The Effects of Hydrologic Residence Time on Consumer-Mediated Nutrient Cycling and Algal Growth

BIOS 35502-01: Practicum in Field Biology

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ABSTRACT

Nutrient cycling dynamics have great influence over primary production and food webs in lake ecosystems. Zooplankton are a trophic level above phytoplankton, meaning that their nutrient demands will effect the rest of the upper trophic levels. Ecological stoichiometry is a fairly new concept that can be used to link the nutrient ratios required by certain zooplankton to the nutrient ratios in their environment. Hydrologic residence time is another factor that plays an important role in determining the amount of nutrients available for consumption, depending on how often water is replenished from outside the system. The goal of this research was to explain the effects of hydrologic residence time on consumer-mediated nutrient recycling and algal growth in mesocosms of a lake ecosystem.

Calanoid copepods and the cladoceran *Daphnia spp.* were used in this experiment because copepods are known to have high intrinsic N:P while cladocerans have a much lower N:P. The results showed that both the type of consumer and residence time had significant effects on the rate of change of chlorophyll concentrations, a proxy for algal growth. Phosphorus limitation in the cladoceran treatments could not be seen directly through the rates of change of phosphorus concentration, but it could be inferred by the drastic difference in chlorophyll concentrations between copepod and cladoceran treatments. Chlorophyll concentrations in copepod treatments also decreased as residence time increased, due to less nutrient replenishment and increased consumer-mediated recycling.

Keywords: Ecological stoichiometry, hydrological residence time, nutrient cycling, zooplankton

INTRODUCTION

The cycling of nutrients through food webs is one of the most influential factors over the structure and function of a lake ecosystem (Sorf et al. 2015). Primary production is the base of all aquatic food webs, most of which comes from algal growth that is commonly constrained by phosphorus or nitrogen availability. Freshwater ecosystems tend to be limited by phosphorus, but this limitation can switch between nitrogen and phosphorus seasonally or based on anthropogenic nutrient inputs (Elser et al. 1988). Zooplankton communities play a large role in determining the amount of nutrients available for algae and higher trophic level organisms (Elser et al. 2000a). This means that the zooplankton community composition can determine the food web structure of the lake as a whole.

This idea that organisms can influence the amount of nutrients available in their environment and vice versa can be analyzed using ecological stoichiometry. Ecological stoichiometry is a fairly new concept that concerns the relative amounts of certain elements in organisms and the environment, linking nutrient cycling to the interactions between zooplankton communities and algal growth. It uses the idea that different levels of biology, from molecules all the way up to organisms, have different elemental compositions that influences their function and their external environment (Elser and Urabe 1999).

Zooplankton are at the bottom of the food web, making their stoichiometric demands very influential in determining nutrient concentrations available for higher trophic levels. Their community composition varies between aquatic systems, and different consumers have different nutrient requirements. Two groups of zooplankton found in lake systems are crustaceans in the orders Calanoida and Cladocera, commonly called copepods and cladocerans. Both of these organisms need nitrogen and phosphorus to grow and reproduce, but they use these elements in different ratios relative to their environment and each other. Cladocerans preferentially retain more phosphorus as biomass than nitrogen, while copepods retain more nitrogen than phosphorus, causing them to recycle nitrogen and phosphorus with different efficiencies (Elser and Urabe 1999). Previous studies have shown that these stoichiometric constraints cause consumers to change the ratio of nitrogen to phosphorus, N:P, in the environment and the amount of nutrients available to algae (Elser et al. 2000a). Copepods would allow for more available phosphorus and cladocerans would allow for more available nitrogen for other organisms to use. The limiting nutrient in an ecosystem can be determined as a result of these trophic interactions.

Hydrologic residence time defines the average amount of time water stays in a particular aquatic system, and it is another factor that can influence nutrient cycling and availability.

Residence time is determined by the environmental conditions surrounding a system, including hydrological processes and geographical features. It depends heavily on rates of water loss to the ground or atmosphere, rates of water input from precipitation and runoff, and the geological nature of the bedrock (Dunn et al. 2007). Bodies of water with shorter residence times could mean that nutrients are being replaced more frequently by runoff, lessening the nutrient limitations of organisms. As the hydrologic residence time increases, nutrients are not replaced as often from outside the system, so internal nutrient recycling and its associated trophic interactions become significantly more important. The frequency of nutrient replenishment is how hydrologic residence time may influence the importance of consumer-mediated nutrient recycling.

