EVALUATING TOP-DOWN EFFECTS OF AQUATIC MACROINVERTEBRATES ON THE NUTRIENT CYCLE VIA MACROPHYTES AND BIOFILM

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ABSTRACT

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Fertilizers rich in nitrogen and phosphorus have been implicated in toxic algal blooms and the eutrophication of Lake Erie. One method for mitigating nutrient runoff is the use of wetlands. Wetlands sequester and process nutrients via biogeochemical processes, decreasing the concentrations of nutrients that eventually reach a large body of water. Research on nutrient cycling in aquatic systems has mainly focused on the sediment, the plants, and the water. Few consider the potential impacts of animals in the system despite the evidence that animals play an important role in nutrient cycling in freshwater systems. Animals can directly move nutrients in and out of aquatic systems, as well as indirectly affect the nutrient budget by altering the ecosystem. The combined direct and indirect effects of animal-mediated nutrient cycling in a wetland system have not been adequately assessed. A few wetland mesocosm experiments have examined the influence of animals on wetland nutrient cycling, but most focus on one functional feeding group. In this study, I evaluated the role of aquatic macroinvertebrates from two functional feeding groups in wetland nutrient sequestration using in-field mesocosms containing macrophytes, in the recently constructed H2Ohio wetland at Oakwoods Nature Preserve (Findlay, OH, USA). Nitrogen and phosphorus content of the water column was measured over six days in response to the presence of each invertebrate. Six replicates of three treatments (snails, crayfish, or control) were installed for a total of 18 mesocosms. A nutrient pulse was added to mesocosms at the end of the experiment to mimic natural nutrient dynamics in an agricultural-adjacent wetland system and nutrient uptake was measured. The results suggest that the crayfish treatment altered nutrient cycling, increasing total nitrogen and total phosphorus

levels and a decreasing light transmission. These changes could be attributed to bioturbation as the crayfish create burrows, suspending sediment and nutrients. The nutrient pulse was fully taken up within 24 hours, preventing the sampling approach used here from detecting differences between treatments. The results suggest differences in invertebrate presence and identity can influence nutrient cycling. This should be considered for a full accounting of wetland nutrient budgets and a better understanding of potential effects of management.

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1. INTRODUCTION

Water quality concerns, including eutrophication and harmful algal blooms (HABs), have continued to be a priority for the public and for local governments due to worsening conditions and the negative economic, health, and ecological impacts. The primary cause is excessive amounts of nutrients, including nitrogen and phosphorus (Qin et al., 2013). In 2014, a HAB in Lake Erie centered near the Toledo drinking water intake system caused local authorities to issue a "do not use" water advisory for the city of Toledo and the surrounding area for 3 days in an event now known as the Toledo Water Crisis. Lake Erie is calculated to generate $7 billion in revenue a year in the local economy (USDA-NRCS, 2005). For the year of 2014, the International Joint Commission estimated there was a total economic loss of $65 million due to the HAB (IJC, 2015). $20 million of this loss is attributed to a decrease in tourism, recreation, and local housing stock (International Joint Commision, 2014). $5 million is attributed to reduced angler activity and purchased fishing licenses (Wolf et al., 2017). In addition, lakeshore properties near algal-infested waters have seen a 22% drop in property values with a long-term loss of $51 million dollars (Wolf & Klaiber, 2017). Decreases in economic value and profits are attributed to the health risks and the decreased aesthetic value of the body of water.

Contact with a HAB via the skin, inhalation, or ingestion can cause severe illness or even death (Carmichael & Boyer, 2016). Ecological impacts include decreased dissolved oxygen leading to fish die-offs (Smith et al., 1999), decreased biodiversity (Dudgeon et al., 2006), decreased abundance (Sakurai, 1990) and biodiversity (James et al., 2005) of submerged plants, and increased turbidity (De Jonge et al., 2002). These impacts will continue to worsen and occur more frequently as the effects of climate change worsen (Chapra et al., 2017). Research strongly points towards agricultural run-off as the major source of the nutrients nitrogen and phosphorus

that have been implicated in the eutrophication and HABs of Lake Erie (Anderson et al., 2012; Baker et al., 2014; Michalak et al., 2013). Managing this issue requires a multi-pronged approach that aims to reduce the amount of these nutrients entering the lake.

