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The effect of cyanobacteria and their chemical cues on the surface area of the third thoracic limb of Daphnia

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THE EFFECT OF CYANOBACTERIA AND THEIR CHEMICAL CUES ON THE
SURFACE AREA OF THE THIRD THORACIC LIMB OF *DAPHNIA*

BY

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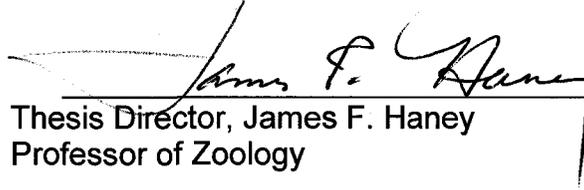
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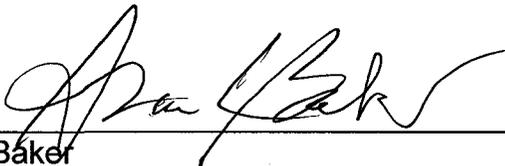
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THE EFFECT OF CYANOBACTERIA AND THEIR CHEMICAL CUES ON THE SURFACE AREA OF THE THIRD THORACIC LIMB OF *DAPHNIA*

by

W. Travis Godkin

University of New Hampshire, December 2007

Changes in the filter appendage surface area (FSA) of *Daphnia* in response to cyanobacteria were examined in an effort to learn more about the relationship between *Daphnia* and extracellular cues exuded by cyanobacteria. The filtering appendage areas of two strains of *D. pulex* were measured after feeding on high and low concentrations of a mixture of *Nanochloropsis spp.* and toxic *Microcystis aeruginosa* for one generation. *Daphnia* were also raised in a filtrate of this same *M. aeruginosa* and given high and low concentrations of food to determine the cause of increased FSA in the presence of cyanobacteria: low amounts of nutritious phytoplankton or response to chemical cues produced by the cyanobacteria. I observed an increased FSA in response to increased proportions of *M. aeruginosa*. However, there was no change in FSA as the amount of *M. aeruginosa* filtrate increased and food levels remained constant, thus suggesting that the lack of nutritious food is the most proximate cause of increased FSA.

Patterns consistent with laboratory experiments were observed in *Daphnia* from eight lakes of varying trophic status. The *Daphnia* from the most

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oligotrophic lakes had the largest FSA and *Daphnia* from eutrophic systems with

an abundance of cyanobacteria had a greater FSA than more mesotrophic

systems.

CHAPTER I

INTRODUCTION

Background

Phytoplankton and zooplankton grazers are important components of lake

ecosystems. Understanding of the interactions between these two trophic levels,

in particular the phenotypic plasticity exhibited in zooplankton, is essential.

Daphnia (Order: Cladocera) are small (~0.5-3 mm) crustaceans that often make

up a considerable proportion of a lake's zooplankton community. Examining

Daphnia's phenotypic responses to seasonal changes in phytoplankton, and

specifically toxin-producing cyanobacteria, enables us to understand one of the

mechanisms by which they adapt to and survive in a fluctuating environment

(Fulton and Paerl 1987; Haney et al. 1994; Trubetskova and Haney 2006).

Several studies have demonstrated that the surface area of the primary filtering

appendages of *Daphnia* (Fig. 1) increase when concentrations of edible grazing

material are low (Geller and Müller 1981; Repka et al. 1999). Ghadouani and

Pinel-Alloul (2002) examined the effects of natural assemblages of cyanobacteria

on the surface area of the filtering appendages, finding increased surface area

when exposed to toxin-producing cyanobacteria. However, the relationship

between surface area changes and food type are not yet fully understood.

Unanswered is the question: is this plastic response induced by a lack of food or

by chemicals produced by the cyanobacteria? This study was designed to address this question.

Koza and Korinek (1985) and Lampert (1994) reported that the total surface area of the filtering appendages increased when *Daphnia* were reared in low concentrations of phytoplankton. As the filtering rate is primarily dependant on the filter screen area (FSA), an increase enhances filtering efficiency without increasing thoracic limb beat frequency, thus allowing the *Daphnia* to increase phytoplankton consumption (Egloff and Palmer 1971). Furthermore, there may be an associated increase in feeding capacity too, as Lampert (1994) observed increases in ingestion rate. Additionally, a decreased amount of digestible material is a prominent trigger for the increase in FSA. When Repka et al. (1999) increased total particle concentration with inert clay particles, while maintaining low phytoplankton levels, *Daphnia* still showed an increase in FSA .

Daphnia exposed to a natural phytoplankton assemblage containing increased amounts of non-ingestible filamentous and colonial cyanobacteria responded as it does to low food concentrations: FSA increased along with filter mesh size (Ghadouani and Pinel-Alloul 2002). Though the quantity of phytoplankton present was large, it was mostly inedible and possibly toxic. *Daphnia* feeding upon cultures of *Microcystis* have exhibited increased mortality, decreased growth, delayed maturation, and decreased offspring production (Trubetskova and Haney 2000). The increase in FSA observed by Ghadouani and Pinel-Alloul (2002), which resulted in the increase in filtering capacity, is presumably an adaptation to capture and consume more edible material. The

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greater mesh size may be due to the increased size of seston, as daphnids from environments dominated by smaller diameter seston feed more effectively with a finer filtering screen (Geller and Müller 1981). The change may also be caused, in part, by the detection of the dissolved chemical, which would accompany the dominant cyanobacteria. These studies suggest that, in nature, the relative size of the *Daphnia* filtering area may shift in response to changes in the phytoplankton size and abundance, such as might occur in lakes with a range of trophic conditions.

Hypotheses

The present study examines the relationship between concentration and composition of phytoplankton and the FSA of *Daphnia* based on field data and laboratory experiments. Specifically, I hypothesize:

1. The filter screen area (FSA) of laboratory cultured *Daphnia pulex* collected from an oligotrophic lake (Russell Pond) as well as a eutrophic lake (Old Durham Reservoir) will increase when reared in low concentrations of the highly edible phytoplankton *Nanochloropsis spp.* as well as when exposed to a unicellular form of toxin-producing *Microcystis aeruginosa*.

2. There will be an increase in the FSA of both of these strains of *Daphnia* when exposed to a filtrate of *M. aeruginosa*, but the increase will be greater in the *Daphnia* from the eutrophic Old Durham Reservoir.

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3. FSA will be larger in *Daphnia* collected from oligotrophic and eutrophic lakes, environments with little edible phytoplankton, while those collected from mesotrophic environments with sufficient amounts of edible phytoplankton, will have a relatively smaller FSA.

Rationale and Assumptions

Hypothesis 1: Low concentrations of edible phytoplankton are encountered in lakes that are oligotrophic, as well as in eutrophic environments dominated by inedible filamentous and colonial cyanobacteria (Ghadouani and Pinel-Alloul 2002). These two scenarios are typical of the environments encountered by the Russell Pond (Haney and Ikawa 2000) and Old Durham Reservoir (*Personal Obs.*) strains of *Daphnia*, respectively. Increased FSA would permit the *Daphnia* to filter a larger volume of water, thus increasing the probability of ingesting more edible material. Previous studies have shown that FSA will increase with exposure to colonial or filamentous forms of cyanobacteria (Ghadouani and Pinel-Alloul 2002). However, it is still unclear if the changes in FSA are a response to the physical or chemical inhibition created by these forms of cyanobacteria. By using a microcystin-producing strain of *M. aeruginosa* that is unicellular and therefore ingestible by *Daphnia*, an increase in FSA should not be due to starvation caused by physical inhibition, but some other factor associated with the *M. aeruginosa*.

Hypothesis 2: I expect the *Daphnia* exposed to the *M. aeruginosa* filtrate will increase their FSA in response to effects produced by extra cellular chemical

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cues present in the filtrate. Exposure to cyanobacteria filtrate alone causes decreases in thoracic limb beat frequency, increased postabdominal rejection of food collected, and decreased filtration rates (Forsyth et al. 1992; Haney et al. 1994; Haney et al. 1995); these behavioral responses may extend to phenotypic changes as well. The cue present in the filtrate has not been identified and could be a number of chemicals produced by cyanobacteria. In addition to microcystin (Bishop et al. 1959), *Microcystis* has been shown to produce anatoxin, a neurotoxin (Namikoshi and Rinehart 1996), aeruginosin, a serine protease inhibitor (Murakami et al. 1995), and microviridin, a depsipeptide (Okino et al, 1995; See also: Ikawa and Sasner, 1990 and Namikoshi and Rinehart, 1996). Furthermore, *Daphnia* life history traits vary in response to stress (Boersma et al. 1999), there are intraspecies differences in tolerance of cyanobacteria (Sarnelle and Wilson 2005), and *Daphnia* may become tolerant to cyanobacteria (Gustafsson and Hansson 2004). Therefore, I expect the change in FSA in response to chemical cues to be greater in the Old Durham Reservoir *Daphnia* because as they have adapted to exposure to *M. aeruginosa* and are more likely to recognize this chemical cue when re-exposed.

Hypothesis 3: I expect the relationship of increased FSA in the presence of decreased phytoplankton will be observed in *Daphnia* collected from both oligotrophic and eutrophic lakes. Oligotrophic lakes typically have low food levels. Eutrophic lakes tend to have greater amounts of phytoplankton, particularly a higher percentage of large (> 50 μm) phytoplankton or colonial and filamentous cyanobacteria (Sommer et al. 1986; Paerl 1988). Thus, decreased

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levels of edible grazing material in these lakes, indicated by the relative amounts

of cyanobacteria, should require greater efficiency in food collection, which can

be accomplished through an increase in FSA.

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CHAPTER II

METHODS

Laboratory Experiments

Experimental *Daphnia* consist of two strains of *D. pulex* from different environments: Old Durham Reservoir (ODR) and Russell Pond (RP). Old Durham Reservoir (Durham, NH) is a eutrophic lake, which often contains an abundance of cyanobacteria, including *M. aeruginosa* and *Anabaena sp.* Russell Pond (Woodstock, NH), in contrast, is an oligotrophic lake which contains a low density and relative abundance of cyanobacteria, and almost no *M. aeruginosa.* *Daphnia* from each lake were collected with an 80 μm plankton net and had been in culture at UNH's Center for Freshwater Biology for approximately 1 year prior to this study.

Feeding effects

The *Daphnia* were cultured in aerated well water and fed *Nanochloropsis spp.* for at least one month prior to the beginning of the experiment. Neonates (< 16 h old) were harvested, using 500 μm and 250 μm sieves, and transferred to the experimental vessels, which contained 10 *Daphnia* each in 60 mL of aerated well water. To test the effect of *M. aeruginosa* on FSA, *Daphnia* were exposed to high (1.0 mg C L^{-1}) and low (0.25 mg C L^{-1}) food concentrations of a 0% (control), 25%, 50%, 75%, and 100% *M. aeruginosa* content mixture of *M. aeruginosa*

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(UTEX 2385; cultured in ASM-1 medium at the UNH Center for Freshwater Biology) and *Nanochloropsis spp.* (Instant Algae Nanno 3600, Reed Mariculture) in 60 experimental vessels. Carbon content was assumed to be 50% dry weight of the phytoplankton cells. Each treatment consisted of three replicates. The entire water volume was replaced and re-inoculated with the appropriate mixture and concentration of algae every 24 h.

These concentrations of phytoplankton, determined by cell counts using a hemacytometer, have been shown to be well above and below the incipient limiting concentration (Rigler 1961) as well as the concentration limiting growth in *Daphnia* (Lampert 1977, 1978). *Nanochloropsis spp.* was chosen for three principle reasons: it is a good source of nutrition for *Daphnia*, it is similar in size and shape to *M. aeruginosa* (both are spherical and are similar in size, approximately 5 μm in diameter) and, like *M. aeruginosa*, sinks slowly and allows for relatively constant algal concentrations throughout the experimental vessel at all times.

Clearance Rates- Clearance rates were measured in each container by monitoring the initial and final concentrations of algae during the final 2-4 h of each treatment (depending on the number of surviving individuals and food concentration). Clearance rate (CR; ml h^{-1} ind.$^{-1}$) was calculated according to Downing and Rigler (1984) as follows:

$$CR = \frac{(\ln (C_0 + \text{cell growth}) - \ln C_t) * V}{t * \text{ind.}}$$

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where V is the volume of water in the container, C_0 is the initial food concentration and C_t is the food concentration at time t (hours). Cell growth was determined using a control consisting of aerated well water inoculated with the appropriate concentration and mixture of algae. Clearance rates were then adjusted to a standard body length for *Daphnia* of 1.5 mm using the average body length in each replicate and correcting with the regression equation for body length and filtering rate from Burns (1969).

Filter Screen Measurement- *Daphnia* were removed from the experimental vessels when they had reached their maximum body length and shown visible signs of egg production. The age of full grown *Daphnia* varied between treatments as those in the high algal concentration grew at a faster rate than in the low algal concentration. Upon removal, the *Daphnia* were preserved in sucrose formalin (Haney and Hall 1972). This preservation technique does not affect body length or appendage size as all measured parts are made of chitin (Campbell and Chow-Fraser 1995).

The average filter screen area (FSA) for the third feeding appendage was measured and related to the body length of the individual. Body length (top of head to the base of the tail spine) and body width of each daphnid was measured and the thoracic filtering appendages were removed from the animal. The screens were mounted on a slide using glycerin and a coverslip and sealed after 24 h with clear nail polish. A digital image of the magnified (100-400x) filter was

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captured and the FSA was measured using the polygon tool combined with the area measurement feature in NIH Image J (version 1.28u).

Each treatment was compared using ANCOVA (Systat 10.0), regression analysis and ANOVA (Sigma Plot 9.0). In all cases $\alpha = 0.05$ and all reported r^2 values are adjusted for the number of independent variables (degrees of freedom). Body length values used in ANCOVA analysis were transformed by raising the value to the power of 2.22 (Egloff and Palmer 1971), thus improving the linear relationship. Additionally, the Relative Filter Surface Area was calculated using the following equation:

$$RFSA= FSA/Body\ Length^{2.22}$$

Data were additionally transformed using the natural logarithm in instances of non-normal distribution.

Chemical Cue

The methods used to measure the response to *Nanochloropsis spp.* in cyanobacteria filtrate were also as described in the feeding effects experiments. *Daphnia* were fed a high ($1.0\ mg\ C\ L^{-1}$) and low ($0.25\ mg\ C\ L^{-1}$) concentration of *Nanochloropsis spp.* as noted above; however, the phytoplankton cells were suspended in a filtrate of *M. aeruginosa*. This filtrate was created by filtering (Whatman GF/C, 47 mm) a volume of cyanobacterial suspension adjusted to the same high concentration as above. Filtered, aerated well water was combined with the filtrate to make 0% (control), 25, 50, 75 and 100% *M. aeruginosa* filtrate suspensions. Microcystin content was analyzed using concentrated water

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samples and ELISA (Envirologix QuantiPlate kit for microcystins, EP 022) (Haney and Ikawa 2000). The high and low concentrations (1.0 and 0.25 mg C L^{-1}, respectively) of *Nanochloropsis spp.* cells were added to this filtrate to create treatments of high and low concentrations of *Nanochloropsis spp.* in high *M. aeruginosa* concentration filtrate. Again, all methods for rearing, calculating feeding rates, harvesting, and measurements of FSA were the same as above. As all treatments were not performed concurrently the dates and duration of each experimental treatment are provided in Appendix A (Table A-1).

Natural Populations

In addition to the controlled laboratory experiments, I also examined field populations of *Daphnia* in order to determine the overall effect of trophic status on the FSA. Plankton samples were collected *via* vertical tows through the entire water column and preserved in 4% sucrose formalin as part of the 50 lakes study conducted by Haney and Ikawa (2000) as well as lakes visited by the 2003 Field Limnology Class (UNH). The trophic status of each lake was determined by chlorophyll a concentrations measured at the time of collection and the trophic classification scheme of Forsberg and Ryding (1980).

Eight different lakes were used to obtain a variety of trophic conditions. These lakes include: York Pond, Otternic Pond, Horseshoe Pond (eutrophic), Barbadoes Pond, Swains Lake, Bow Lake, (mesotrophic), Christine Lake, and Russell Pond (oligotrophic). York Pond and Christine Lake data were from the University of New Hampshire Field Limnology class and the remaining lakes from the 50 lakes study (Haney and Ikawa 2000)(Table 1).

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<u>Data Collection</u>- *Daphnia* from each lake were individually identified (An Image-Based Key To The Zooplankton Of The Northeast (USA), Version 2.0), measured (body length and width), and digitally photographed. The third filtering appendages were removed, mounted, and measured as above. FSA measurements were made from 9 to 16 individuals from each lake, covering the maximum in *Daphnia* body length present in the lake. These body length measurements were transformed and all data were analyzed as outlined above.

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CHAPTER III

RESULTS

Laboratory Results

Feeding effects

There was a significant difference in mortality between Russell Pond and Old Durham Reservoir strains at the same, as well as at differing, food levels (ANOVA; $p < 0.001$; Holm-Sidak pairwise comparisons). Russell Pond *Daphnia* experienced greater overall mortality than Old Durham Reservoir *Daphnia*. Additionally, Russell Pond *Daphnia* mortality increased with increased exposure to *M. aeruginosa* in high food concentrations suggesting that the individual *Daphnia* were subjected to food related stress (Fig. 2).