My research seeks to describe the relationship between this consumer-mediated nutrient recycling and a system's hydrologic residence time, something that has not been extensively studied in previous literature. My research questions include: how will the presence of different consumers alter the availability of nutrients for algal growth? How will this nutrient availability be affected across a hydrologic residence time gradient? To test these questions, I used experimental mesocosms that manipulated hydrologic residence time and the type of consumer in the system. I hypothesized that the dominant presence of cladocerans will result in a phosphorus-limited system, while the dominant presence of copepods will result in a nitrogen-limited system. Consumer-mediated nutrient recycling becomes more important as hydrologic residence time increases because nutrients are not replenished as frequently. At long residence times, the system will become more dependent on nutrient cycling between algae and zooplankton, and the presence of certain consumers will dictate nutrient limitation. I would expect to observe more severe nutrient limitation and less algal growth as residence time increases, than at shorter residence times where the external nutrient supply is more important.

MATERIALS AND METHODS

Study Lake

Bay Lake is ~170 acres in area and reaching 14 meters in depth. Water and zooplankton from Bay Lake were used in the experimental mesocosms. Like all lakes on the UNDERC property, Bay Lake is limited by phosphorus. The ratio of N:P found naturally is 46:1, 398.44 μg/L of nitrogen and 19.32 μg/L of phosphorus. Bay Lake also has a zooplankton community consisting largely of calanoid copepods and *Daphnia spp.*, the two organisms of interest.

Methods

Experimental Design – Mesocosms were used to allow for control over both the zooplankton community and hydrologic residence time, and to prevent any confounding variables. They were set up using 18, 5-gallon buckets filled with 10 liters of water from Bay Lake that was filtered through a 153-micron sieve to remove any zooplankton. Nutrients were added to each mesocosm to ensure that any changes in concentrations could be detected. 225.63 μg/L of nitrogen were added in the form of ammonium nitrate, and 19.98 μg/L of phosphorus were added using monopotassium phosphate. Plankton tow nets were used to collect calanoid copepods and cladoceran *Daphnia spp.* from Bay Lake. Copepods were added to nine mesocosms while cladocerans were added to the other nine, according to twice the density found in 10 liters of natural lake water. Twice the natural density was used instead of equal density to help account for death and displacement.

Hydrologic Residence Times –Three different hydrologic residence times were tested during this experiment: 2 days, 5, days, and 10 days. Each of these residence times were tested on copepods and cladocerans separately, creating six unique treatments. Each treatment was replicated three

times, totaling 18 individual mesocosms. Two treatments, three mesocosms containing copepods and three containing cladocerans, represented a residence time of two days, where five liters of water were replaced everyday, so that the 10 liters of water was replaced every two days. Two treatments represented a five-day residence time, where two liters of water were replaced everyday, and the last two represented a ten-day residence time, where one liter of water was replaced daily. In addition to the water that was replaced, the fraction of the initial nutrient spike removed with the water was also replaced (Table 1). Every week, one half ambient density of zooplankton was added to their respective mesocosms to account for any organisms lost during water exchange.

Sampling – The experiment ran for a duration of 21 days. Nutrient samples were taken every three days to measure the concentrations of phosphorus and nitrate. Chlorophyll samples were taken at the beginning, end, and midpoint of the experiment.

Analysis

Phosphorus – Phosphorus concentration was determined by reacting water samples with a reagent consisting of molybdic acid, ascorbic acid, sulfuric acid, and potassium antimonyl-tartrate. The sample absorbance at 885 nm was then determined by spectrophotometer, and phosphorus concentration was calculated from a standard curve (Wetzel and Likens 1991).

Nitrate – Nitrate concentration was determined using a spectrophotometer to measure the absorbance of each sample at multiple wavelengths between 200 and 250 nm. The maximum of

the second derivative of the eight wavelengths recorded was used to calculate the concentration of nitrate from a standard curve (Olsen 2008).

Chlorophyll – Chlorophyll *a* was extracted using methyl alcohol and analyzed using a fluorometer.