Targeting the source of the nutrient influx is one way to decrease the flow of nutrients into large bodies of water. Wetland habitats are natural systems that can sequester and process nutrients before reaching a large body of water. Water flow slows in a wetland allowing nutrients bound to sediments to settle, be taken up by macrophytes and algae (Picard et al., 2005), and to be processed into slow-releasing forms of the nutrients. Some wastewater treatment facilities utilize constructed wetlands as an efficient technology to treat water due to their low cost and maintenance (Kivaisi, 2001).

Studies of wetland nutrient cycling have mainly focused on nutrients in the water column, macrophytes, algae, and sediments, hereafter referred to as "nutrient pools", but little work has investigated the influence of the wildlife community inhabiting wetland habitats (Vanni et al., 2002). The role of animals in nutrient cycling has been shown to be important in terrestrial, marine, and freshwater systems (Vanni, 2002). These effects can be categorized in two ways: (1) as direct versus indirect effects or (2) as top-down versus bottom-up effects.

Direct effects are those where nutrients go through a physiological transformation within the animals' body. The consumption of nutrients can be stored in the mass of an animal (growth) or re-enter the system as a metabolic or gastrointestinal waste products (excretion or egestion, respectively) (Sterner & Elser, 2002; Vanni, 2002). Excretion from animals can be an important source of nutrients for primary producers, though this importance varies temporally and geographically (Sharitt et al., 2021). Sharitt et al. (2021) studied the phosphorus demand that was met by the excretion of gizzard shad in a eutrophic reservoir. They found that, on average, the

fish supported 7-27% of the P demand over the growing season with a higher proportion of support in the summer compared to the spring.

Indirect effects include an animal's impact on their resource populations, prey behavior, or surrounding physical habitat structures (Hawlena & Schmitz, 2010; Vanni, 2002). Williams et al. (2002) studied the effects of stocked fish species on macrophyte loss in a shallow, nitrogen-poor, phosphorus-rich lake through a mesocosm experiment. Declines in macrophytes were attributed to an increase in periphyton growth due to the increase in bioavailable nitrogen from fish excretion.

Bottom-up effects are those that affect primary producers due to limited resources (Bunnell et al., 2014). For instance, the ratio of inorganic to organic forms of nutrients has a direct impact on the rate of uptake and, therefore, the relative composition of bacteria and phytoplankton in an aquatic system (Glibert, 1998).

Top-down effects are those that affect primary producers due to pressure by consumers (Vanni et al., 1997). Winton & Richardson (2017) studied the top-down effects of waterfowl on methane emissions and nitrogen cycling in wetland ecosystems. They found that herbivory inhibited the oxidation of methane and prevented nitrification, increasing emissions.

Studies of nutrient budgets for wetland systems have examined certain nutrient pools such as water, sediment (Raisin et al., 1999), and vegetation (Hoagland et al., 2001; Venterink et al., 2002), while others have looked at a more complete set of nutrient pools but only looked at N or P (Noe & Childers, 2007) or focused on system-wide nutrient input and outputs (Meuleman et al., 2003). Research on nutrient cycling in aquatic systems has mainly focused on the sediment, the plants, and the water but few consider the potential impacts of animals in the system (Atkinson et al., 2017). The combined direct and indirect effects of animal-mediated nutrient

cycling in a wetland system have not been adequately assessed. Few wetland mesocosm experiments involving animal mediated nutrient cycling have been conducted, and those that have mainly focus on one functional feeding group such as grazer-scrapers (Fang et al., 2010). Further research is needed to examine the effects of animals and different functional feeding groups on animal mediated nutrient cycling in wetlands.

This study examines the effects of aquatic macroinvertebrates on the nitrogen and phosphorus nutrient cycles using field mesocosms in a recently constructed wetland in northwest Ohio. Emergent wetland macrophytes and microbial growth within the field mesocosms were exposed to a different treatment of aquatic macroinvertebrates – snails (grazers), crayfish (omnivores), or no invertebrates (control). Following approximately a week of measurements, to mimic natural nutrient dynamics in an agricultural-adjacent wetland system, a nutrient pulse, mimicking concentrations in a spring streamflow, was added to each mesocosm. Water samples were collected throughout the experimental period to measure nitrogen and phosphorus levels. Macrophyte and microbial growth samples were collected at the end of the experiment to measure their dry masses.