There were significant differences in the relationship between body length and FSA in all treatments of the feeding response (ANCOVA; Table 2). The differences between treatments were best elucidated by examining the Relative Filter Surface Area (RFSA), which removes much of the variation due to body length differences (Fig. 3). In 10 of 17 of treatments, higher percentages of *M. aeruginosa* led to greater RFSA. In the Russell Pond high food treatments (Fig 4a), exposure to greater amounts of *M. aeruginosa*, as in the 100% *M. aeruginosa* treatment, produced greater RFSA values compared to treatments with less *M. aeruginosa* (Table 2). Russell Pond *Daphnia* in the low food (Fig 4c) did not survive in the 50, 75, and 100% *M. aeruginosa* treatments and the RFSA

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for the 25% *M. aeruginosa* was greater than the 0% *M. aeruginosa*, though this difference was not significant (Table 2).

Similar trends were found for the Old Durham Reservoir *Daphnia*. In the high food treatment (Fig 4b), RFSA values were greatest in high concentrations of *M. aeruginosa* (Table 2). In the low food treatment (Fig. 4d), the average RFSA for the *Daphnia* fed the most *M. aeruginosa* was larger than all other treatments, although only significantly different from the 0% *M. aeruginosa* control and 25% *M. aeruginosa* (Table 2).

There were significant differences in RFSA between high and low food concentrations within *Daphnia* strains as well. The Old Durham Reservoir *Daphnia* consistently exhibited larger RFSA values when fed a lower amount of food (Fig 5); these differences were significant in all but the 25% *M. aeruginosa* treatment (Table 2). Additionally, the average clearance rate for Old Durham Reservoir *Daphnia* raised on low amounts of food was greater than the other treatments, though not significantly, and decreased with increasing proportions of *M. aeruginosa* (Fig. 6). Russell Pond *Daphnia* RFSA did not differ significantly in the high and low food in either of the surviving treatments (Table 2), and there was no change in clearance rate (Fig. 6).

Chemical Cue

Russell Pond and Old Durham Reservoir *Daphnia* exposed to *M. aeruginosa* filtrate instead of *M. aeruginosa* cells did not exhibit the same trends seen in the feeding effects experiments. Though mortality patterns were similar,

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with greater mortality in the low food treatments (Fig 7; ANOVA, p< 0.001), there was no significant difference in FSA or RFSA (which was again used to reduce the effects of body length on FSA; Fig. 8) as the concentration of *M. aeruginosa* filtrate increased (ANCOVA, fig. 9 and ANOVA fig. 10, respectively; Table 3). Similarly, there was no significant difference in mean clearance rate between treatments (Fig 11). Additionally, there was no difference in Russell Pond *Daphnia* RFSA between high and low food treatments and very little difference in Old Durham Reservoir *Daphnia* RFSA value (Fig. 12).

Natural Populations

When *Daphnia* from all natural populations sampled were pooled together, the filter surface area (FSA) of the third feeding appendage was positively correlated with body length ($r^2 = 0.81$, $p < 0.001$; Fig. 13). Data were transformed by raising the body length to the exponent of 2.22, increasing the r^2 values for four of the lakes (Christine Lake, York Pond, Swains Lake, and Russell Pond; Table 4). The highly correlated relationship between FSA and body length was seen in each of the individual lakes as well, with the strongest correlation found in Swains Lake and the weakest significant correlation in Otternic Pond (Table 5; Figs. 14 and 15, respectively). Although *D. catawba* generally had greater FSA values than *D. dubia* in Horseshoe Pond, there was no significant difference in FSA of the 3rd limb between the two *Daphnia* species (Figure 16, ANCOVA, p = 0.566) so they have been grouped together and presented as Horseshoe Pond *Daphnia*. Individual regressions between body length and FSA

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for Christine Lake, York Pond, Barbados Pond, Bow Lake, and Russell Pond can be found in Appendix A (Figures A-1 through five, respectively).

The largest of the *Daphnia* from Christine Lake, Otternic Pond, Russell Pond, Swains Lake, and York Pond had a greater FSA than *Daphnia* from the others lakes (Fig. 17). The relationship between FSA and body length for these five lakes was significantly different from those of Barbadoes Pond, Bow Lake, and Horseshoe Pond (Fig. 17; ANCOVA; p = 0.000), but not from each other (Fig. 17; ANCOVA, p = 0.247). Similarly, there was no significant difference in this relationship between Barbadoes Pond, Bow Lake, and Horseshoe Pond (Fig. 17; ANCOVA; p = 0.419).

The RFSA was again used to examine differences between lakes. The regression of average body length for each lake with the average RFSA indicated that this method successfully reduces the effect of body length on RFSA (Fig. 18; p = 0.985; r^2 = 0). However, when individual body lengths for all *Daphnia* in the study were regressed by their RFSA, there was a significant relationship with a very low r^2 value of 0.089 (Fig. 19; p = 0.001) indicating considerable interlake variability.

The RFSA of Russell Pond *Daphnia* was significantly greater than all other lakes (Fig. 20; ANOVA, p < 0.001; Holm-Sidak pairwise comparison). This pattern was not seen in results obtained using ANCOVA, in which Russell Pond was significantly different from only Barbadoes Pond, Bow Lake, and Horseshoe Pond.

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The relationships between chlorophyll a concentration and the area of the filtering appendages of *Daphnia* were examined to elucidate some of the factors influencing the differences between each of the lakes. The RFSA of *Daphnia* from more oligotrophic lakes, especially Russell Pond, were greater than more eutrophic lakes such as Horseshoe Pond (Fig. 20). There was an increase in the RFSA of the most eutrophic lake: York Pond. There was no significant linear relationship between RFSA and chlorophyll a concentration. However, regression analysis using exponential models (RFSA = 0.026 + 0.051$^{-0.658* \, Chl \, a}$ $^{(\mu g/L)}$ + 0.0003 * Chl a (μg L^{-1}) revealed a significant relationship (Figure 21; p = 0.01, r^2 = 0.712).

Similarly, level of microcystins present in each of the lakes was not linearly correlated with the RFSA (Fig. 22; p = 0.105, r^2 = 0.274), even when Russell Pond, which appeared to be an outlier, was removed. The relationship between microcystin and RFSA fits an exponential pattern (RFSA= 0.037+ 0.240 $^{-0.687 *}$ $^{WLW \, Microcystin \, (pg/ml)}$) similar to the chlorophyll a concentration and RFSA relationship (Fig. 22, p = 0.013, r^2 = 0.752).

CHAPTER IV

DISCUSSION

Laboratory Studies

Feeding effects

Mortality rates differed greatly between treatments, but also within replicates (Fig. 2) within the feeding effects study. Increased concentrations of *M. aeruginosa* did not significantly increase the mortality in the feeding effects or chemical cue experiments (ANOVA, interaction term p-value = 0.169 and 0.166, respectively). This was unexpected, as numerous researchers have reported increased mortality with increased cyanobacteria exposure (Forsyth et al. 1992; Reinikainen et al. 1994; Hietala et al. 1997), and could be due to the low sample size used in this study. Larger sample sizes may have permitted the detection of statistical differences, but would have also hindered the experimental progress.

Daphnia exposed to greater amounts of *M. aeruginosa* had greater FSA and RFSA values (Figs. 9 and 4, respectively). Old Durham Reservoir *Daphnia* exposed to high amounts of food had greater filtering areas at all concentrations of *M. aeruginosa* compared to the control with no *M. aeruginosa*, although this effect was only significant at 75% *M. aeruginosa* (Fig. 4b). Similar trends were seen in the low food concentration. All treatments, except 25% *M. aeruginosa*, were greater than the control, although none were significantly different (Fig. 4d). As expected, this increase in RFSA was greater in the lower food concentrations

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as the actual amount of edible food was greatly reduced, especially in the 75% and 100% *M. aeruginosa* treatments (Fig 5; Table 2). Increases in FSA coupled with decreasing food levels have been reported in numerous studies, and was therefore an expected outcome (Lampert 1994). Conversely, clearance rate decreased as the proportion of *M. aeruginosa* increased in the Old Durham Reservoir low food treatments, which shows that these *Daphnia* were consuming less food. This decrease in clearance rate is typical of exposure to cyanobacteria and not low food levels, as clearance rate is expected to increase in the latter case. This may indicate that poor nutrition, resulting from decreased consumption and not only decreased *Nanochloropsis spp.* availability, was the cause of the increase in FSA in these treatments.

Russell Pond *Daphnia* exhibited similar trends: in the high food experiment, the 50, 75 and 100% *M. aeruginosa* treatments had greater RFSA values relative to the control, though the difference was not significant (Fig. 4a). In the low food concentration, mortality prevented similar comparisons. The mean RFSA in the 25% *M. aeruginosa* treatment is greater than the control, but not significantly. No Russell Pond *Daphnia* survived in the 50, 75 or 100% *M. aeruginosa* treatments (Fig. 4c) indicating this strain of *D. pulex* was probably less well adapted to exposure to toxin-producing *M. aeruginosa*. Furthermore, the clearance rate in the 25% *M. aeruginosa* low food treatment was greater than in the control, which is typical of a low food response and the opposite of documented responses to cyanobacteria (Forsyth et al. 1992; Haney et al. 1994; Haney et al. 1995). Additionally, the clearance rate in the 0% *M. aeruginosa*

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control is the same in the high and low food levels, indicating that these *Daphnia* are filtering at an elevated capacity even when food is plentiful. This absence of response to the *M. aeruginosa* is to be expected as Russell Pond does not have high levels of microcystins or *M. aeruginosa* (Haney and Ikawa 2000) while Old Durham Reservoir often has blooms of toxin-producing cyanobacteria, including *M. aeruginosa* (*Personal Obs.*). In a similar situation, Sarnelle and Wilson (2005) examined clones of *Daphnia pulicaria* from environments with and without high levels of cyanobacteria. They found that the clones from lakes with abundant cyanobacteria grew faster and were less inhibited by a diet of 100% *M. aeruginosa* than clones from more oligotrophic systems. Additionally, Hairston et al. (2001) compared *Daphnia* hatched from pre- and post-eutrophication sediments and found that modern *Daphnia* had evolved a phenotypically plastic response to the cyanobacteria that had become prevalent in the lake.

RFSA of the Russell Pond *D. pulex* did not differ significantly between high and low food treatments (Fig. 5, Table 3). However, this result was likely due to the high mortality rate in treatments with 50% or more *M. aeruginosa*, which is where differences between high and low food RFSA were most often found. Perhaps it was a lack of change in FSA that led to the increased mortality in the treatments with less *Nanochloropsis spp.* If there had been an increase in FSA, filtering rates would have likely increased and these *Daphnia* would have been able to ingest more *Nanochloropsis spp.* (Lampert 1994).

These results presented above support hypothesis one that *Daphnia* FSA increases in conditions of decreased food abundance. I expected the greater

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concentrations of *M. aeruginosa* to produce higher FSA values than the 0% *M. aeruginosa* treatment, and this is largely what occurred. When the total amount of phytoplankton was high, but the amount of nutritious material was low, as in the 75 and 100% *M. aeruginosa* treatments, there was an increase in RFSA. Ghadouani and Pinel-Alloul (2002) advanced the "reaction norm" hypothesis (Stearns 1989) that changes in filter area could be a function of food scarcity, speculating that this response could be similar to cyclomorphic predator induced changes (Dodson 1974; Dodson 1989; Tollrian 1990, 1993). My results, given the gradient in levels of *Nanochloropsis spp.*, support this hypothesis. Additionally, changes in clearance rate suggest that a decrease in consumption is causing the increase in FSA. However, it may have been the ingestion of the *M. aeruginosa* that may be causing the increase in FSA, not the depleted amounts of *Nanochloropsis spp.*

Chemical Cue

Daphnia exposed to *M. aeruginosa* filtrate did not respond to the same extent as those exposed to the whole cells. It appears as if the amount of *M. aeruginosa* filtrate had no effect on the FSA of either strain of *Daphnia*, unlike in the feeding effects experiments; there was no increase in RFSA coupled with the increase in *M. aeruginosa* filtrate (Fig. 10). In the feeding effects experiments, as the percentage of *M. aeruginosa* increased, the percentage of *Nanochloropsis spp.* decreased. In the chemical cue experiments, however, the only variable between treatments was the proportion of *M. aeruginosa* filtrate and the amount

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of *Nanochloropsis spp.* remained constant across treatments. The consistent RFSA, in conjunction with the static clearance rate, suggests that FSA was not influenced by the detection of extra cellular chemicals produced by this strain of *M. aeruginosa* but by the levels of edible food present.

Other researches, though, have reported effects of cyanobacteria filtrate on feeding in *Daphnia*. Forsyth et al. (1992) reported decreases in the thoracic limb beat frequency of *Daphnia carinata* when exposed to cyanobacteria filtrate. Both of these studies, however, used naturally occurring populations and densities (15-20 mg dw L^{-1}) of *Anabeana minutissima* (Forsyth et al. 1992) or *M. aeruginosa* (Haney et al. 1995). Perhaps the *Daphnia* in these experiments were responding to cues that were not produced by my laboratory reared strain of *M. aeruginosa*, or perhaps the greater density of cyanobacteria (my experiments used 2 mg dw L^{-1} of *M. aeruginosa* to create the filtrate) produced enough of these cues to surpass a threshold of stimulation. Conversely, the lack of response to the filtrate could also indicate that ingestion of the *M. aeruginosa* cells is necessary for response. *Daphnia* reared in water containing purified microcystin-LR have shown either no response or response only to amounts much greater than those found naturally (Lürling and Van Der Grinten 2004).

Additionally, there were no significant differences between high and low food concentrations within *Daphnia* strains (Fig. 12). Old Durham Reservoir *Daphnia* produced greater RFSA at the low food treatments than at the high food treatments, but the difference was significant only in the 0% *M. aeruginosa* control and 100% *M. aeruginosa* filtrate treatments. The differences were even

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more subtle in the Russell Pond *Daphnia*, as none of the low food treatments yielded significantly greater RFSA values than the control.

One explanation for this result is that Old Durham Reservoir *Daphnia* from the feeding effects experiments, as previously stated, exhibited an increase in RFSA as the percentage of *M. aeruginosa* increased while the percentage of *Nanochloropsis spp.* decreased. In the chemical cue experiments, though, the food level remained constant and the control exhibited a significantly greater increase in RFSA in the low food treatment. The remaining chemical cue treatments did not see a continuously greater disparity in RFSA between high and low food levels because there was not a continuous change in actual food level. The same is true for Russell Pond *Daphnia*: there was no significant difference in RFSA between high and low food levels in the feeding effects control so it should not be surprising that there was no difference in the chemical cue control, and therefore the remaining treatments as well. In fact, Russell Pond *Daphnia* may have actually received more food in the low food treatments than they would encounter in their natural habitat.

Natural Populations

Filter screen area was correlated with body length, with only one instance of non-correlation: *D. catawba* from Horseshoe Pond. When the two Horseshoe Pond species were combined, though, the relationship was significant (Fig 16). Additionally, the larger *Daphnia* from Christine Lake, Swains Pond, Otternic Pond, York Pond, and especially Russell Pond had proportionally larger filter

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screens (Fig. 17). The observed difference may be due to larger *Daphnia*, in general, in these lakes (i.e.: the other lakes not included in this group did not have *Daphnia* in the same size range). However, the difference in body size should have been accounted for in the ANCOVA analysis, which indicates that these lakes had a greater FSA. This suggests that some factor, probably predation, was limiting the size of *Daphnia* in Barbadoes Pond, Bow Lake, and Horseshoe Pond (Brooks and Dodson 1965). The only detectable factor that grouped these lakes was, however, body length. The body length of Christine Lake *Daphnia* was significantly greater than all lakes excluding Swains Lake and York Pond, and Swains Lake *Daphnia* were larger than all of the other lakes. Otternic Pond *Daphnia* were the smallest, significantly smaller than Christine Lake and Swains Lake (Fig. 23; ANOVA, $p < 0.001$, Holm- Sidak pairwise comparison). Russell Pond *Daphnia* had an intermediate average body length and was not significantly different from any of the other lakes. Otternic Pond *Daphnia* may be grouped with these larger *Daphnia* due to lake effects. The proportion of net cyanobacteria (>50μm) in this lake was high (80%), with the next greatest percentage found in York Pond (42%; Fig. 24). In this case, the smallest *Daphnia* seem to have a proportionally large FSA, which supports my hypothesis that large amounts of cyanobacteria results in a greater FSA, despite the high concentrations of chlorophyll a (a surrogate for general phytoplankton only). This same response was seen by Ghadouani and Pinel-Alloul (2002) where the highest nutrient enclosure had a peak chlorophyll a level of 112 μg L^{-1} but approximately 30 times more inedible than edible phytoplankton, which

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resulted in an increase of FSA. Similar studies have looked at life history responses to increased concentrations of *M. aeruginosa* and have also found increased negative affects with increased proportions of *M. aeruginosa* (Reinikainen et al. 1994; Lürling and Van Der Grinten 2004).

Additionally, Horseshoe Pond and Barbadoes Pond *Daphnia* may have had smaller FSA values due to the levels of microcystin, which is known to retard somatic growth in *Daphnia* (Hietala et al. 1995; Hietala et al. 1997; Lürling 2003), found in those lakes (Fig. 22 and 25). These two lakes had the highest amount of whole lake water microcystin, respectively, of every lake in this study. Additionally, Bow Lake had the greatest amount of microcystin relative to the amount of cyanobacteria present (Fig 26), suggesting that the relatively high levels of microcystin could be affecting *Daphnia* growth in this lake. York Pond and Otternic Pond, in contrast, have some of the lowest levels of microcystin relative to the amount of cyanobacteria.