Statistical Tests

A two-way analysis of variance (ANOVA) was used to determine the significance of the type of consumer, hydrologic residence time, and the interaction between these two variables on the mean rates of change of nutrient and chlorophyll concentrations. The mean rates of change were calculated by finding the slope between each set of time points and taking the average of all the slopes. A separate ANOVA was used for nitrate, phosphate, and chlorophyll.

RESULTS

Three separate, two-way ANOVAs were run on the six treatments to determine the effects of consumer type and hydrologic residence time on the rates of change of phosphorus, nitrate, and chlorophyll concentrations. A significance level of 0.05 was used for all statistical tests.

Phosphorus - There was no significant effect of residence time ($p = 0.216$) or consumer type ($p = 0.985$) on the rate of change of phosphorus concentration. All phosphorus concentrations began high and dropped steeply between the first two sampling days (Figure 1). All concentrations stayed between 0 – 10 μg/L for the rest of the experiment. The mean rates of change in phosphorus concentrations were all negative, ranging from 0 to -4 μg/L per day and averaging between 0 and -2 μg/L per day (Figure 7).

Nitrate – There was a significant effect of residence time on rate of change of nitrate concentration, $F(2, 12) = 9.938$, $p = 0.00285$. A multiple pairwise-comparison between residence times showed that this significance was found between residence times of 5 days and 10 days ($p = 0.0161$) and between 2 days and 5 days ($p = 0.0030$), but not between 2 days and 10 days ($p = 0.6244$). All rates of change were negative, and the 5-day residence time had a higher rates of change than both the 2-day and 10-day residence times (Figure 8). There was no significance in the relationship between consumer and rate of change in nitrate concentration ($p = 0.2975$), nor was there a significant interaction between consumer and residence time. This can be seen in the varying nitrate concentrations over time for all treatments over the entire length of the experiment (Figures 3 and 4).

Chlorophyll – There was a significant effect of consumer type on rate of change of chlorophyll concentration, $F(1, 12) = 38.729$, $p = 0.0000443$. Residence time was not statistically significant at $\alpha = 0.05$ but was at $\alpha = 0.1$ ($p = 0.0527$). A multiple pairwise-comparison between residence times showed that this significance was found between residence times of 2 days and 10 days ($p = 0.0465$) but not between 5 and 10 days ($p = 0.6452$) or between 2 days and 5 days ($p = 0.2111$) (Figure 6). There was also a significant interaction between consumer type and residence time on rate of change of chlorophyll concentration, $F(2, 12) = 4.039$, $p = 0.0456$. Chlorophyll concentrations continuously increased in the copepod treatments, also increasing with decreasing residence time (Figure 5). Cladoceran treatments did not exhibit this same pattern as their concentrations increased slightly between the first two sample and then decreased again between the last two.

DISCUSSION

My hypothesis was partially supported by the statistically significant interaction between consumer type and residence time on the rate of change in chlorophyll concentrations. Final chlorophyll concentrations were much higher in the copepod treatments than in the cladoceran treatments (Figure 5), as well as rates of changing chlorophyll concentration (Figure 6). Because algal growth is usually limited by phosphorus, this supports the hypothesis that copepods recycle phosphorus with more efficiency than cladocerans. Algal growth was higher in the copepod treatments because the organisms allowed there to be more phosphorus available to be used relative to the cladoceran treatments. Additionally, *Daphnia* may have limited algal growth by being one of the most non-selective and powerful filter feeding groups of zooplankton, consuming a lot of the algae that managed to grow (Kasprzak and Lathrop 1997). Residence time was also a factor because of its significant interaction with the zooplankton. Copepods were already recycling phosphorus with more efficiency than cladocerans, and at short residence times, even more phosphorus was being added and immediately used for algal growth. As less water was being frequently replaced, algal growth in copepod treatments decreased but was still higher than all cladoceran treatments. Residence time did not play as much of a role in cladoceran treatments because any phosphorus that was added was immediately being held by the *Daphnia* and was therefore unavailable for algae.