We hypothesize that for the snail treatment, H1: snails will increase nutrient levels in the water column due to biofilmconsumption, this top-down effect would cause a decrease of microbial growth in the system lowering nutrient uptake rates by the microbial growth, H2: snails will decrease nutrient levels in the water column due to positive feedback grazing, where consumption of the biofilm leads to compensatory increased growth of biofilm and, therefore, their nutrient consumption, or H0: snails will have no effect on water column nutrient levels. We hypothesize for the crayfish treatment, H1: crayfish will increase nutrient levels in the water column due to their bioturbation behavior, the physical disturbance of the sediment will release

nutrients bound into the sediment into the water column, H2: crayfish will decrease nutrient levels in the water column due to positive feedback grazing, where consumption of the microbial growth leads to an increase of microbial growth and, therefore, their nutrient consumption, H3: crayfish will increase nutrient levels in the water column due to their consumption of the macrophyte decreasing nutrient uptakes rates of the macrophyte, or H0: crayfish will have no effect on water column nutrient levels.

2. METHODS

To test my hypotheses, I conducted a field mesocosm experiment, manipulating presence of different aquatic macroinvertebrates, and measuring the nitrogen (N) and phosphorus (P) levels of the water column. Samples of macrophytes and microbial growth were collected to measure dry mass. Nutrient pools sampled and the terms used for each can be found in Table 1. Additional samples from the water column were collected to measure the algal community composition. In addition, nutrients were added to mimic a spring nutrient pulse to measure each mesocosm's response to an influx of nutrients.

2.1 FIELD

2.1.1 Study Site

The mesocosm field experiment took place at a recently restored wetland within the Oakwoods Nature Preserve (Findlay, Ohio USA) (41.024619, -83.685119). The wetland was constructed in 2021 by creating over forty vernal pools of varying shapes and depths. Mesocosms were installed in pools 26 and 40 in the eastern project area (Figure 1) from June to July 2022.

2.1.2 Mesocosm Installation and Design

Field mesocosms were installed in the wetland to measure the effect of aquatic macroinvertebrates on nutrient cycling. Eighteen mesocosms were installed in the wetland in six groups of three (triplicates), where one mesocosm within each triplicate received each treatment. Triplicates were evenly distributed between two pools (Figure 2) with three triplicates in pool 26 and three triplicates in pool 40. Triplicates were installed in a line parallel to the shoreline at the time of installation, from 7.62-21.59 cm water depth (Table 2). Mesocosms were constructed of plexiglass acrylic tubes with a height of 61.0 centimeters and a 24.4 centimeter diameter. Tubes

were installed six inches into the sediment with the remaining 45.7 centimeters above the ground. Outside of each tube, a 83.8 centimeter tall tomato cage and gardening insect barrier netting were added to the mesocosms. The netting was attached to the tomato cages and tubes using a combination of rubber bands and binder clips (Figure 3). Within each mesocosm a single Sweet Flag (*Acorus americanus*) plant and American Water Horehound (*Lycopus americanus*) plant were planted, a HOBO logger (HOBO Data Loggers) recording temperature and light measurements was added, and two unglazed tiles to measure biofilm growth were added (Figure 4).

Mesocosms and the macrophytes were installed and planted from June 21-23, 2022, three weeks prior to adding invertebrates for a plant acclimation period. Installation of the mesocosms caused high turbidity delaying the installation of the HOBO loggers and unglazed tiles until the sediment settled (July 5 and July 8).

L. americanus in several mesocosms were dead or dying during the plant acclimation period. On July 12, *L. americanus* was removed from each mesocosm leaving one plant, *A. americanus*, within each mesocosm.