As in the laboratory experiments, additional analysis was performed using the RFSA, which removed the effect of body length. When the average RFSA of *Daphnia* from each lake was regressed against each lake's average body length, there was no significant correlation (Fig. 18; $p = 0.985$; adjusted $r^2 = 0$). When each individual RFSA measurement, irrespective of the lake of origin, is regressed against the corresponding body length, there was a significant relationship (Fig 19). This relationship, though, was very weak, as only about 9% ($r^2 = 0.089$) of the variation in RFSA was caused by body length, further indicating that this analysis greatly reduced the effect of body length.

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The relationships indicated by the ANOVA analysis, however, differ from those suggested by ANCOVA (Figs. 20 & 17, respectively). Analysis using the relative surface area, which satisfactorily reduced the effect of body size, indicated interesting patterns with Russell Pond, Bow Lake, and York Pond. Russell Pond had the greatest mean RFSA, which could be explained by the fact that it has the lowest concentration of chlorophyll a of all of the lakes as well as the lowest amount of cyanobacteria and microcystins. Russell Pond was different from most of the other lakes, with the greatest mean RFSA per unit of chlorophyll a (Fig. 27). Additionally, the mean RFSA for York Pond was greater than the RFSA for some of the lakes with lower levels of chlorophyll a (Fig. 28; Table 1). Bow Lake, too, had a greater mean RFSA than other lakes with similar chlorophyll levels and the Otternic Pond *Daphnia*'s RFSA was greater than those of Horseshoe Pond, even though there was a greater concentration of chlorophyll a (Fig. 27). This is unexpected when considering the hypothesis that RFSA decreases with increasing concentrations of chlorophyll a. However, this pattern would be expected under my hypothesis that RFSA increases with a decrease in edible phytoplankton; as the percentage of cyanobacteria increases, the relative amount of edible, nutritious phytoplankton decreases. Even though York Pond had a high level of chlorophyll a (Fig. 28, Table 1), a large proportion of that was composed of inedible, less nutritious cyanobacteria (Fig. 24). A similar situation existed in Otternic Pond, which also had a large concentration of chlorophyll a (Fig. 28, Table 1) and the highest percentage of cyanobacteria (Fig 24). Again, this is consistent with Ghadouani and Pinel-Alloul's (2002) enclosure

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experiments (high chlorophyll a levels but also high proportions of

cyanobacteria), yet these results were from a natural system.

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CHAPTER V

CONCLUSION

I hypothesized that the filter surface area of *Daphnia* exposed to decreased amounts of edible food would increase. This hypothesis was supported: when fed decreased amounts of nutritious phytoplankton, Old Durham Reservoir *Daphnia* showed an increase in FSA in all treatments. Russell Pond *Daphnia* did not survive exposure to concentrations of *M. aeruginosa* greater than 50% when coupled with low amounts of *Nanochloropsis spp.* There was, though, an increase in RFSA of the low compared to the high food treatment *Daphnia* in the 25% *M. aeruginosa* treatment, suggesting that surviving individuals may have experienced the same increase in greater proportions of *M. aeruginosa*. Alternatively, the similarity in RFSA between the high and low food control could indicate that these *Daphnia* were already producing the largest FSA possible, as these *Daphnia* are accustomed to living in oligotrophic systems. Moreover, both strains of *Daphnia* exhibited an increase in FSA when exposed to greater proportions of *M. aeruginosa* in both the high and low food experiments. While these differences were not consistently significant, the trend still exists.

My second hypothesis, which stated that FSA would also increase with exposure to *M. aeruginosa* filtrate alone, and that this increase would be greater in the *Daphnia* from Old Durham Reservoir, was not supported. Exposure to

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filtrate containing greater amounts of the chemical cues produced by *M. aeruginosa* did not affect FSA in either high or low food experiments. However, there were interesting differences in the responses of the two strains of *Daphnia*, even though they were observed in the feeding effects experiment. Old Durham Reservoir *Daphnia* exhibited a decrease in clearance rate coupled with an increase in FSA. The change in clearance rate is a typical response to the presence of cyanobacteria and may have lead to increased starvation, thus triggering the increase in FSA. An increase in FSA, though, should also increase the amount of cyanobacteria ingested. This outcome may be avoided by limiting the gape of the carapace in response to the normally colonial cyanobacteria (Young et al. 1997). The Russell Pond *Daphnia*, however, showed an increase in clearance rate. This response indicates that these *Daphnia* were responding primarily to the decrease in *Nanochlopsis spp.* by increasing instead of decreasing filtration to limit ingestion of the *M. aeruginosa*. It may have been this increase in *M. aeruginosa* consumption that led to the high mortality in these treatments. These clearance rate results, combined with the changes in FSA, suggest that the increases seen in the *Daphnia* feeding experiments was largely the result of decreased amounts of the nutritious *Nanochloropsis spp.* and not induced as a response to the chemicals exuded *M. aeruginosa* and that the Old Durham Reservoir *Daphnia* are better equipped to survive exposure to *M. aeruginosa*.

My final hypothesis, that *Daphnia* from oligotrophic and eutrophic lakes would have a greater FSA that *Daphnia* from mesotrophic lakes, was also

supported. The *Daphnia* from the most oligotrophic lake, Russell Pond, had the greatest FSA values and there was an increase in the FSA of York Pond *Daphnia* over those from less oligotrophic lakes. Additionally, Otternic Pond *Daphnia* had a large RFSA value coupled with the second greatest chlorophyll a level and the greatest proportion of cyanobacteria in the study, providing further supporting evidence in support of this hypothesis.

Each of these studies provides evidence supporting the hypothesis that it is the lack of edible food that stimulates an increase in the filter surface area of *Daphnia* feeding appendages and not chemical cues produced by cyanobacteria. Specifically, the laboratory studies have shown that chemical cues contained in filtrate of the UTEX 2385 strain of *M. aeruginosa* do not affect the FSA of *Daphnia* that did show a response to the whole cell form of this strain of *M. aeruginosa*. Furthermore, my study of eight natural ecosystems supports the hypothesis that the level of nutritious food affects FSA and that changes in FSA may be linked to amounts of phytoplankton in proportion to cyanobacteria as a whole, and not just the strain of *M. aeruginosa* used in this study. This conclusion, though, could be bolstered by incorporating more lakes to obtain a greater variety of trophic status.

This experiment does not definitively rule out cyanobacterial cues as a trigger for the increase in FSA. Future studies should expand upon these conclusions by incorporating different varieties of cyanobacteria. Cyanobacteria filtrate made from high densities of naturally occurring cyanobacteria elicited behavioral responses, and may also affect FSA more than the filtrate used in my

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experiment. Furthermore, changes in FSA could still be triggered by the ingestion of cyanobacteria. This scenario could be investigated through similar experiments using treatments composed entirely of decreasing concentrations of *Nanochlorosis spp.* or *M. aeruginosa*. Finally, it would be interesting to see if other zooplankton, such as *Bosmina* or calanoid copepods, possess the ability to alter the size of their feeding appendages.

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TABLES

Table 1: Physical and chemical data on lakes sampled in the Natural Populations analysis. Values in parentheses indicate standard error of the mean, N=3.

Lake	Collection Date	Location (Town, NH)	Max Depth (m)	Total Phosphorus (μg L^{-1})	Total Nitrogen (μg L^{-1})	Chlorophyll a (μg L^{-1})	Mean SDD (m)
Christine	26-Sep-2003	Stark	19.50	4.07 (0.09)	203.00 (11.02)	1.57 (0.09)	7.35 (0.0)
York	25-Sep-2003	Berlin	5.20	46.3 (0.67)	843.33 (18.48)	39.43 (1.04)	0.85 (0.0)
Russell Pond	28-Jun-1999	Woodstock	23.7	3.66 (0.56)	174.66 (5.67)	1.09 (0.12)	13.97 (0.07)
Swains Lake	13-Jul-1999	Barrington	8.8	10.6 (0.67)	294.66 (12.81)	3.18 (0.06)	3.63 (0.15)
Barbadoes	19-Jul-1999	Madbury	14.6	11.1 (0.36)	469.0 (50.09)	1.93 (0.06)	3.92 (0.17)
Horseshoe Pond	10-Aug-1999	Merrimack	6.1	19.9 (0.67)	410 (17.62)	18.4 (0.52)	2.60 (0.03)
Otternic Pond	10-Aug-1999	Hudson	4.8	52.9 (3.16)	734 (46.46)	22.17 (0.19)	1.88 (0.17)
Bow Lake	21-Jul-2000	Strafford	28.3	12.03 (0.27)	164.66 (13.22)	2.7 (0.58)	4.83 (0.03)

Table 2: ANCOVA and ANOVA results for all significant relationships in the feeding effects experiments using Old Durham Reservoir (ODR) and Russell Pond (RP) *Daphnia*. (*) indicates the data were Ln transformed.

Treatment	p-value, ANCOVA	p-value, ANOVA
ODR, High Food	0.001	0.004*
ODR, Low Food	0.023	0.025*
RP, High Food	< 0.001	0.011
RP, Low Food	< 0.001	0.242 (t-test)
ODR, High vs. Low Food	---	< 0.001*
RP, High vs. Low Food	---	0.176

Table 3: ANCOVA and ANOVA results for all significant relationships in the chemical cue experiments using Old Durham Reservoir (ODR) and Russell Pond (RP) *Daphnia*.

Treatment	p-value, ANCOVA	p-value, ANOVA
ODR, High Food	0.483	0.905
ODR, Low Food	0.766	0.671
RP, High Food	0.407	0.441
RP, Low Food	0.180	0.679
ODR, High vs. Low Food	---	0.018
RP, High vs. Low Food	---	0.633

Table 4: Change in adjusted r^2 values when transforming body length.

Lake	Original r^2 value	Transformed r^2 value
Barbados Pond	0.717	0.616
Bow Lake	0.975	0.953
Horseshoe Pond	0.752	0.717
Russell Pond	0.09	0.934
Christine Lake	0.876	0.891
Otternic Pond	0.868	0.854
York Pond	0.782	0.789
Swains Lake	0.907	0.958

Table 5: Adjusted r^2 values obtained through regression analysis of body length ($mm^{2.22}$ and filter surface area (FSA; mm^2) of *Daphnia* collected from eight NH lakes.