The high chlorophyll concentrations can also explain why the effects of residence time and consumer type on rate of change of phosphorus concentrations were insignificant. The phosphorus in the initial nutrient spike was immediately taken up either by *Daphnia* in the cladoceran treatments or by algae in the copepod treatments, and then the concentrations leveled off between 0 and 10 micrograms/L for the rest of the experiment (Figures 1 and 2). The rates of change in

phosphorus concentrations were also relatively similar in all treatments (Figure 7). A significant difference in the amount of phosphorus used by the zooplankton themselves cannot be seen because any phosphorus recycled by them was used for algal growth.

The results of the two-way ANOVA did not show a statistically significant effect of consumer type on the rate of change in nitrate concentrations, contrasting my hypothesis that copepods would cause nitrogen limitation (Figure 8). Figures 4 and 5 show that nitrate concentrations varied for all treatments for the entire length of the experiment. This result was surprising because the intrinsic N:P ratio of copepods ranges from ~30-50:1 compared to that of *Daphnia* which is ~14:1 (Elser and Urabe 1999). Both the copepod and cladoceran treatments had approximately twice the amount of zooplankton naturally found in 10 liters of lake water, so it is unlikely that a skewed abundance of either organism could explain the lack of nitrogen limitation. However, it is possible that *Daphnia* were able to grow and reproduce much faster than anticipated, therefore holding more nitrate than expected and decreasing the difference in nitrate concentrations between the treatments.

There are two, more plausible explanations for the nitrate concentrations that were observed. First, Bay Lake is already very limited by phosphorus, with a 46:1 ratio of N:P. In an attempt to relieve some of that extreme limitation and to be able to detect changes in phosphorus concentration, nitrogen and phosphorus were added to all of the treatments. In a study done by Kasprzak and Lathrop (1997) on phytoplankton assemblages, when 200 μg/L of nitrogen were added to an already eutrophic lake, nutrient limitation did not occur. In this study, 225 μg/L of nitrogen were added, so it is possible that the amount of copepods in each mesocosm just did not need as much nitrogen as they were given. This could also explain why residence time still had a significant effect on the rate of change of nitrate concentrations. Nitrate concentrations remained

high at short residence times when more nitrogen was added to nitrogen that most likely had not been used instead of replenishing nitrogen concentrations like I hypothesized it would. As the residence time increased, less nitrogen was added, giving the zooplankton a chance to use more of it, even though still not as much as expected. The second explanation that I believe to be the most likely reason has to do with the form of nitrogen most preferred by zooplankton. Nitrogen was added to the mesocosms in the form of ammonium nitrate salt. Most phytoplankton and zooplankton prefer nitrogen in the form of ammonium when present (McCarthy et al. 1977). Given this information, the copepods were most likely using the ammonium provided to them and leaving the nitrate behind, explaining why there was no difference in the nitrate concentrations between the copepod and cladoceran treatments. If total nitrogen had been analyzed, a larger difference in the rates of change of nitrate may have been observed.

CONCLUSION

The theory behind ecological stoichiometry, that different classes of zooplankton recycle nutrients with different efficiencies and strongly influence algal growth, was supported by the rates of change of chlorophyll concentrations observed in this experiment. Although differences in the rate of change of phosphorus concentrations could not be seen directly, phosphorus limitation in the cladoceran treatments can be inferred from significantly lower chlorophyll concentrations, and algal growth, than in the copepod treatments. Residence time was also shown to be influential for algal growth by determining how often nutrients were replenished and available for use. Future research should use a different method to analyze total nitrogen, in order to better address nitrogen limitation and determine if it too is effected by consumers and hydrologic residence time.

TABLES

Consumer	**Copepods**			**Cladocerans**		
Residence Time	2 days	5 days	10 days	2 days	5 days	10 days
Amount of Water Replaced per Day	5 liters	2 liters	1 liter	5 liters	2 liters	1 liter
Concentration of Nutrients added per day	9.991 μg/L P 112.500 μg/L N	3.996 μg/L P 45.000 μg/L N	1.998 μg/L P 22.500 μg/L N	9.991 μg/L P 112.500 μg/L N	3.996 μg/L P 45.000 μg/L N	1.998 μg/L P 22.500 μg/L N

Table 1. Amount of water replaced and amount of nutrients added each day for different residence times.