2.1.3 Experiment Setup and Timeline

Mesocosms were exposed to different aquatic macroinvertebrates, representing different feeding strategy groups. One Rusty crayfish (*Orconectes rusticus*), representing the omnivore feeding group, was added to one mesocosm within each triplicate. Five Rams-horn snails (*Helisoma* spp.), representing the grazing feeding strategy group, were added to one mesocosm within each triplicate. Snails were collected from the experimental pools and the crayfish were collected from the Portage River in Bowling Green, OH, USA (Latitude: 41.3169, Longitude: -83.6083) by seining. All crayfish were Form 2 (non-reproductive) reproductive state females.

The numbers of crayfish and snails were chosen to achieve similar wet masses of invertebrates at the start of the experiment. The order of the three treatments (control – no invertebrate, snail, and crayfish) was stratified within each triplicate, such that each treatment was in each location within a triplicate (left, center, and right) twice (Figure 5). All invertebrates were marked with white nail polish for easier monitoring and collection at the end of the experiment. Crayfish had a dot placed at the center of their carapace and snails on the center of the widest whorl so that when the snail was moving parallel to the ground, the dot was facing upward (Figure 6).

The experimental period was seven days, labeled Day 0-6 (July 19-25). No field work was conducted on July 23 (Day 4) for safety reasons due to inclement weather. On day 0, aquatic invertebrates were added to the mesocosms. Mesocosms were monitored on days 0-5 for the effect the invertebrates had on the system called the "Monitoring Period". On day 5, a nutrient pulse was added to each mesocosm to mimic a spring pulse (See 2.2 Nutrient Pulse). Mesocosms were monitored on days 5-6 for how the system responded to the nutrient pulse given any changes that may have occurred from the presence of the invertebrates. This will be called the "Nutrient Pulse Period".

2.1.4 Abiotic Sampling and Measurements

Percent dissolved oxygen, pH, conductivity, and temperature were recorded using a YSI multiparameter sonde (Pro DSS) with three sensors: pH and oxygen reduction potential, conductivity and temperature, and dissolved oxygen. These measures were recorded from the center of the mesocosms in the middle of the water column. Water chemistry data were collected from every mesocosm during the three-week plant acclimation period and every day of the experiment. Water levels were measured every 3-4 days during the plant acclimation period and every day of the experiment. A photo of each mesocosm and *A. americanus* was taken to

document the condition of the mesocosms throughout the plant acclimation and experimental periods.

A 50 mL water sample was collected from each mesocosm to measure the suspended plankton group composition and light transmission of each mesocosm. Samples were collected from the center of the mesocosms in the center of the water column. Water samples were collected on June 30, July 8, and July 15 during the plant acclimation period. Samples were collected daily during the experimental period (July 19-25). Samples were analyzed using a Fluoroprobe III (bbe Moldaenke) within 36 hours of collection. Samples collected from July 24-25 (Day 5-6) were not analyzed due to issues with the fluoroprobe. A fluoroprobe is a multi-wavelength fluorometer that measures excitation spectra of chlorophyll *a* fluorescence to infer the composition and abundance of phytoplanktonic communities and concentration of chromophoric dissolved organic matter (CDOM) (Harrison et al., 2018).

2.1.5 Water Nutrient Pool Sampling

To measure total phosphorus and total nitrogen, water samples from the water column were collected from each mesocosm each day of the experiment. Samples were collected in plastic specimen cups from the center of the mesocosm. Samples were stored in a cooler with ice packs while in the field and then frozen once returned to the lab.

To measure dissolved phosphorus and nitrogen, a water sample was collected from each mesocosm on days 0, 5, and 6 of the experiment. Samples were collected following H2Ohio protocols for dissolved nutrient collection (H2Ohio LEARN Wetland Monitoring Program – Sample Collection). Using a 60 mL plastic syringe, water was collected from the center of the mesocosm and then 10 mL of the water was filtered through a 0.45 μm filter. The filtered water was stored in a plastic specimen cup which was stored in a cooler with ice packs while in the

field and then refrigerated once returned to the lab. Samples were delivered to the campus facility in the Midden lab for nutrient analysis within 24-48 hours of collection.

To measure the algal community composition, water samples from the water column were collected from each mesocosm each day of the experiment. Samples were collected in plastic specimen cups from the center of the mesocosm. Samples were stored in a cooler with ice packs while in the field and then stored in the refrigerator until processed in the lab.