Lake	Species	Abbreviation	Adjusted r^2 value
Russell Pond	*D. pulex*	RP	0.935
Christine Lake	*D. laevis*	CL	0.892
Barbadoes Pond	*D. rosea*	BP	0.647
Bow Lake	*D. catawba*	BL	0.954
Swains Lake	*D. schødleri*	SL	0.958
Horseshoe Pond	*D. catawba*	---	0.596
Horseshoe Pond	*D. dubia*	---	0.875
Horseshoe Pond	All	HP	0.717
Otternic Pond	*D. catawba*	OP	0.855
York Pond	*D. catawba*	YP	0.779
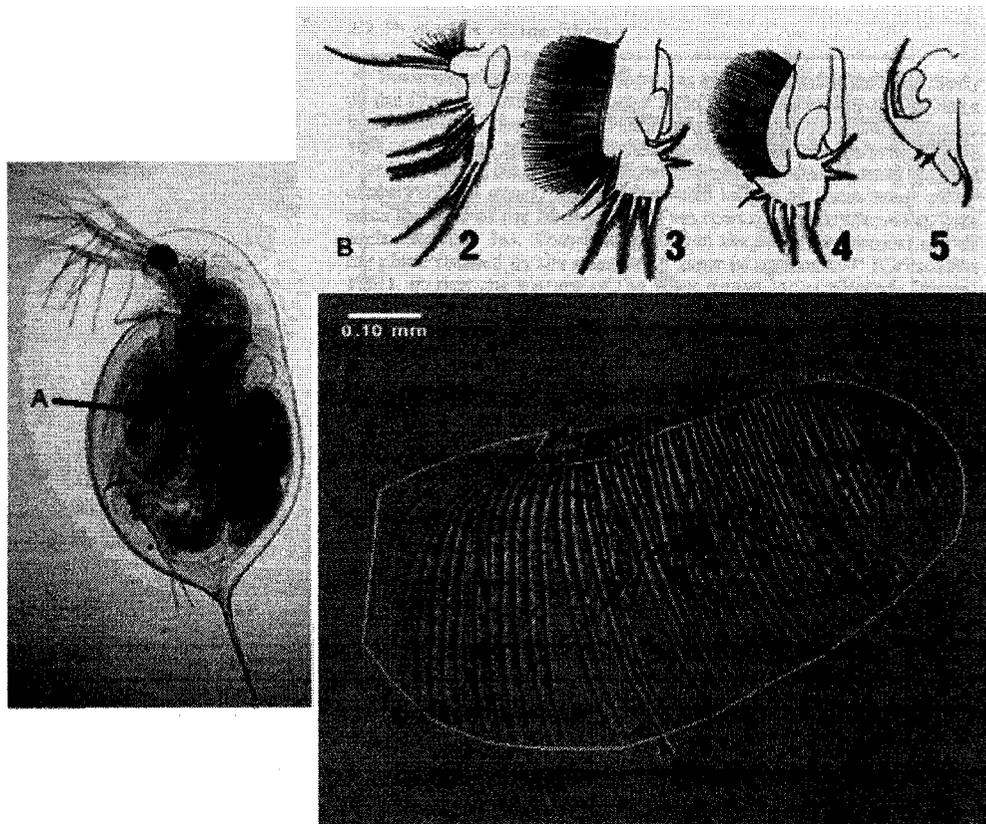
Figure 1: Images of a *Daphnia* with the 3rd filtering appendage clearly visible (A) (from An Image-Based Key To The Zooplankton Of The Northeast (USA) Version 2.0); a diagram of thoracic appendages two through five (B); from Lampert 1987, and a dissected and mounted filter screen from the third feeding appendage with the measured surface area indicated (C).
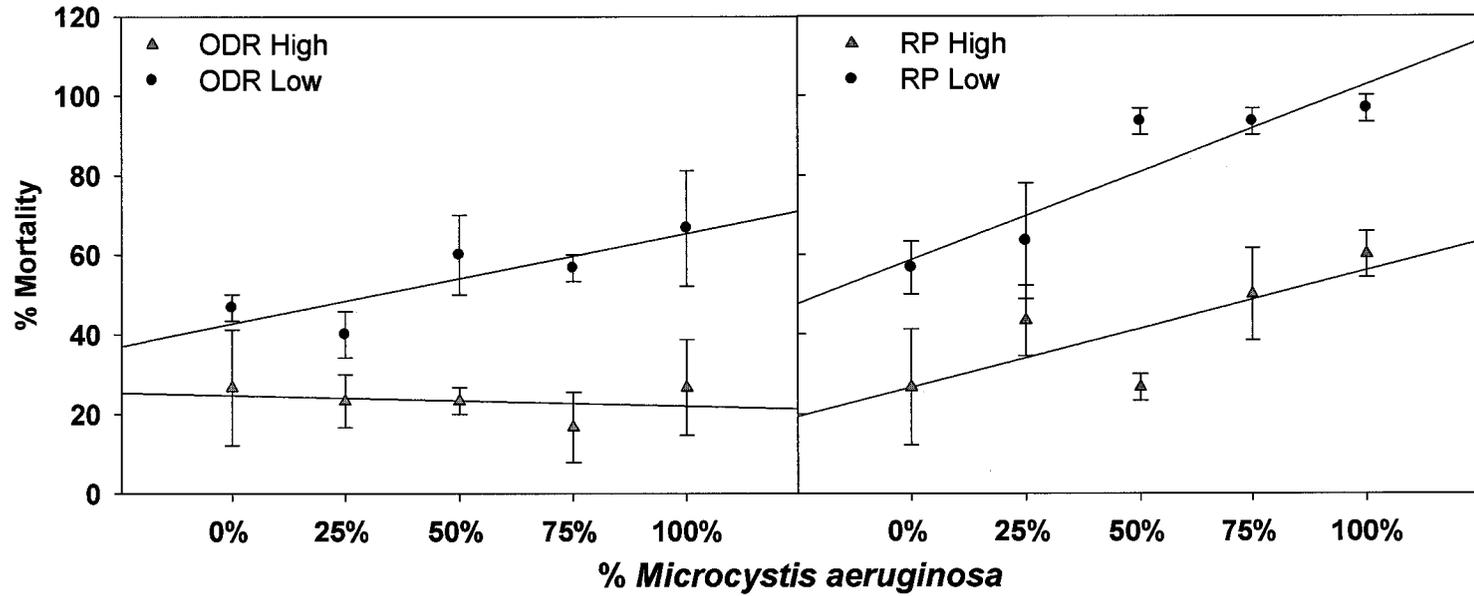
Figure 2: Average mortality (±1 SE) after five days in each feeding effects treatment for Old Durham Reservoir (ODR; left) high food (1 mg C L^{-1}; adj. r^2 = 0.00, p = 0.67) and low food (0.25 mg C L^{-1}; adj. r^2 = 0.61, p = 0.07) and Russell Pond (RP) high (adj. r^2 = 0.50, p = 0.11) and low (adj. r^2 = 0.78, p = 0.03) food *Daphnia pulex*. Concentrations of *Microcystis* range left to right from 0-100%, n=3 in all cases.
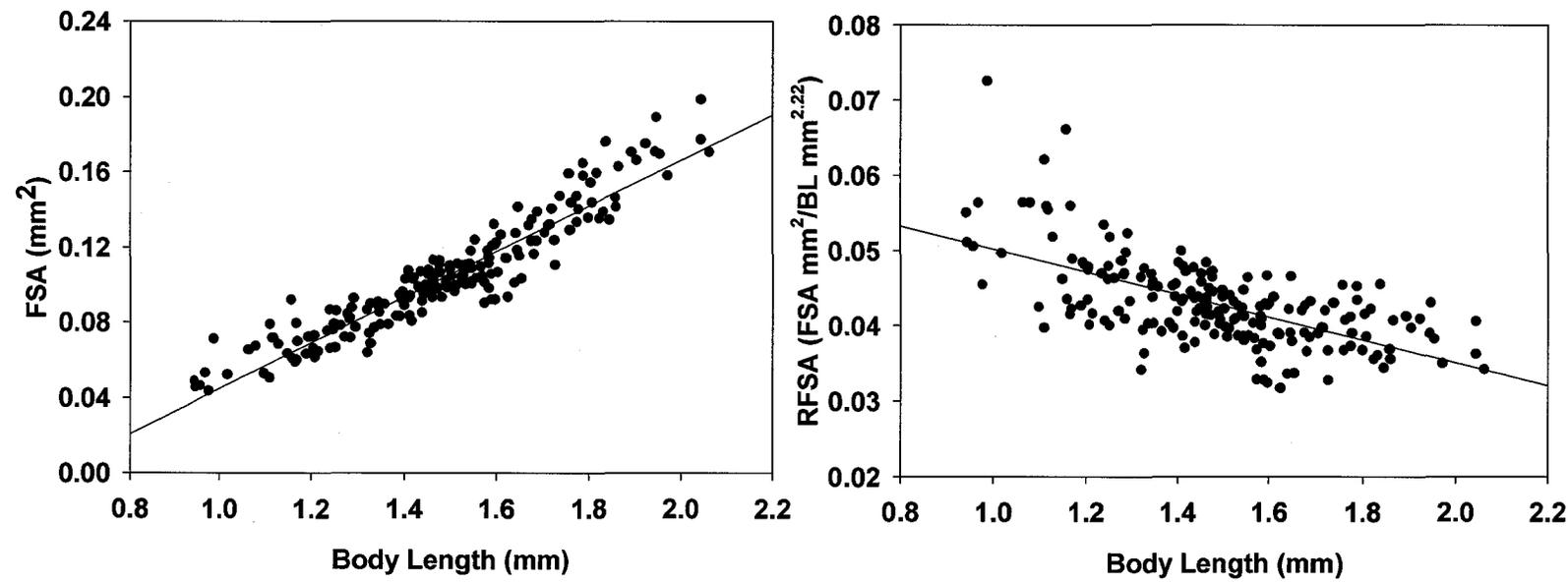
Figure 3: Body length vs. Filter Screen Area (FSA; left, adjusted r^2 = 0.885, p<0.001, n = 197) and Relative Filter Surface Area (RFSA; right, adjusted r^2 = 0.388, p<0.001, n = 197), RFSA (FSA/body length $mm^{2.22}$) used to reduce the effect of body length in the feeding effect experiments.
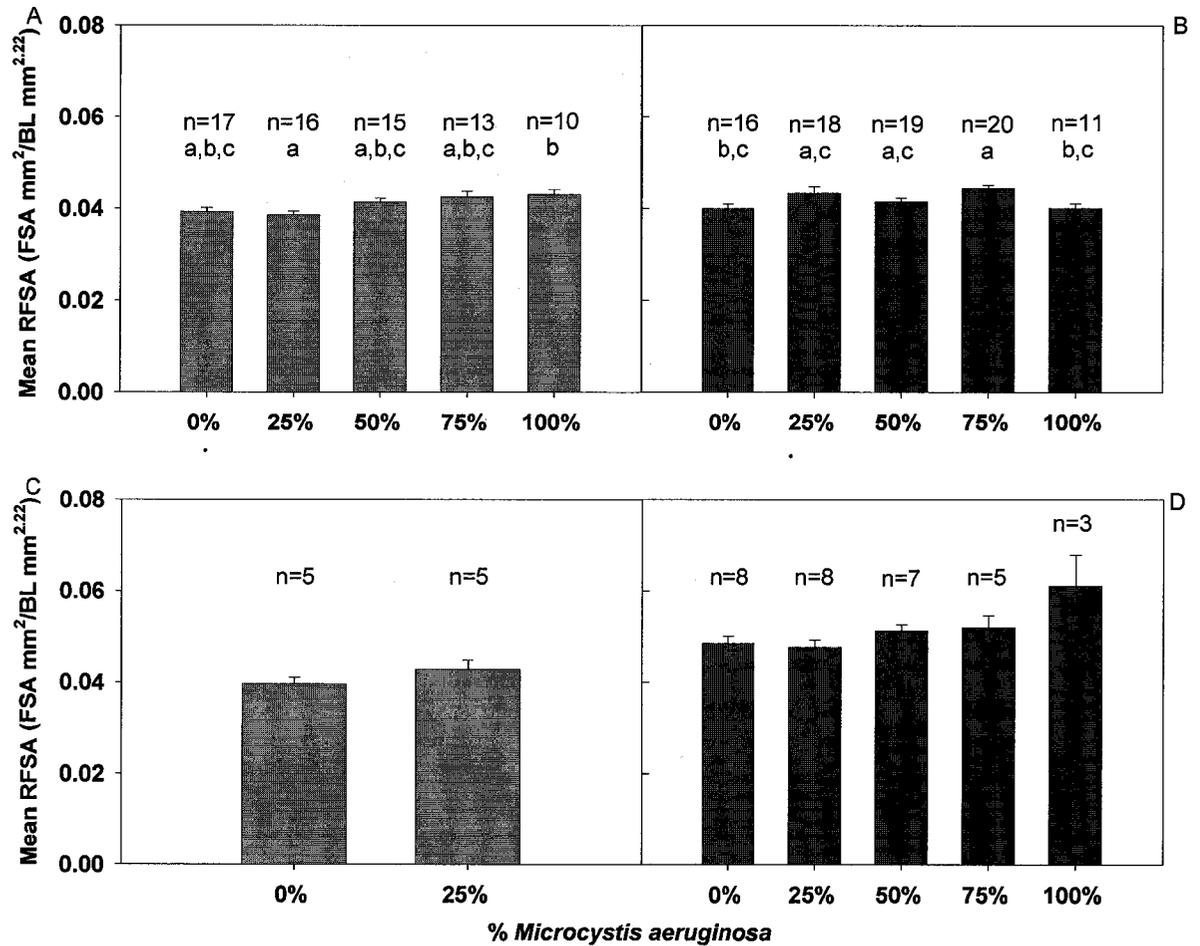
Figure 4: Mean Relative Filter Surface Area (RFSA; +1 SE) for each food treatment in the feeding effect experiment for Russell Pond (RP; a) High (1.0 mg C L^{-1}) food and c) Low (0.25 mg C L^{-1}) food) and Old Durham Reservoir (ODR; b) High food and d) Low food) *Daphnia*. Different letters signify significant difference ($p < 0.05$, ANOVA), no letters indicates no significant difference exists. Concentrations of *Microcystis* range left to right from 0-100%.

40
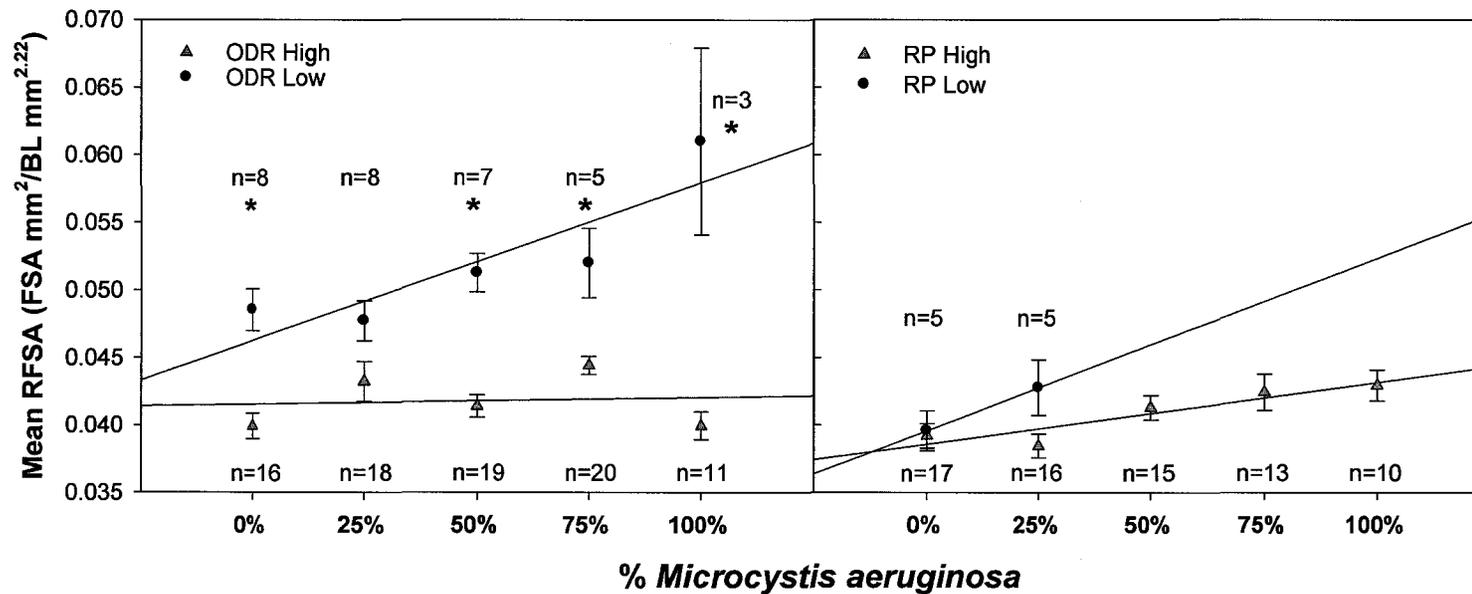
Figure 5: Comparison of mean Relative Filter Surface Area (RFSA; ±1 SE) in high (1.0 mg C L^{-1}) and low food (0.25 mg C L^{-1}) treatments for Old Durham Reservoir (ODR; left) and Russell Pond (RP; right) *Daphnia pulex* in the feeding effects experiments. (*) indicates significant difference between high and low food RFSA in that treatment (ANOVA, p < 0.001). Concentrations of *Microcystis* range left to right from 0-100%. Regression statistics are as follows: ODR High: adj. r^2 = 0.00, p = 0.87, f= 0.042 + 4.98x10^{-6} * % *M. aeruginosa*; ODR Low: adj. r^2 = 0.69, p = 0.05, f= 0.046 + 1.17x10^{-3} * % *M. aeruginosa*; RP High: adj. r^2 = 0.79, p = 0.03, f= 0.039 + 4.56x10^{-5} * % *M. aeruginosa*.
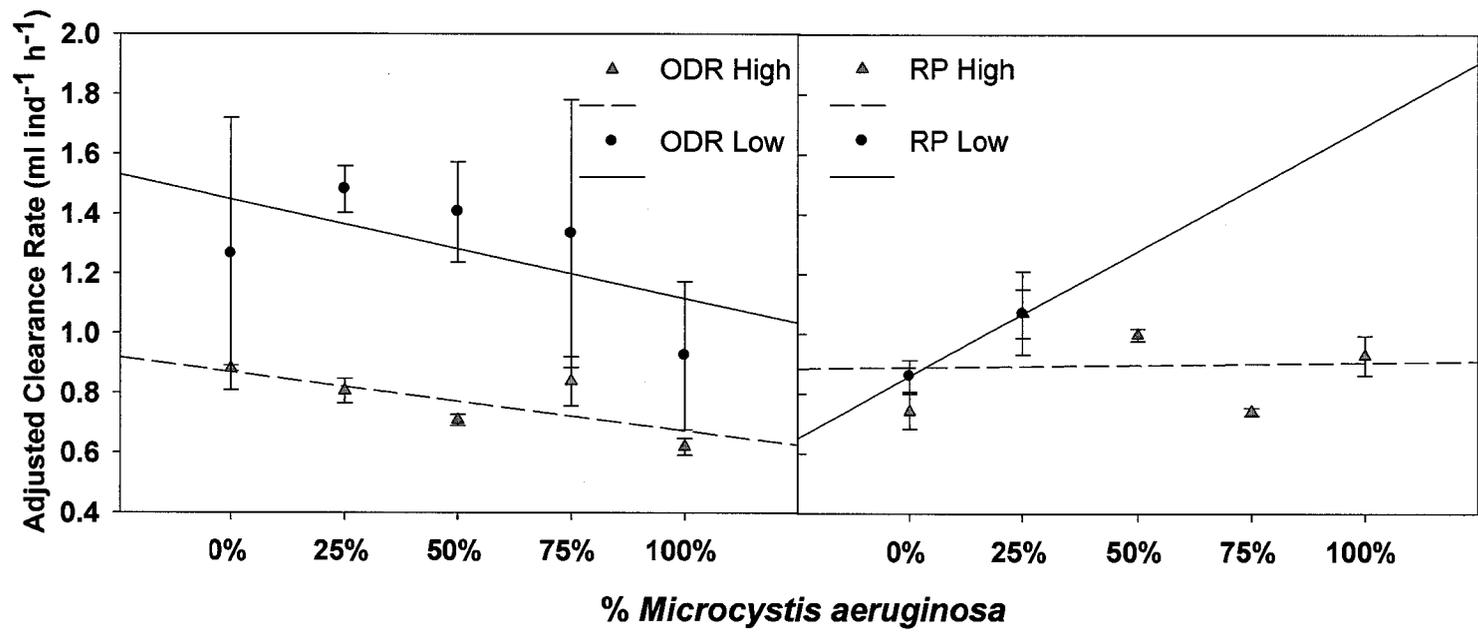
Figure 6: Average clearance rate (±1 SE) adjusted to a body length of 1.5 mm in each feeding effects treatment for Old Durham Reservoir (ODR; left) high food (1 mg C L^{-1}; adj. r^2 = 0.39, p = 0.16) and low food (0.25 mg C L^{-1}; adj. r^2 = 0.16, p = 0.28) and Russell Pond (RP) high (adj. r^2 = 0.00, p = 0.94) and low food *Daphnia pulex*. Concentrations of *Microcystis* range left to right from 0-100%, n=3 in all cases.
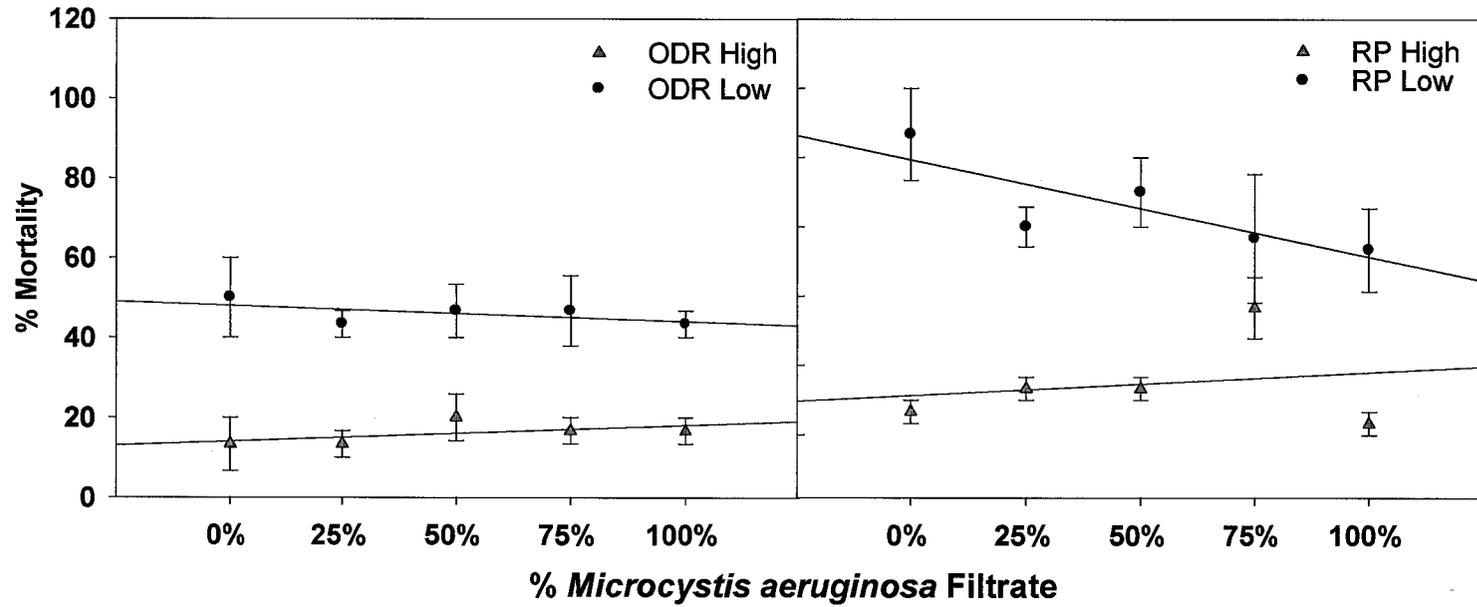
Figure 7: Average mortality (±1 SE) at day five for *Daphnia pulex* from Old Durham Reservoir (ODR; left) high food (1 mg C L^{-1}; adj. r^2 = 0.10, p = 0.32) and low food (0.25 mg C L^{-1}; adj. r^2 = 0.10, p = 0.32) and from Russell Pond (RP) high (adj. r^2 = 0.00, p = 0.74) and low (adj. r^2 = 0.57, p = 0.09) food. Concentrations of *Microcystis* range left to right from 0-100%, n=3 in each case.
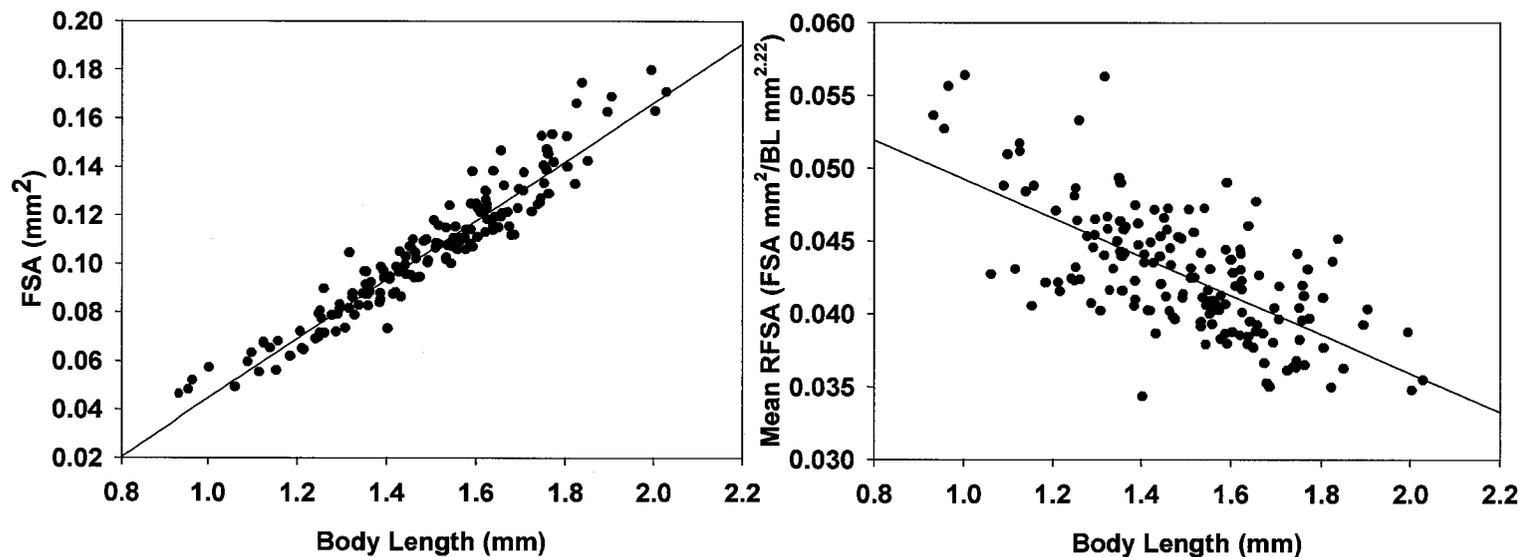
Figure 8: Body length vs. Filter Screen Area (FSA; left, adj. r^2 = 0.913, p<0.001, n=158) and body length versus the Relative Filter Surface Area (RFSA = FSA / body length$^{2.22}$; right, adj. r^2 = 0.441, p<0.001, n=158) in the chemical cue experiments.
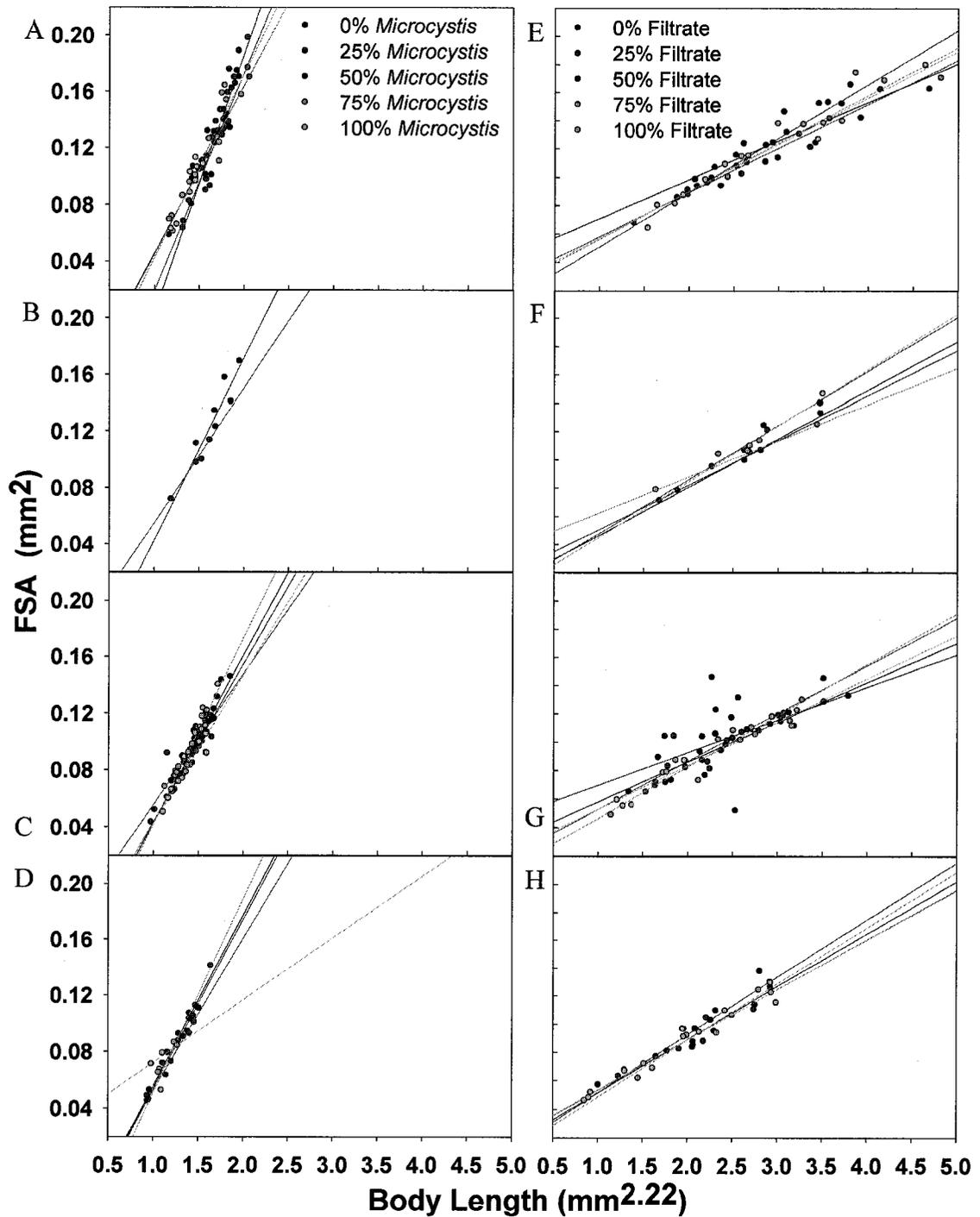
Figure 9: Corrected body length vs. Filter Surface Area (FSA) for *Daphnia pulex* from (left; A) Russell Pond high (1.0 mg C L^{-1}) food, B) Russell Pond low (0.25 mg C L^{-1}) food, C) Old Durham Reservoir high food, D) Old Durham Reservoir low food) and chemical cue (right; E) Russell Pond high (1.0 mg C L^{-1}) food, F) Russell Pond low (0.25 mg C L^{-1}) food, G) Old Durham Reservoir high food, H) Old Durham Reservoir low food) experiments.

