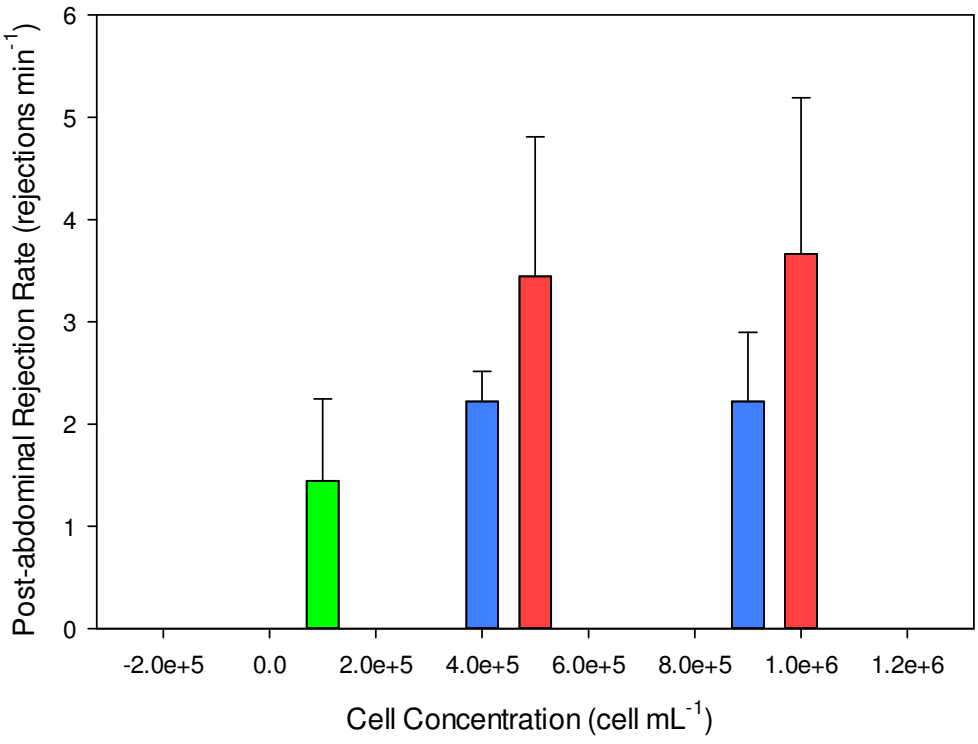

Figure 4: Post-abdominal rejection rate of *Daphnia pulex* on *Nannochloropsis, Microcystis aeruginosa,* and filtered-aerated water at 0, $5*10^5$, and $1*10^6$ cell mL^{-1}. As cell concentration increases, post-abdominal rejection rate increases very little. Post-abdominal rejection rate increases dramatically from *Nannochloropsis* to *M. aeruginosa*.