2.1.6 Macrophyte Nutrient Pool Sampling

The full macrophyte (above and below ground) was removed from each mesocosm and stored in a plastic bag. Samples were stored in a cooler with ice packs in the field and then refrigerated once they were returned to the lab.

2.1.7 Microbial Growth Nutrient Pool Sampling

At the beginning of the experiment, day 0, one unglazed tile was collected from each mesocosm to measure microbial growth during the plant acclimation period. Tiles were stored in plastic bags which were stored in a cooler with ice packs in the field and then frozen once returned to the lab.

On day 6, microbial samples were collected from five sources or sub-nutrient pools: tile biofilm, mesocosm wall biofilm, epiphytic plant biofilm, floating algal mat, and suspended plankton. The remaining unglazed tile was collected as described above to measure biofilm growth during the experimental period. A subsample of the floating algal mat, if present, was collected by bare hand and stored in plastic bags. These samples were used only for biomass and nutrient content, so contamination from hands was likely minimal. A subsample of the suspended plankton were collected by dipping a 60 mL specimen cup into the center of the mesocosm into the center of the water column. The biofilm growing on the inside of the

mesocosm walls was subsampled by scraping two portions of the inner wall, one facing North and the second facing East. Scrapings were collected using a flat rubber spatula (width 5.87 centimeters). Scraped biofilm samples were stored in a plastic specimen cup. Epiphytic plant biofilm samples were collected in the lab from the collected macrophyte. All microbial growth samples were stored in a cooler with ice packs in the field and then frozen once returned to the lab.

2.2 NUTRIENT PULSE

On day 5, a nutrient pulse was added to mesocosms to mimic a spring nutrient pulse. Diammonium phosphate (DAP) was added to mesocosms to reach the target concentration of 0.19 mg/L (Figure 7). The volume of DAP added was determined by the volume of surface water within each mesocosm (Table 3). The target concentration was determined using the average soluble reactive phosphate (SRP) nutrient data from the Blanchard River (Findlay, OH) site from the National Center for Water Quality Research (Heidelberg University). High flow data (>1000 cu. ft./sec.) for April to June from 2017-2022 were included.

Water column samples to measure total and dissolved nutrients were collected to monitor the uptake of nutrients from the nutrient pulse at three time points: before the pulse on day 5, 30 minutes after the pulse was added on day 5, and 24 hours after the pulse on day 6. All water samples were stored in a cooler with ice packs in the field. Dissolved nutrient water samples were refrigerated once returned to the lab and then delivered to the campus facility in the Midden lab for nutrient analysis 24-48 hours after collection. Total nutrient water samples were frozen once returned to the lab.

2.3 LAB SAMPLE PROCESSING

2.3.1 Macrophyte Sample Processing

The below-ground portion of the macrophyte was rinsed to remove all sediment. Once cleaned, the macrophyte was stored in a new plastic bag and then frozen. Macrophyte samples were thawed and placed into paper bags. Samples were then placed in the drying oven set to 65° C to be dried for a minimum of 72 hours.

2.3.2 Microbial Growth Sample Processing

Unglazed tiles were thawed, then scraped with a razor to remove the biofilm and rinsed with deionized water into a weigh boat. For floating algal mat, suspended plankton, and mesocosm wall biofilm samples were thawed and placed into weigh boats. All sample weigh boats were then placed in the drying oven set to 50° C to be dried for a minimum of 48 hours.

To collect epiphytic plant biofilm, before the macrophyte was frozen, the above-ground portion of the macrophyte was gently scrubbed with a soft-bristle toothbrush into a weigh boat of deionized water to collect any biofilm growing on the plant surface. Weigh boats containing biofilm samples were placed into a drying oven set to 50° C to be dried for a minimum of 48 hours.

2.3.3 Dried Mass Data Collection

Dried mass data was collected for the total macrophyte dried mass and the total microbial mass (tile biofilm, epiphytic plant biofilm, floating algal mat, suspended plankton, and mesocosm wall biofilm) pools using a balance (ME2002E Mettler Toledo) for the macrophyte samples and a microbalance (XPE56 Mettler Toledo) for the remaining samples.