45
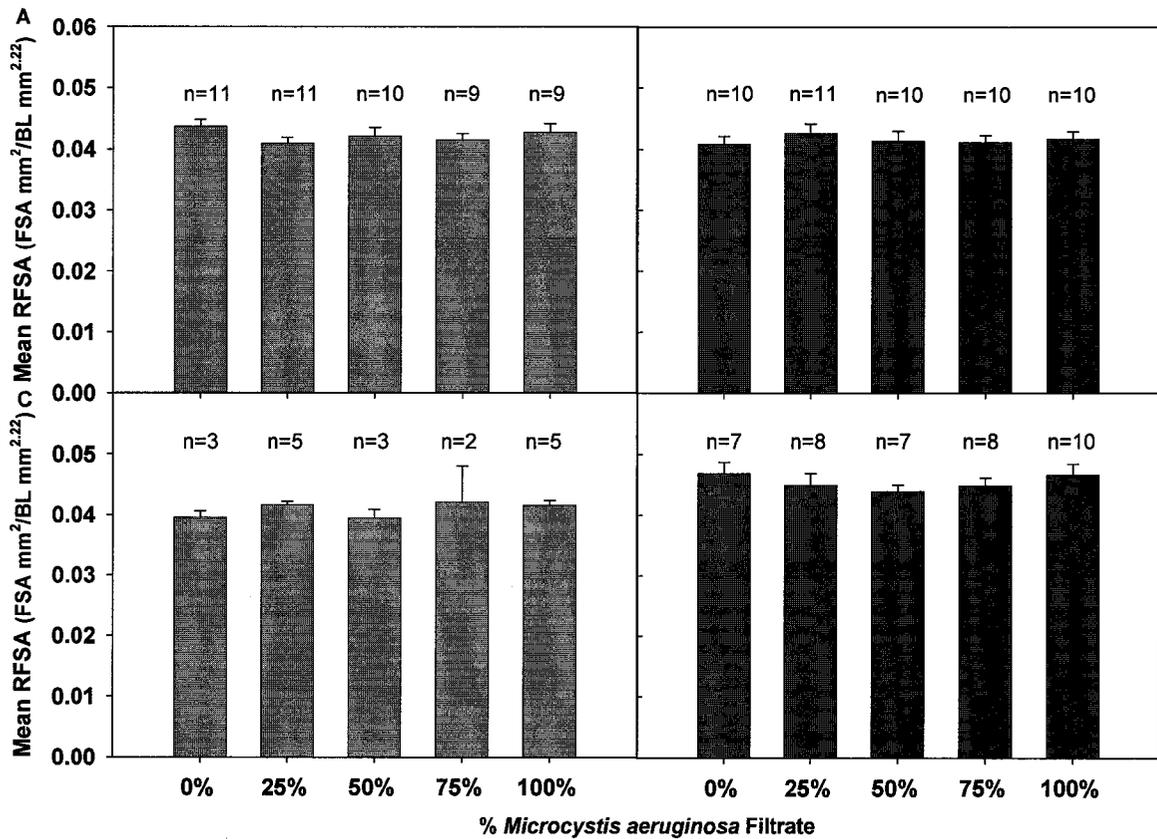
Figure 10: Mean Relative Filter Surface Area (RFSA; +1 SE) for each food treatment in the chemical cue experiment for Russell Pond (RP; a) High (1.0 mg C L^{-1}) food and c) Low (0.25 mg C L^{-1}) food) and Old Durham Reservoir (ODR; b) High food and d) Low food) Daphnia pulex. p > 0.05 in all experiments (ANOVA), concentrations of Microcystis range left to right from 0-100%