FIGURES

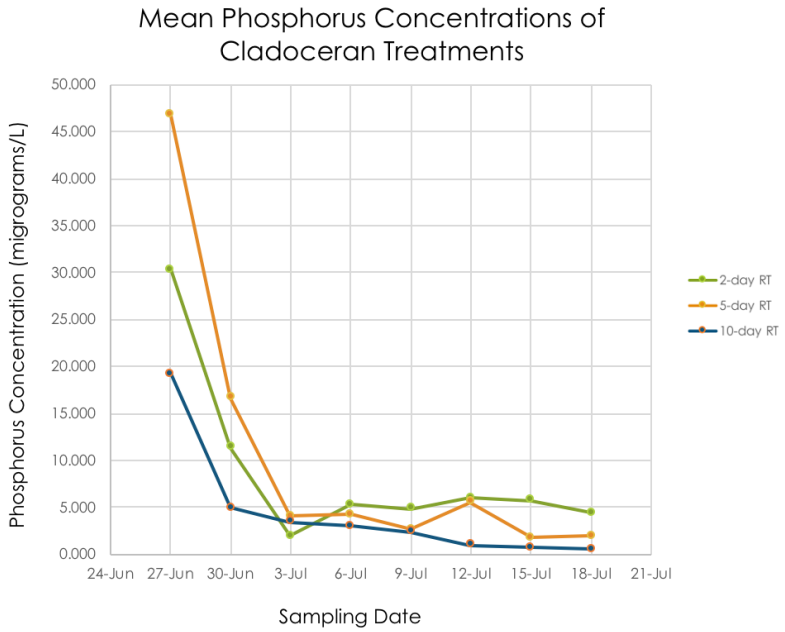

Figure 1. Change in phosphorus concentrations over time for all residence times in cladoceran treatments.

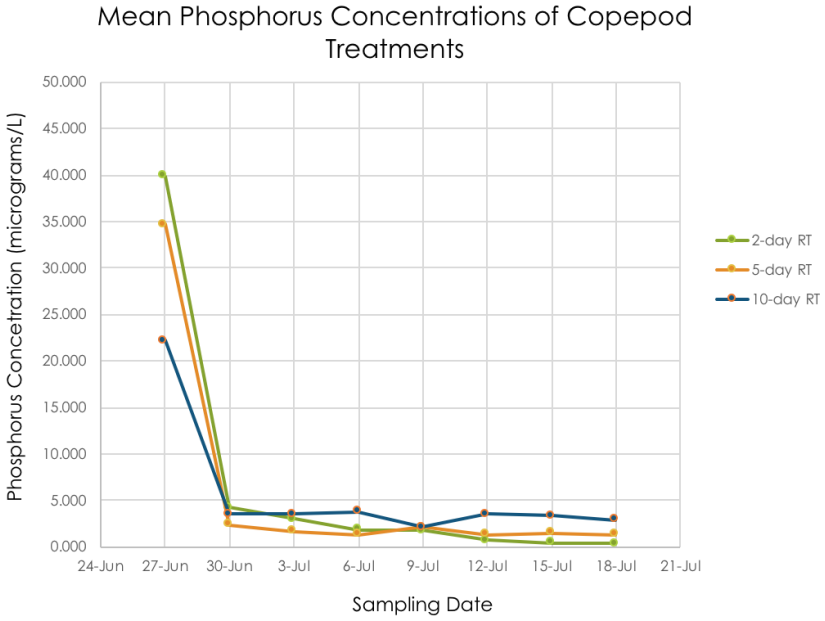

Figure 2. Change in phosphorus concentration over time for all residence times in copepod treatments.