2.3.4 Plankton Group Composition Processing

Water column samples were analyzed for plankton group composition using a Fluoroprobe. Each sample was gently stirred and then poured into a 25 mL glass cuvette with a stir bar added to maintain homogeneity of the sample. Ten readings were taken for each sample and averaged for data analysis.

2.4 NUTRIENT ANALYSIS

Water sample nutrients were determined using methods from the U.S. EPA and the U.S. Geological Survey National Water Quality Laboratory ((Patton & Kryskalla, 2003; United States Environmental Protection Agency, 1993). Methods from Patton and Kryskalla (2003) were used to analyze the total phosphorus and total nitrogen. These methods are similar to the previously described methods for sediment and tissues by digesting samples with the reagent mix and then analyzed with the Seal AQ2 Discrete Analyzer. Dissolved phosphorus and dissolved nitrogen were determined using multiple EPA (1993) techniques (see Table 4: Methods used by the Midden Lab to analyze water samples for dissolved and total nutrients. Brackets indicate the source of the methods from either the U.S. Environmental Protection Agency [EPA] or Patton and Kryskalla [USGS].provided by Midden lab).

2.5 DATA ANALYSIS

Reported results exclude triplicate one which had uniquely low water levels within the mesocosms (Figure 8), but analysis including all triplicates can be found in Appendix 1.

2.5.1 Monitoring Period

Time series data for the monitoring period from Day 0 – 5 were analyzed using RMANOVA (Table 6) with triplicate as a blocking factor and the Greenhouse Geiser correction, when appropriate, using the program *R* (R Core Team 2023) and the *tidyverse* package.

Assumptions of equal variance and normality were assessed via plots of residuals and transformed when necessary.

For data that did not meet assumptions even after transformations, the change was calculated by subtracting the Day 0 value from the Day 5 value. Changes in light transmission and algae group composition measured by the Fluoroprobe were calculated by subtracting the averaged Day 0 value from the averaged Day 3 value, while dried mass data is from field samples collected on Day 6. Values for the change over time or single time points were analyzed using either an ANOVA, a Welch's ANOVA, or a Kruskal-Wallis test, depending on the assumptions that were met for each test (Table 5; Table 6). All contained the triplicate as a blocking factor. Assumptions of equal variance and normality were assessed via plots of residuals and transformed when necessary. Post-hoc Tukey tests and Games-Howell tests were run for ANOVA and Welch's ANOVA tests respectively, for variables with significant p values ($p < 0.05$).

2.5.2 Nutrient Pulse Period

Time series data for the nutrient pulse period consisted of data from Day 5-6. Time series variables were analyzed using ANOVA (Table 5; Table 6) with triplicate as a blocking factor, when appropriate, using the program *R* (R Core Team 2023) and the *tidyverse* package. Assumptions of equal variance and normality were assessed via plots of residuals and transformed when necessary. Nutrient uptake rates were calculated by subtracting the nutrient concentration 30 minutes after the nutrient pulse was added from the nutrient concentration 24 hours after (Day 6) and then dividing the time span between these two collection times. Nutrient pulse uptake rates were analyzed using either an ANOVA, a Welch's ANOVA, or a Kruskal-Wallis test, depending on the assumptions that were met for each test (Table 5; Table 6). All

contained the triplicate as a blocking factor. Assumptions of equal variance and normality were assessed via plots of residuals and transformed when necessary.

2.5.3 Plankton Group Composition

Three dates within the monitoring period were used to analyze the plankton group composition: Day 0, 3, and the change in group composition by subtracting the Day 0 value from the Day 3 value. Data were plotted using NMDS using the program *R* (R Core Team 2023) and the *vegan* package. Data were analyzed using PERMANOVA (Table 5; Table 6) using the program *R* (R Core Team 2023) and the *vegan* package. Assumptions of equal variance and normality were assessed via plots of residuals and transformed when necessary. ANOVA was run as a post-hoc test for taxa that appeared associated with significant group changes ($p < 0.05$), based on PERMANOVA and NMDS plots.