46
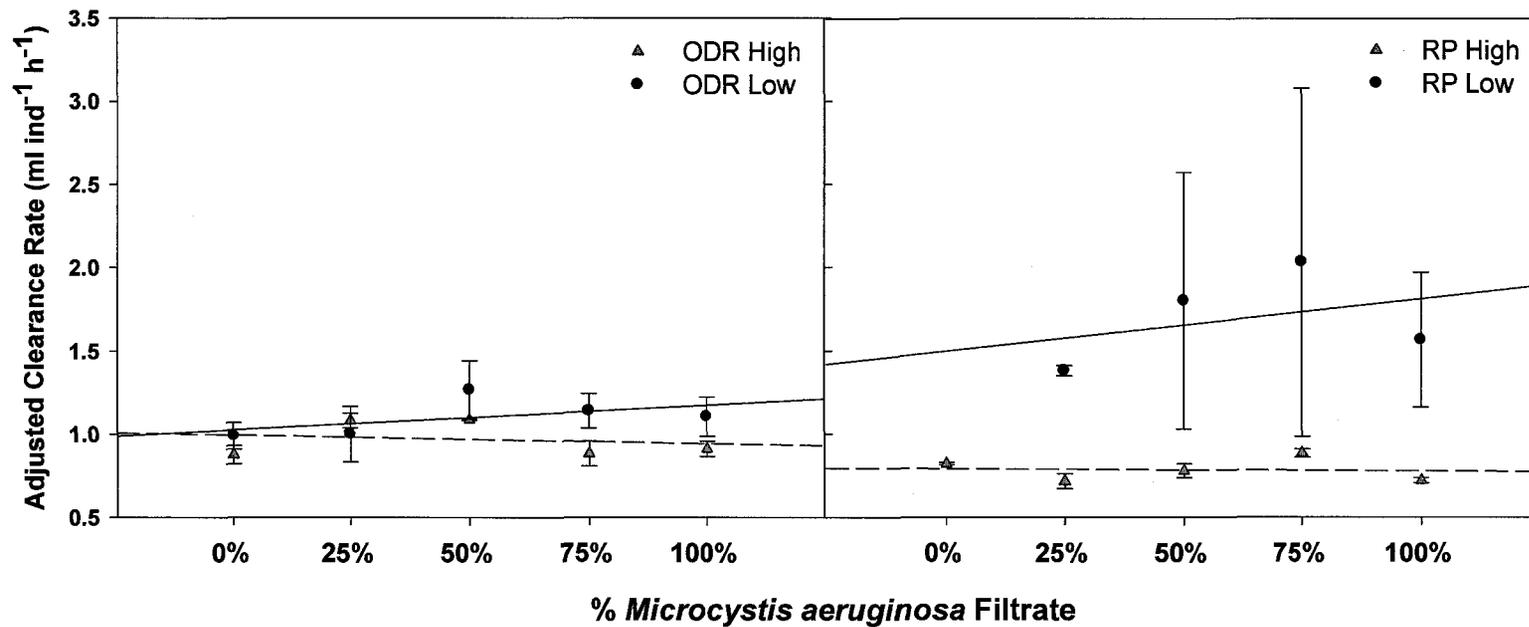
Figure 11: Average clearance rate (± 1 SE) adjusted to a body length of 1.5 mm in each chemical cue effects treatment for Old Durham Reservoir (ODR; left) high food (1 mg C L^{-1}; adj. $r^2 = 0.00$ p = 0.76) and low food (0.25 mg C L^{-1}; adj. $r^2 = 0.02$, p = 0.37) and Russell Pond (RP) high (adj. $r^2 = 0.00$, p = 0.91) and low (adj. $r^2 = 0.00$, p = 0.64) food *Daphnia pulex*. Concentrations of *Microcystis* range left to right from 0-100%, n=3 in each case.
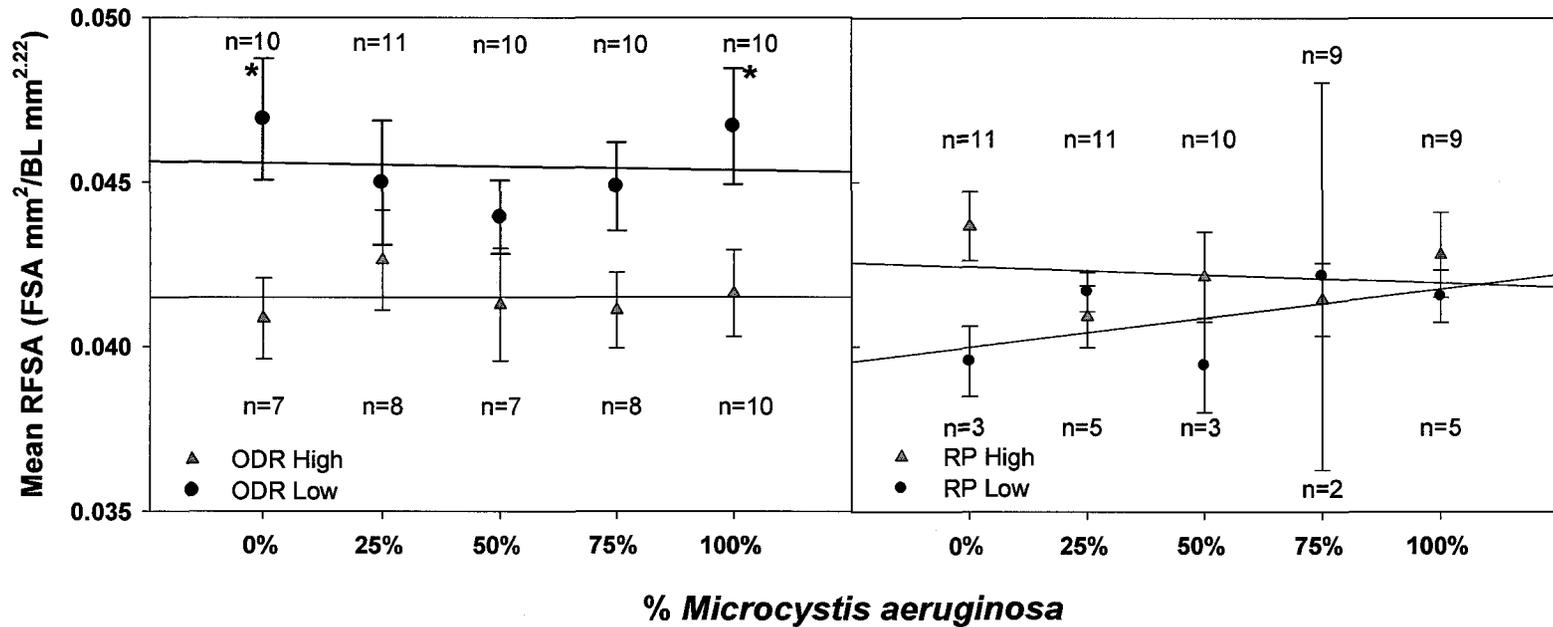
Figure: 12: Comparison of mean Relative Filter Surface Area (RFSA; ±1 SE) in high (1.0 mg C L^{-1}) and low food (0.25 mg C L^{-1}) treatments for Old Durham Reservoir (ODR; left) and Russell Pond (RP; right) *Daphnia pulex* in the chemical cue experiments. (*) indicates significant difference between high and low food RFSA in that treatment (ANOVA, p < 0.001). Regression statistics are as follows: ODR High: adj. r^2 = 0.00, p = 0.99, f= 0.041 + 7.67x10^{-8} * % *M. aeruginosa* ; ODR Low: adj. r^2 = 0.00, p = 0.92, f= 0.046 – 2.12x10^{-6} * % *M. aeruginosa*; RP High: adj. r^2 = 0.00, p = 0.77, f= 0.042 – 4.92x10^{-6} * % *M. aeruginosa*; RP Low: adj. r^2 = 0.07, p = 0.34, f= 0.040 + 1.77x10^{-5} * % *M. aeruginosa*. Concentrations of *Microcystis* range left to right from 0-100%.
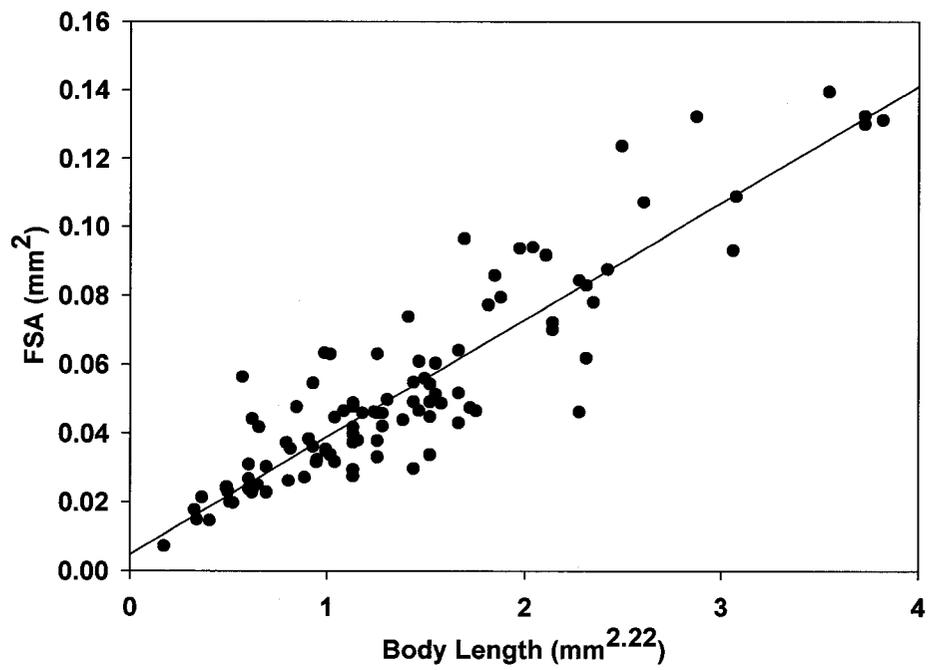
Figure 13: Corrected body length vs. Filter Surface Area (FSA) for all *Daphnia spp.* in the natural populations analysis (adj. $r^2 = 0.82$, $p < 0.001$, n=99).
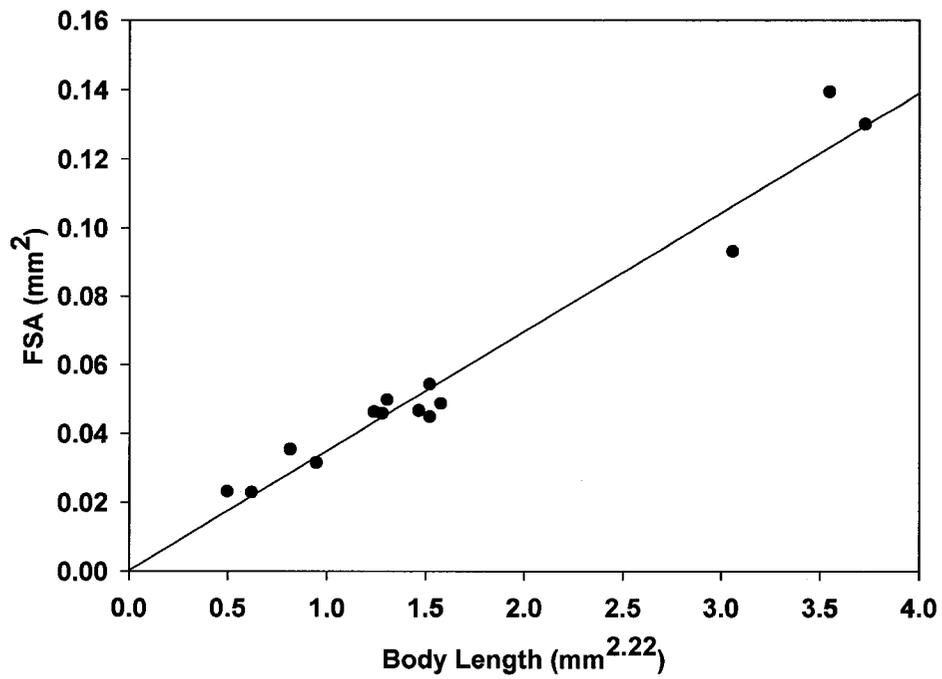
Figure 14: Corrected body length vs. Filter Surface Area (FSA) for Swains Lake *Daphnia schødleri* used in the natural populations analysis (adj. $r^2 = 0.96$, $p < 0.001$, n=14).

50
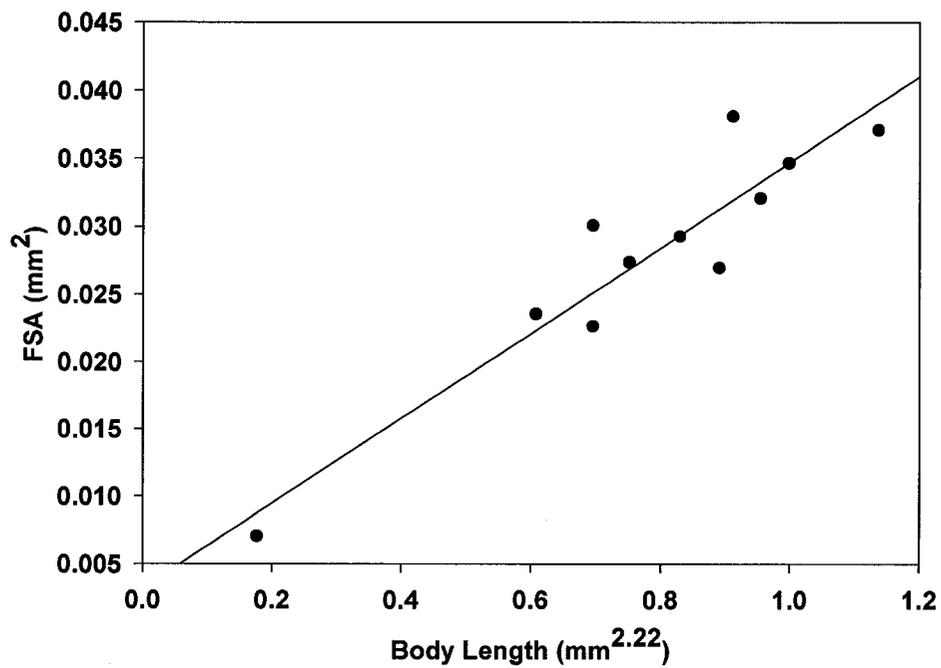
Figure 15: Corrected body length vs. Filter Surface Area (FSA) for Otternic Pond *Daphnia catawba* used in the natural populations analysis (adj. r^2 = 0.85, p < 0.001, n=11).

51
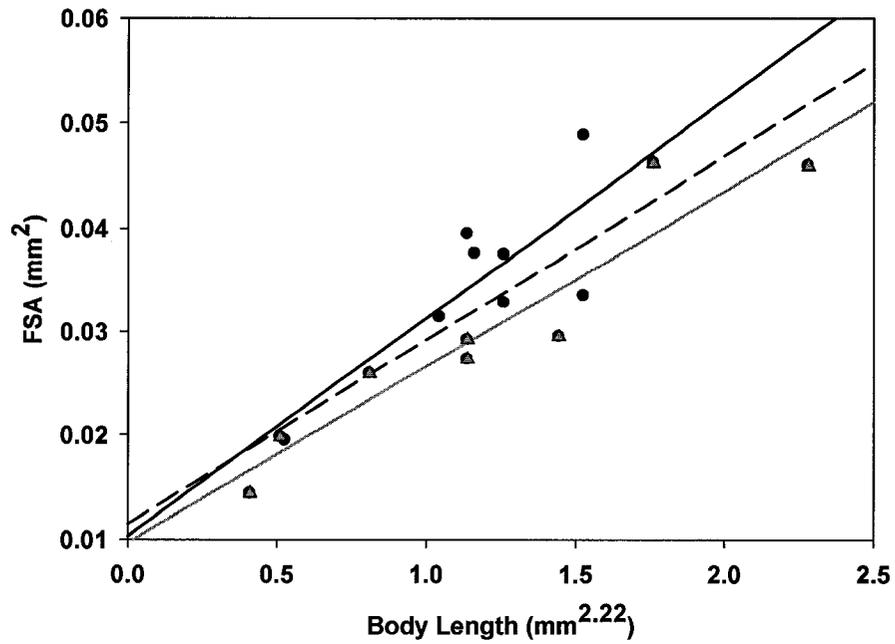
Figure 16: Body length vs. Filter Surface Area (FSA) for each Horseshoe Pond species of *Daphnia*: *D. catawba* (black circles, black line, n=8) and *D. dubia* (grey triangles, grey line, n=8). The dashed line represents the combined *Daphnia* (adj. r^2 = 0.72, p < 0.001, n=16).

52
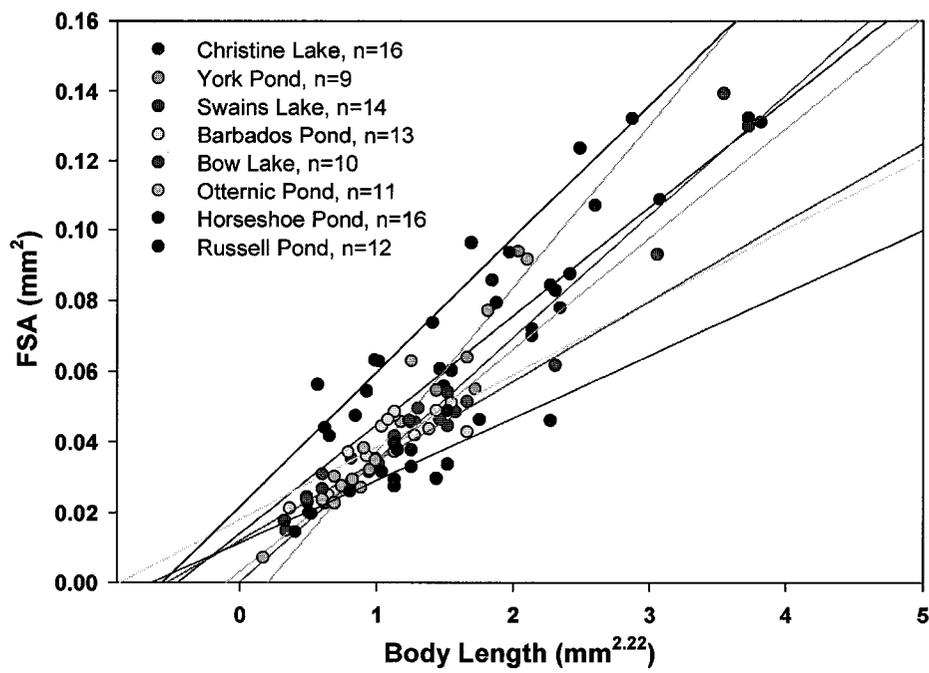
Figure 17: Corrected body length vs. Filter Screen Area (FSA) for all *Daphnia spp.* used in the natural populations analysis, grouped by lake.
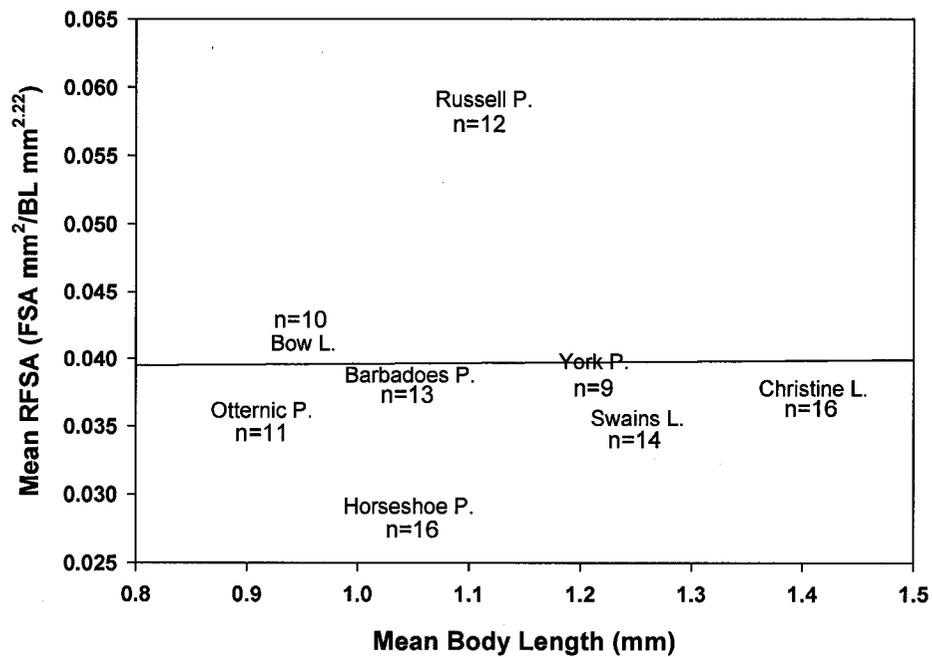
Figure 18: Mean body length vs. Mean Relative Filter Surface Area (RFSA) for all *Daphnia* spp. from each lake in the natural populations analysis (adj. $r^2 = 0$, $p = 0.985$).