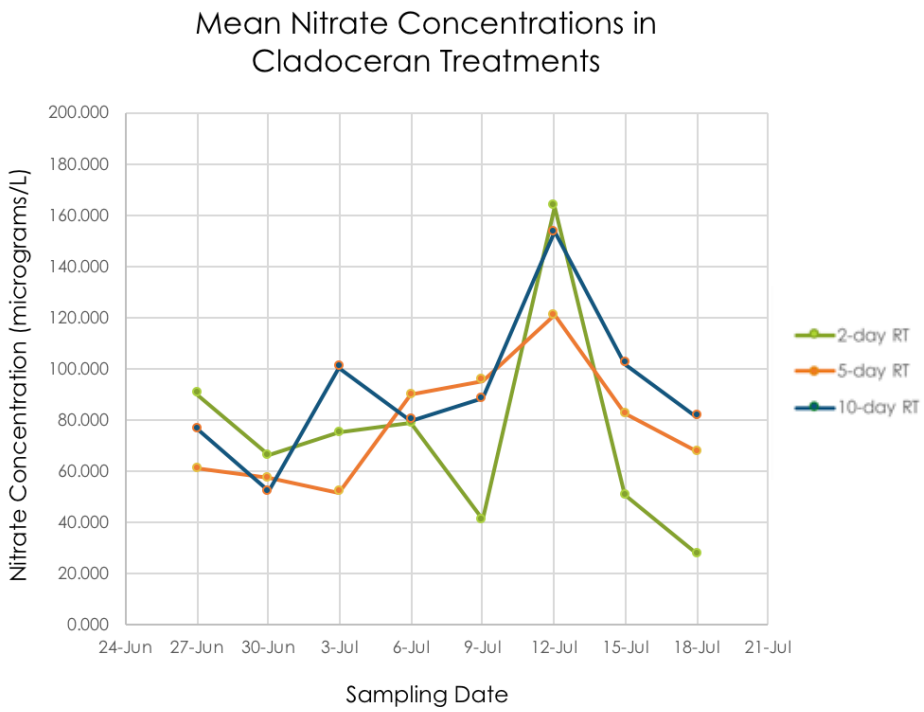

Figure 3. Change in nitrate concentration over time for all residence times in cladoceran treatments.

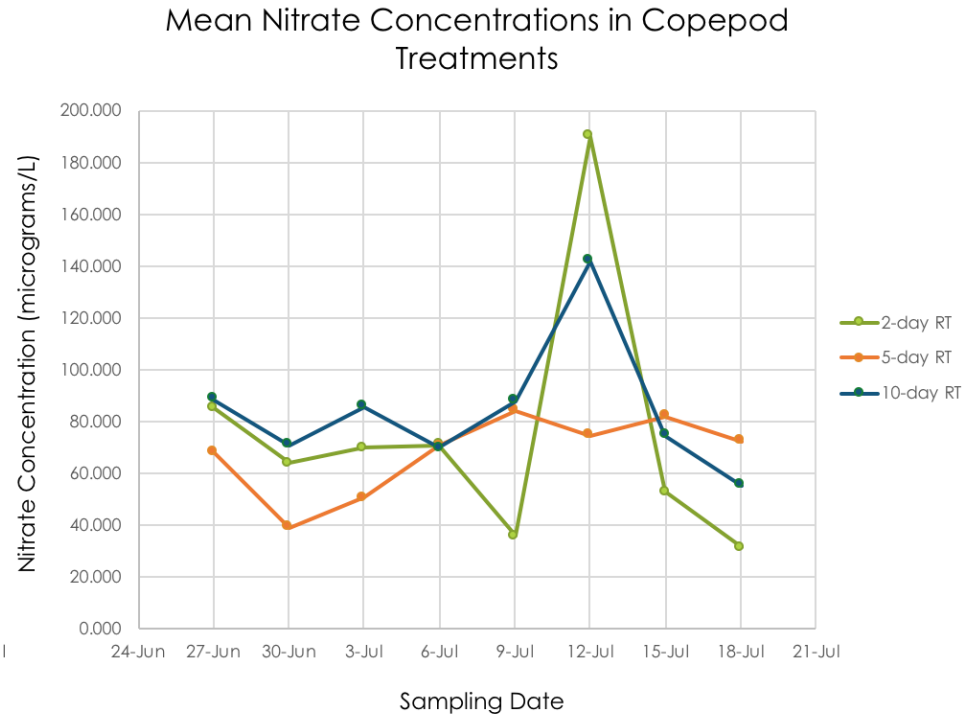

Figure 4. Change in nitrate concentrations over time for all residence times in copepod treatments.