3. RESULTS

Reported results exclude triplicate one which had uniquely low water levels within the mesocosms (Figure 8). Analysis including triplicate one can be found in Appendix 1.

3.1 MONITORING PERIOD (DAY 0-5)

3.1.1 Water Column Nutrients

From Day 0 to Day 5, the crayfish treatment showed an increase in total water column phosphorus, but the control (Tukey's $p = 0.002$) and the snail treatments did not (Tukey's $p = 0.004$; One-way ANOVA, $F = 12.992$, $df = 2, 11$, $p = 0.001$; Figure 9). Similarly, from Day 0 and Day 5, the crayfish treatment showed a greater increase in total water column nitrogen than the control (Tukey's $p = 0.002$) and the snail treatments (Tukey's $p = 0.048$; One-way ANOVA, $F = 11.186$, $df = 2, 11$, $p = 0.002$; Figure 10). Levels of dissolved ammonia within the water column were found to differ between treatments (Welch's One-way ANOVA, $F = 4.995$, $df = 2, 6.881$, $p = 0.0457$; Figure 11) but post hoc tests did not determine which groups were different. There appears to be a slight tendency for higher ammonia levels in the snail treatments, but the pattern is not consistent. Treatment effects were not detected for the dissolved nutrients DRP, NO_3, and NO_2 (Table 7).

3.1.2 Other Water Measurements

All treatments saw a decrease in pH (RMANOVA, $F = 5.517$, $df = 4, 32$, $p = 0.022$; Figure 12) and dissolved oxygen (RMANOVA, $F = 8.810$, $df = 4, 32$, $p = 0.004$; Figure 13) as the experimental period progressed. The interaction between treatment and time influenced dissolved oxygen where the crayfish treatment saw a decrease in dissolved oxygen over time (RMANOVA, $F = 4.984$, $df = 8, 32$, $p = 0.012$; Figure 13) while the control and snail treatments did not show any consistent trends.

The crayfish treatment had lower fluoroprobe light transmission levels than the control (Tukey's p = <0.001) and the snail treatments (Tukey's p = <0.001; One-way ANOVA, F = 169.950, df = 2, 11, p = <0.001; *Figure 14*). No effect of the treatment on temperature or conductivity was detected (Table 7).

3.1.3 Dried Mass

No effect of the treatment on the total microbial growth or total macrophyte dried masses was detected (Table 7).

3.2 NUTRIENT PULSE PERIOD (DAY 5-6)

3.2.1 Water Column Nutrients

Treatment effects were not detected for nutrients in the water columns including total phosphorus, total nitrogen, dissolved NH_3, dissolved NO_3 and NO_2, and DRP (Table 7). For the nutrients total phosphorus, total nitrogen, dissolved NH_3, and DRP, nutrient levels returned to baseline levels 24 hours after the nutrient pulse was added for all mesocosms.

3.2.2 Other Water Measurements

We found lower pH levels in the crayfish treatment than the control (Tukey's p = 0.010) and the snail treatments (Tukey's p = 0.004; One-way ANOVA, F = 7.429, df = 2, 26, p = 0.003; Figure 12). Dissolved oxygen levels were lower in the crayfish treatment than the snail treatment (Tukey's p = 0.045; One-way ANOVA, F = 3.428, df = 2, 26, p = 0.048; Figure 13) but not compared to the control treatment. No effect of the treatment on temperature or conductivity was detected (Table 7).

3.3 PLANKTON GROUP COMPOSITION (DAY 0-3)

Across all mesocosms and treatments, green algae was the most abundant taxa, representing a mean of 70.8% of the community followed by bluegreen algaeX which was 12.6X% of the community. I found that plankton group composition was significantly different between treatments (PERMANOVA, $F = 2.468$, $df = 2, 12$, $p = 0.020$; nMDS see Figure 15). The crayfish treatment showed increases in cryptophyta (One-way ANOVA, $F = 6.639$, $df = 2, 12$, $p = 0.011$; Figure 16) and decreases in yellow substance (One-way ANOVA, $F = 12.840$, $df = 2, 12$, $p = 0.001$; Figure 17), but the control and snail treatments did not show substantial changes. No effect of the treatment on green algae, bluegreen algae, or diatoms was detected (Table 7).