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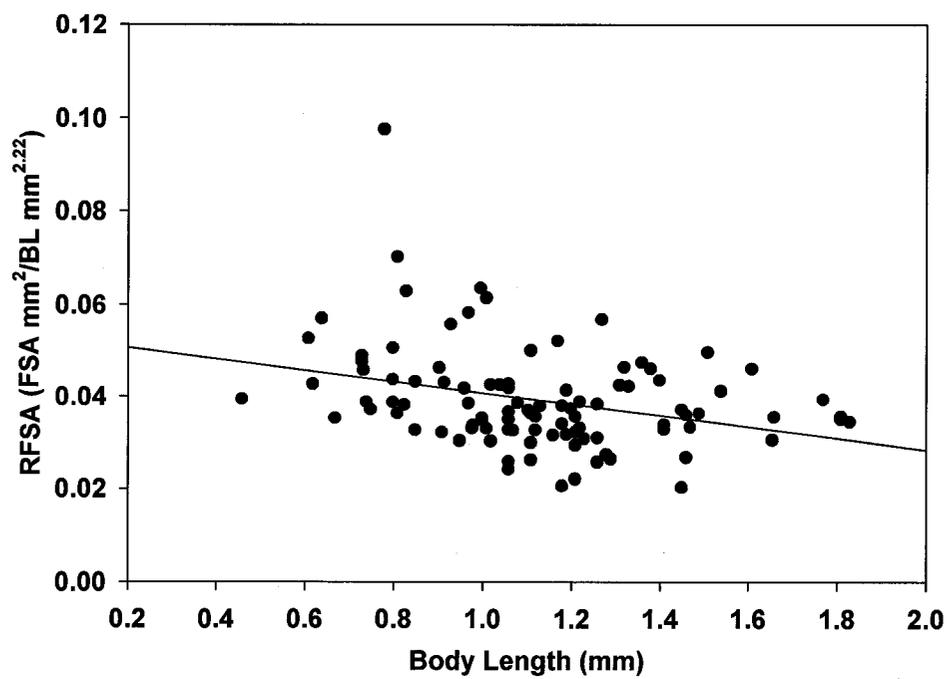
Figure 19: Body length vs. Relative Filter Surface Area (RFSA) for all *Daphnia spp.* included in the natural populations analysis (adj. r^2 = 0.089, p = 0.009, n=99).
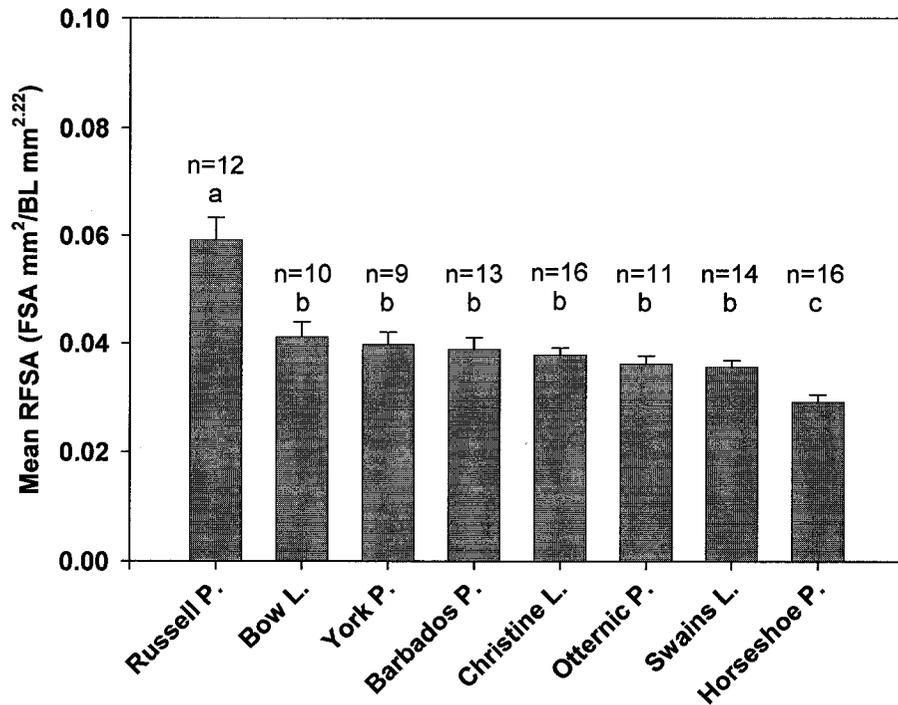
Figure 20: Mean *Daphnia* Relative Filter Surface Area (RFSA; +1 SE) values for each lake included in the natural populations analysis. Different letters indicate significant difference (ANOVA, p < 0.001).
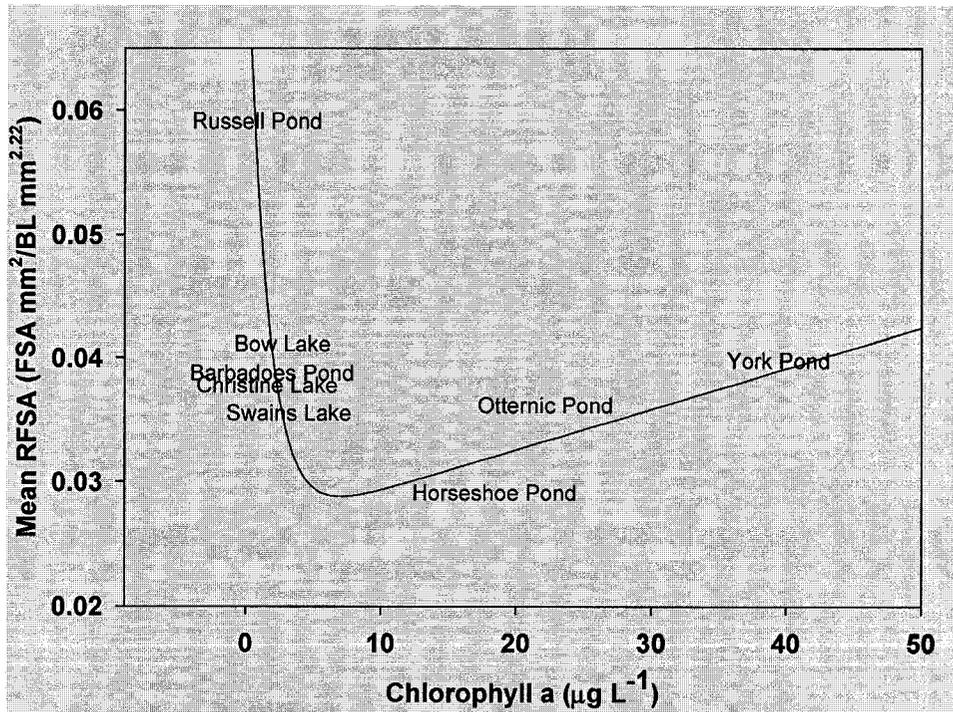
Figure 21: Relationship between Chlorophyll a and the mean Relative Filter Surface Area (RFSA) of each lake in the natural populations analysis using an exponential model (adj. r^2 = 0.88, p = 0.034; f = 0.026 + 0.051$^{-0.658 \, * \, Chl \, a}$ + 0.0003*Chl. a).
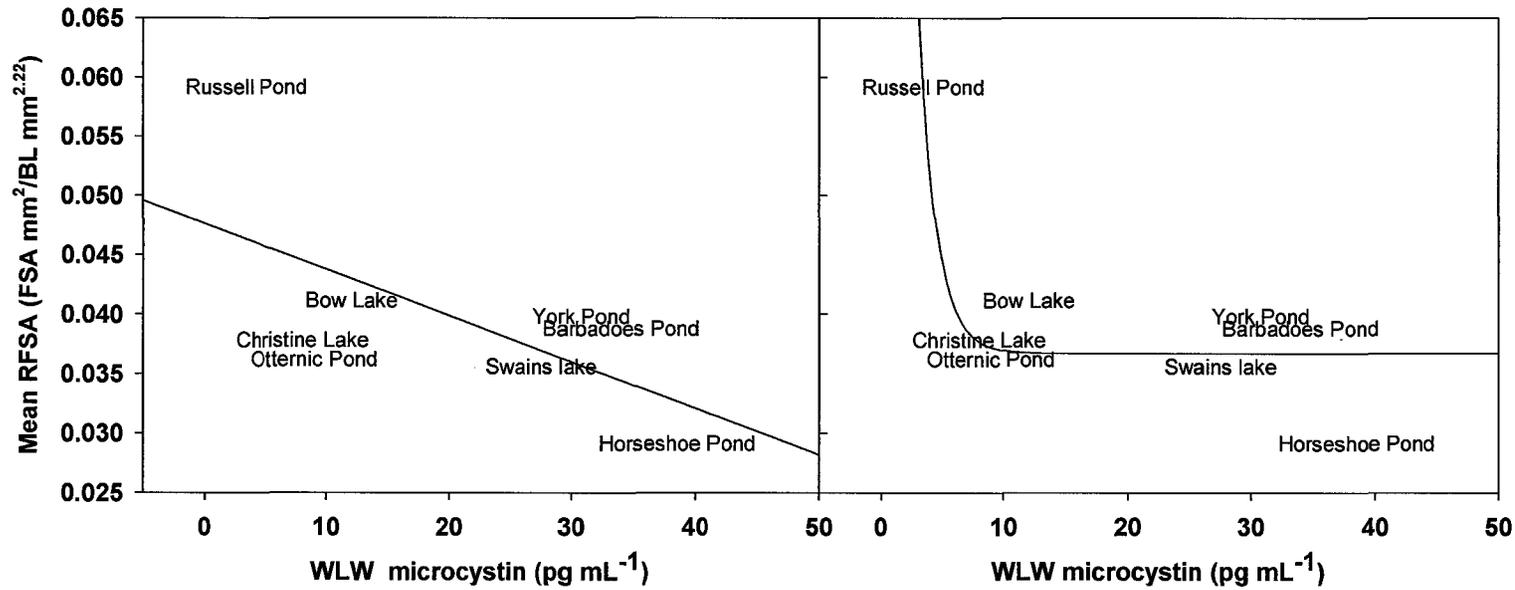
Figure 22: Linear (left; adj. r^2 = 0.274, p = 0.105, f = 0.048 + 0.0004 * WLW microcystin) and exponential (right; adj. r^2 = 0.752, p = 0.013; f = $0.037 + 0.240^{-0.687 \text{ * WLW microcystin.}}$) relationship between whole lake water microcystin levels and mean Relative Filter Surface Area (RFSA) for each lake in the natural populations analysis.
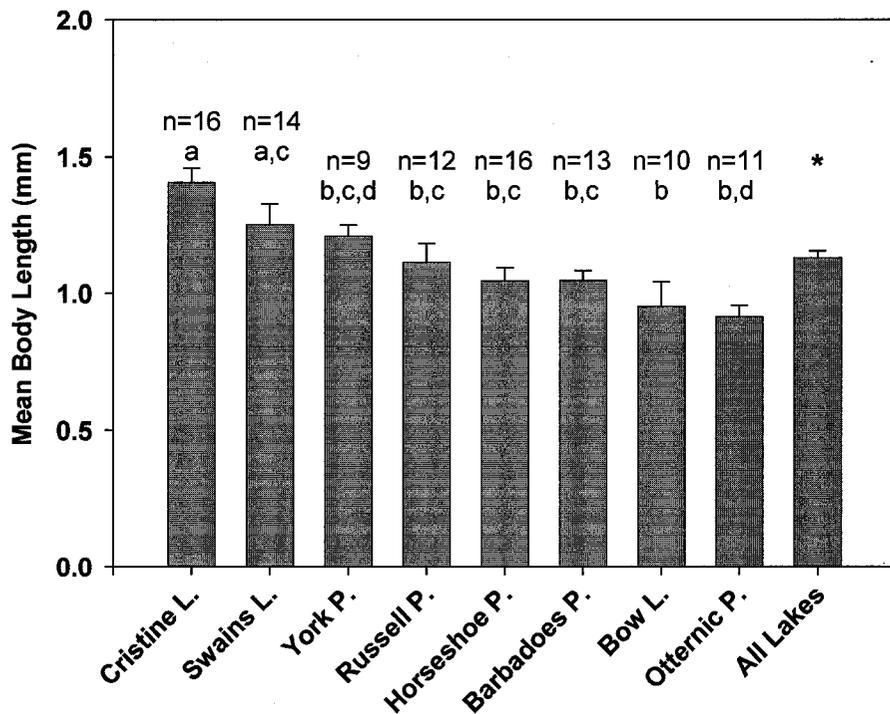
Figure 23: Mean body length of *Daphnia* (+1 SE) from each lake included in the natural populations analysis. Different letters signify significant difference (ANOVA, p < 0.05).
* The mean body length for all lakes was not included in the ANOVA.

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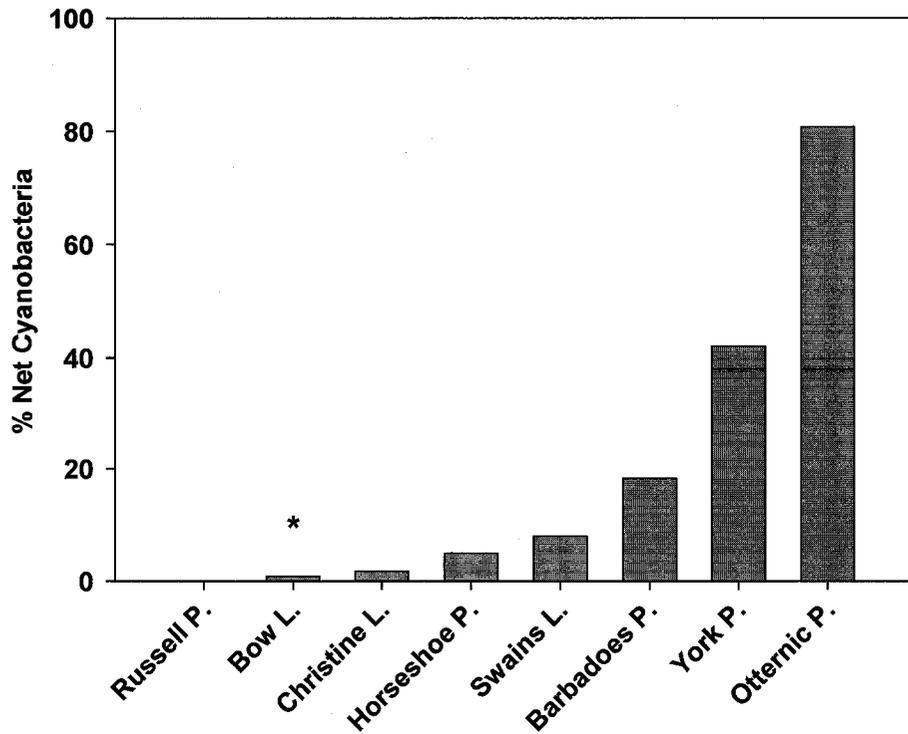
Figure 24: Relative abundance of cyanobacteria in the net phytoplankton (>50μm) in each lake included in the natural populations analysis.
* data for this lake were from a collection date one month earlier in the previous year.

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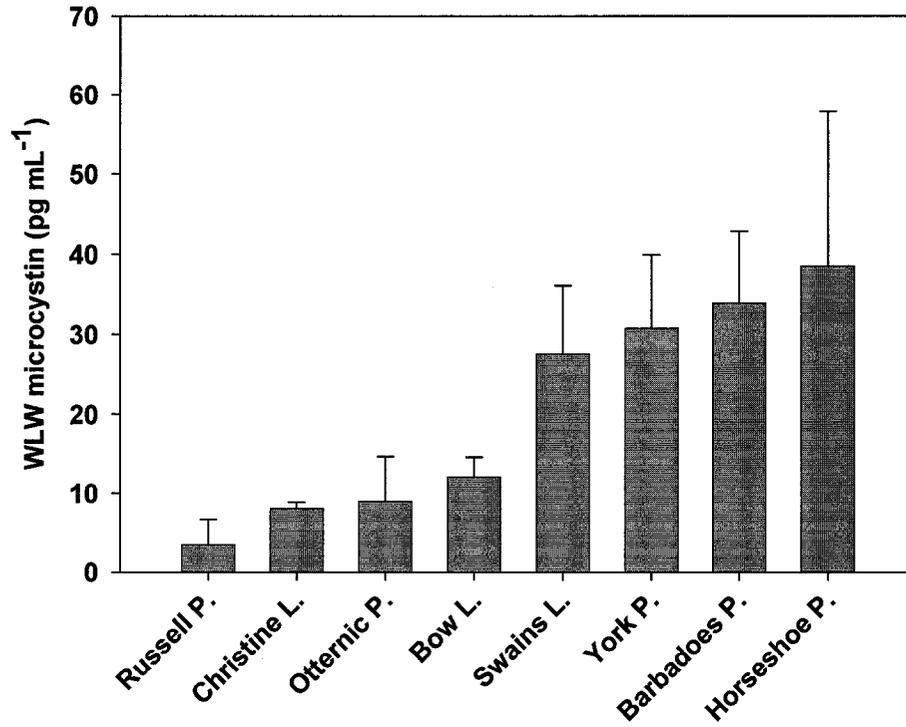
Figure 25: Mean concentration of whole lake water (WLW)
microcystins (+1 SE) in each lake included in the natural populations
analysis. n=3 in each case

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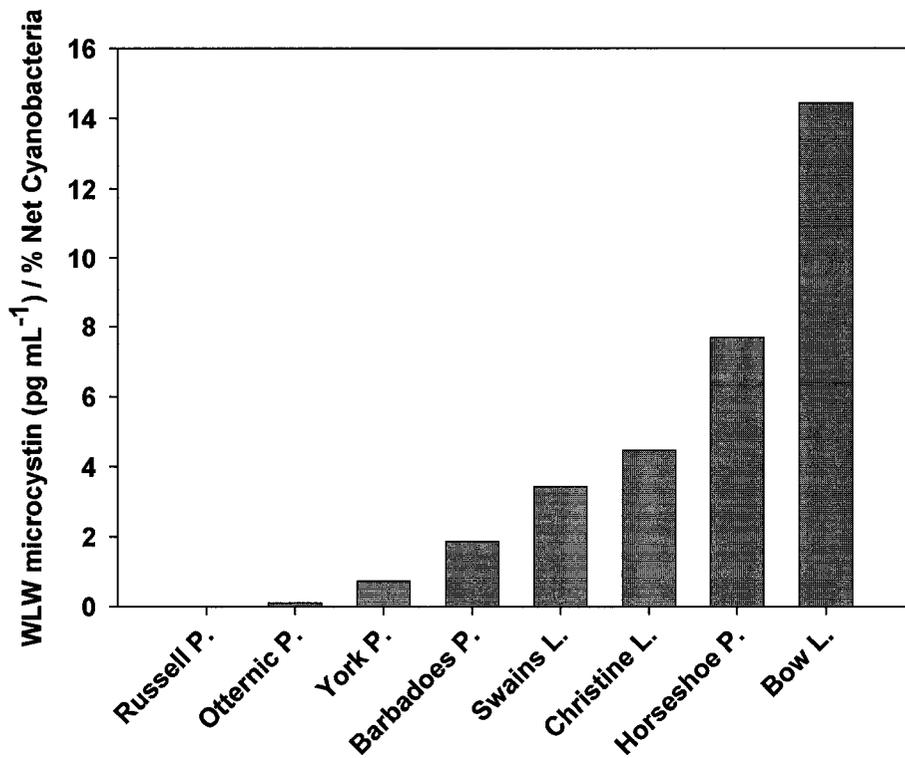
Figure 26: Concentration of whole lake water (WLW) microcystins relative to the proportion of cyanobacteria in the net phytoplankton (<50μm) in each lake.
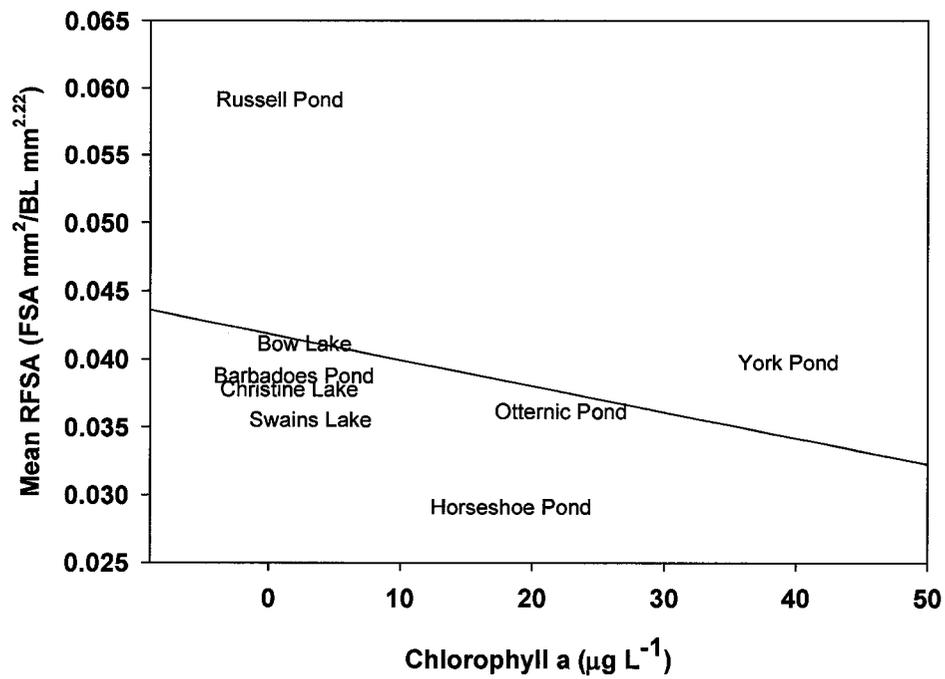
Figure 27: Concentration of chlorophyll a vs. mean Relative Filter Surface Area (RFSA) for all lakes included in the natural populations analysis (adj. r^2 = 0.00, p = 0.45).