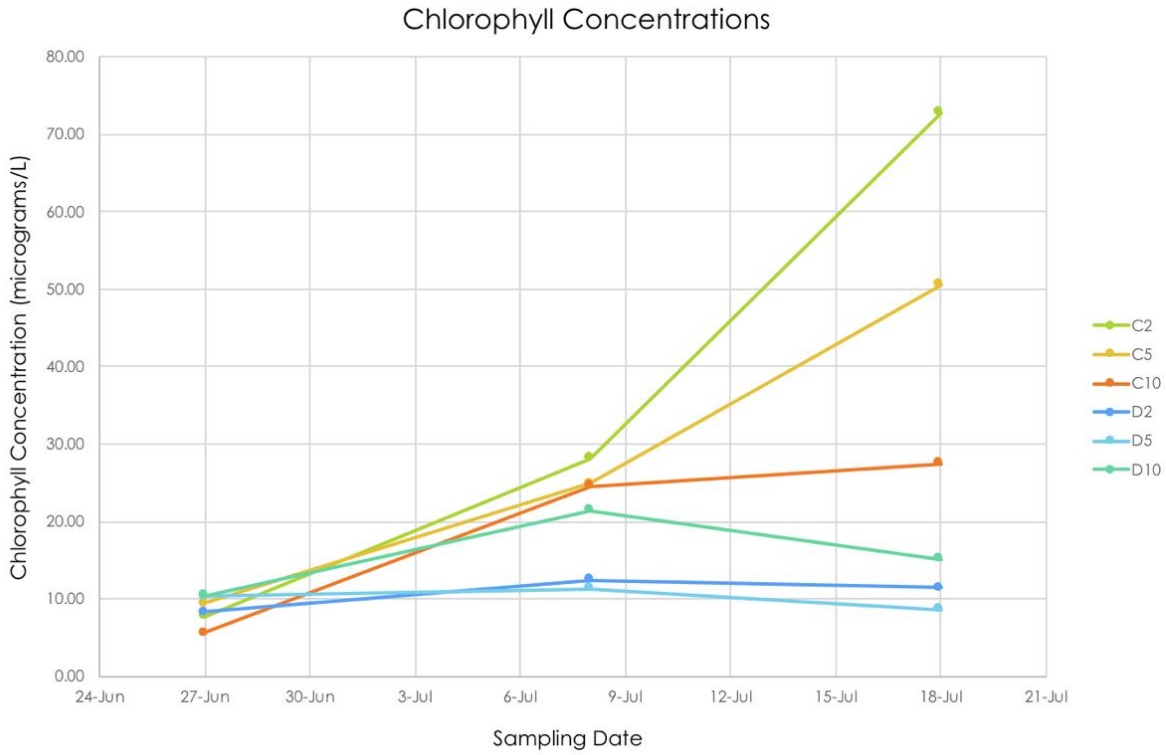

Figure 5. Chlorophyll concentrations over time for all treatments.

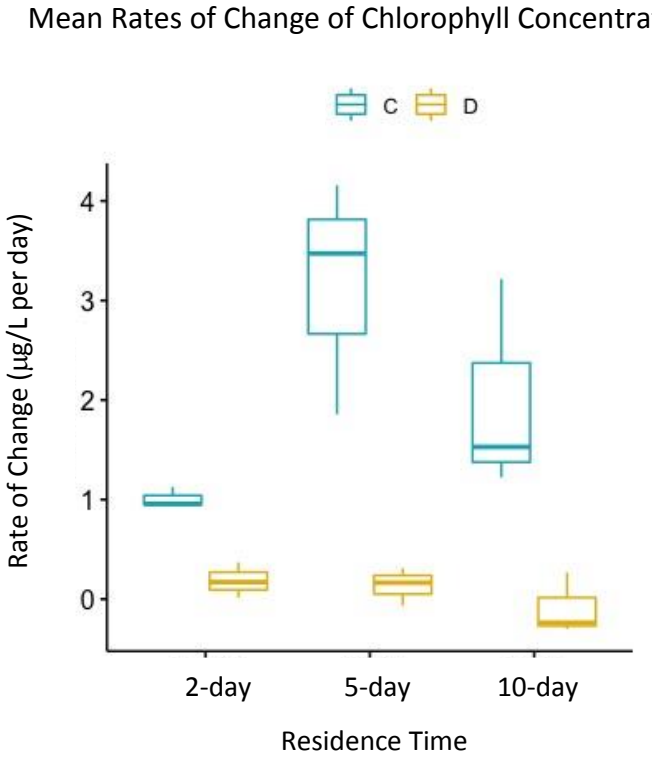

Figure 6. Distribution of the mean rates of change of chlorophyll concentrations for both copepod (C) and cladoceran (D) treatments.

C, 2-day: (3.16, 1.18)
D, 2-day: (0.137, 0.189)
C, 5-day: (1.99, 1.07)
D, 5-day: (-0.0897, 0.311)
C, 10-day: (1.01, 0.103)
D, 10-day: (0.185, 0.178)

Mean Rates of Change of Phosphorus Concentrations

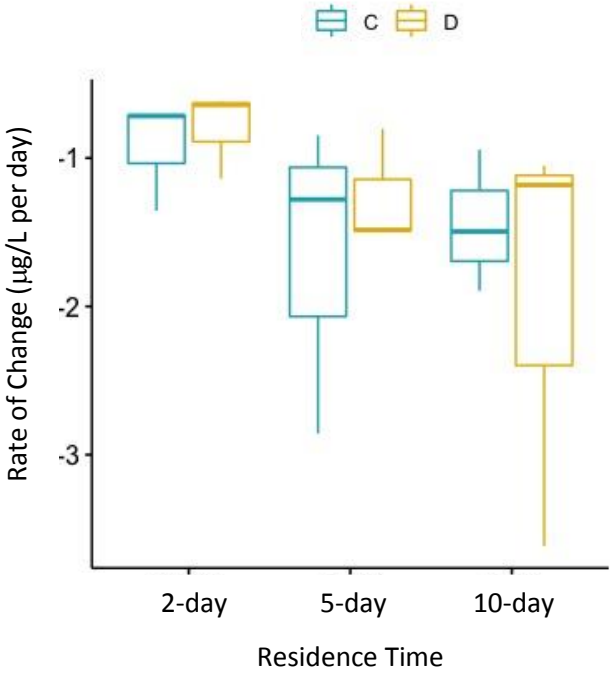

Figure 7. Distribution of the mean rates of change of phosphorus concentrations for both copepod (C) and cladoceran (D) treatments.

C, 2-day: (-1.66, 1.06)
D, 2-day: (-1.26, 0.396)
C, 5-day: (-1.44, 0.478)
D, 5-day: (-1.95, 1.44)
C, 10-day: (-0.926, 0.371)
D, 10-day: (-0.800, 0.294)

Mean Rates of Change of Nitrate Concentrations

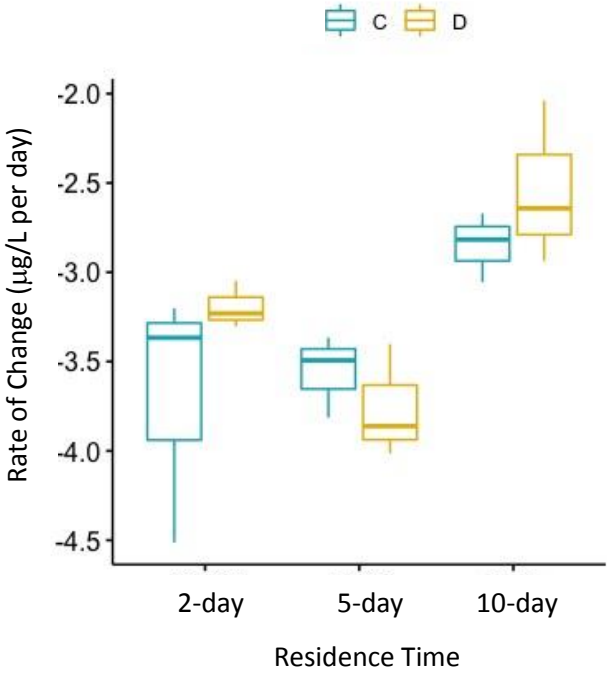

Figure 8. Distribution of the mean rates of change of nitrate concentrations for both copepod (C) and cladoceran (D) treatments.

C, 2-day: (-3.56, 0.231)
D, 2-day: (-3.76, 0.319)
C, 5-day: (-2.85, 0.194)
D, 5-day: (-2.54, 0.457)
C, 10-day: (-3.69, 0.714)
D, 10-day: (-3.19, 0.131)

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