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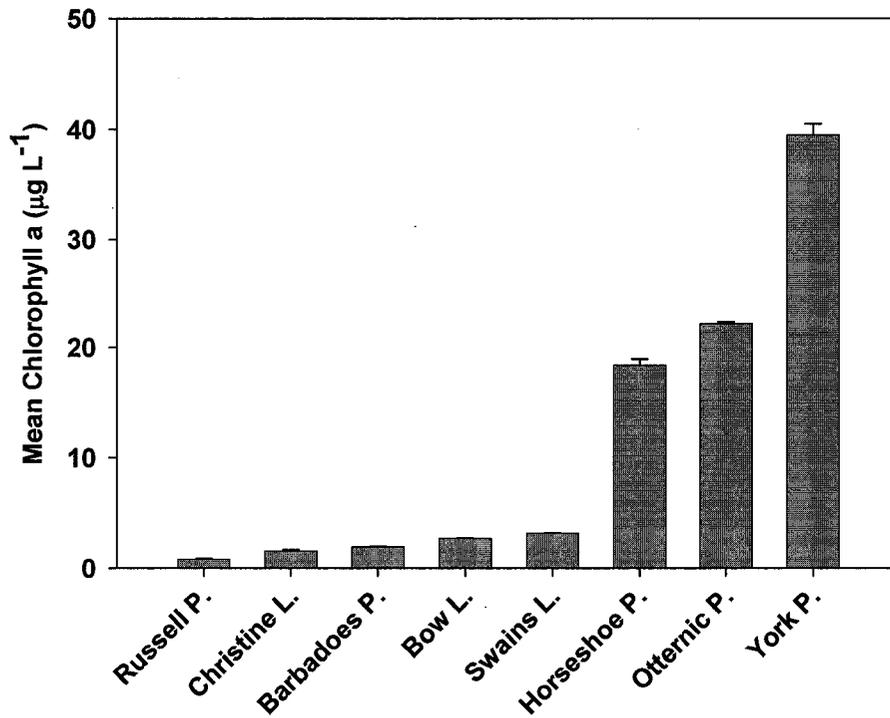
Figure 28: Mean chlorophyll a concentration (+1 SE) for each lake included in the natural populations analysis. n=3 in each case.

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APPENDIX

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APPENDIX

Additional Tables and Figures

Table A1: Dates and duration of experimental treatments.			
Experiment	Treatment	Start Date	End Date
Part 1: *Microcystis*	ODR: High Food	7-Dec-2006	17-Dec-2006
Part 1: *Microcystis*	ODR: Low Food	31-Jan-2007	18-Dec-2007
Part 1: *Microcystis*	RP: High Food	5-Feb-2007	17-Feb-2007
Part 1: *Microcystis*	RP: Low Food	1-Mar-2007	19-Mar-2007
Part 2: *Microcystis* Filtrate	ODR: High Food	11-Apr-2007	23-Apr-2007
Part 2: *Microcystis* Filtrate	ODR: Low Food	25-Apr-2007	9-May-2007
Part 2: *Microcystis* Filtrate	RP: High Food	28-Apr-2007	9-May-2007
Part 2: *Microcystis* Filtrate	RP: Low Food	7-Apr-2007	23-Apr-2007

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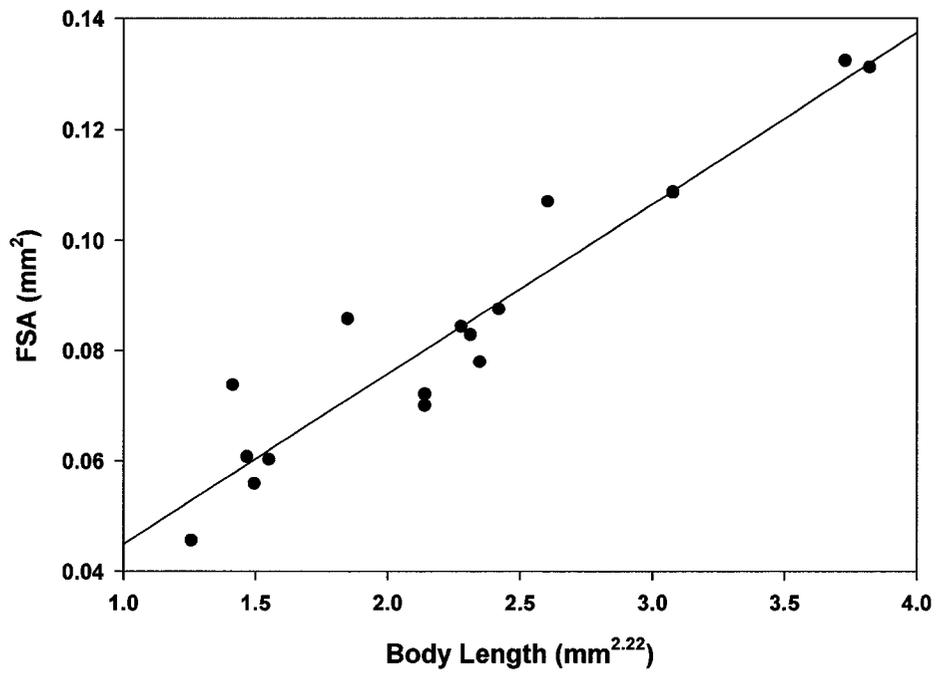
Figure A1: Corrected body length vs. Filter Surface Area (FSA) for Christine Lake *Daphnia laevis* used in the natural populations analysis (adj. r^2 = 0.89, p < 0.0001, n=14).

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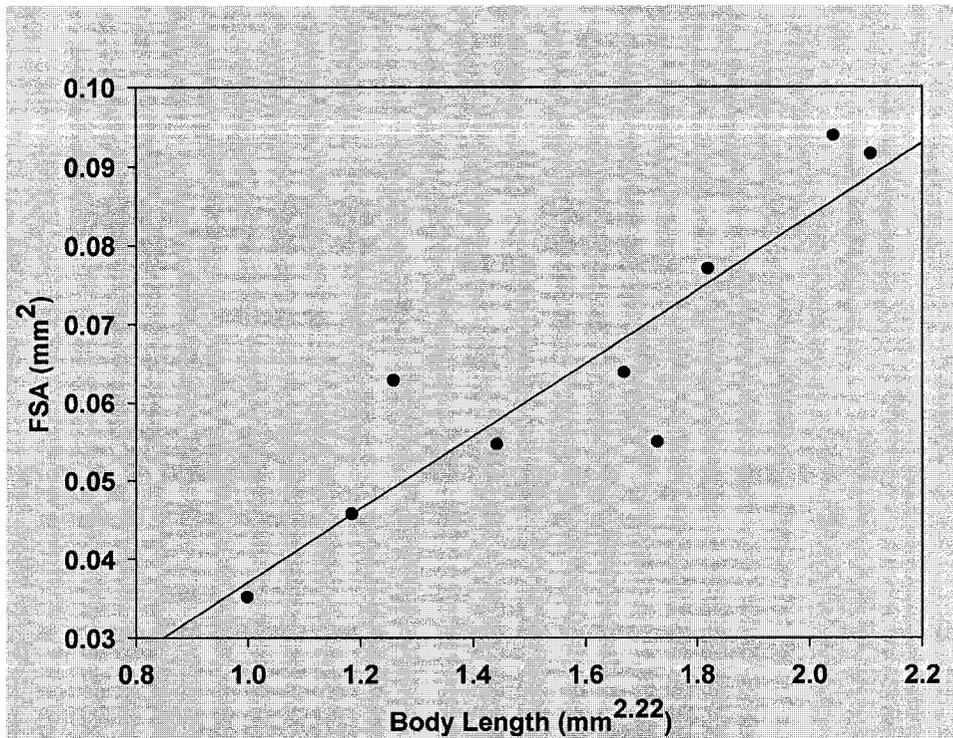
Figure A2: Corrected body length vs. Filter Surface Area (FSA) for York Pond *Daphnia catawba* used in the natural populations analysis (adj. $r^2 = 0.78$, $p < 0.001$, n=9).

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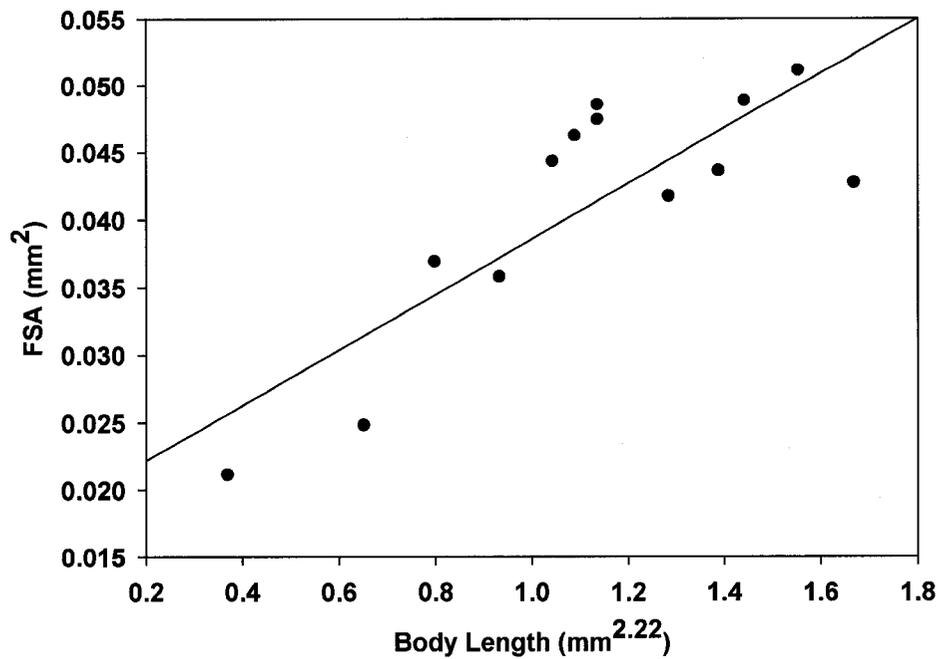
Figure A3: Corrected body length vs. Filter Surface Area (FSA) for Barbadoes Pond *Daphnia rosea* used in the natural populations study (adj. $r^2 = 0.65$, $p < 0.001$, n=13).

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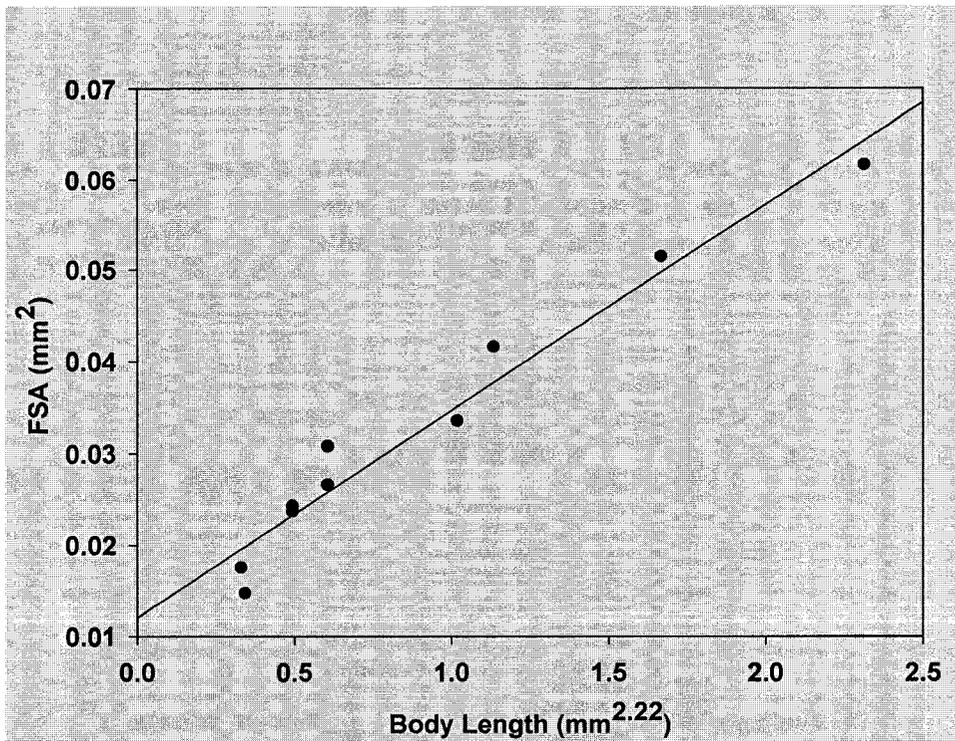
Figure A4: Corrected body length vs. Filter Surface Area (FSA) for Bow Lake *Daphnia catawba* used in the natural populations study (adj. $r^2 = 0.95$, $p < 0.0001$, n=10).
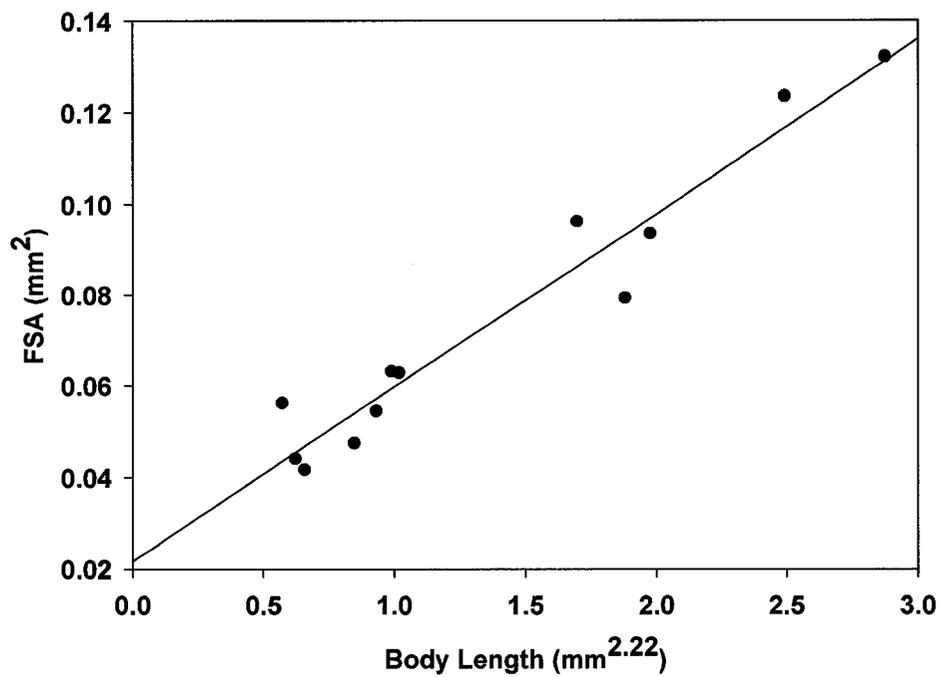
Figure A5: Corrected body length vs. Filter Surface Area (FSA) for Russell Pond *Daphnia pulex* used in the natural populations study (adj. $r^2 = 0.94$, $p < 0.0001$, n=12).