4. DISCUSSION

This experiment provides support for the idea that crayfish can alter wetland nutrient cycling via their bioturbation activity. Crayfish mesocosms saw lowered light transmission, DO, and pH during the monitoring period and nutrient pulse, increases in total water column P and N during the experimental period, and changes in the suspended plankton community. For the crayfish treatment, results support crayfish H1 increasing nutrient levels via bioturbation. Interestingly, no support was found for crayfish altering nutrient cycling through their effects on macrophytes. It could be that this was because *A. americanus* is fibrous while crayfish prefer macrophytes with delicate, filamentous, or finely-branched architecture (Cronin et al., 2002) and prefer submerged macrophytes over emergent macrophytes (Nyström & Strand, 1996). *A. americanus* also may deter predators chemically. The plant rhizomes have medicinal and insecticidal properties (Motley, 1994) and have been used for these purposes by people dating back to the vedic periods (c. 1500 – c. 500 BCE) (Rajput et al., 2014). For the snail treatment, the null hypothesis was not rejected and no consistent changes in the nutrient levels of the water column were observed.

Differences in nutrient uptake rates were not detected, but this was likely because uptake was rapid in all mesocosms, taking up all added nutrients within 24 hours, causing us to miss key time points with differing uptake. Further research needs to be done to investigate potential effects on uptake rates.

Overall, the snail treatment responded similarly to the control. During the monitoring period, the snail treatment may have had an increase in dissolved ammonia levels compared to the crayfish and control treatments, although this was not a strong and consistent pattern. This could potentially be attributed to the excretory waste of freshwater snails which contains

primarily ammonia as its nitrogen-containing excretion product (Dresel & Moyle, 1950; Haggag & Fouad, 1968).

During the monitoring period, the water in the mesocosms containing crayfish saw a greater increase in the total nitrogen and total phosphorus compared to the control and the mesocosms containing snails. This may be attributed to bioturbation as the crayfish create shallow burrows, suspending sediments and nutrients in the process. Angeler et al (2001) also found higher levels of total nitrogen and total phosphorus in treatments containing crayfish, finding that crayfish acted as a nutrient pump, translocating nutrients bound in the sediment into the water column, and increasing total suspended solids. Crayfish have also been found to transform the sediment bound nutrients and release them into the water column via excretion as the dissolved inorganic forms (Angeler et al., 2003). However, dissolved nutrient concentrations were not greater in the crayfish treatment compared to the control and snail treatments. This could be due to binding of dissolved nutrients with suspended sediment particles.

Light transmission in the crayfish treatment was significantly lower compared to the control and the snail treatments. Other laboratory and mesocosm experiments in river habitats have found treatments including crayfish have higher suspended sediment concentrations (Harvey et al., 2014) or turbidity (Welch, 2014) leading to lower light transmission levels (Curran & Novo, 1988; Storlazzi et al., 2015). Bioturbation by the crayfish could explain this. After crayfish were released into the mesocosms, we observed a single shallow burrow within the mesocosm that the crayfish would shelter in, typically along the wall of the mesocosm (personal observation).

Dissolved oxygen decreased in all treatments during the monitoring period with the greatest decrease in the crayfish treatment. Crayfish bioturbation resuspends sediment and

organic matter into the water column decreasing light transmission levels. This could reduce photosynthesis rates of the macrophytes and biofilm (Wen et al., 2007) decreasing DO. Resuspension of sediment and organic matter also moves the material from the anoxic layer to an oxic area in the water column. Aerobic bacterial activity increases with this newly available resource, accelerating the decomposition of organic nutrients (Tambo, 2018). This increase in decomposition increases oxygen demands by those decomposers, decreasing the levels of dissolved oxygen in the water column. Along with this decrease in DO, an increase in aerobic respiration by bacteria would increase the levels of CO2 shifting the carbonate equilibrium towards CO2 and H2O leading to a decrease in the pH (Nairn & Mitsch, 1999). During the monitoring period, all treatments saw a decrease in pH, with no difference by treatment. However, it is important to note that pH levels for the crayfish treatment mesocosms at the end of the monitoring period were variable, with some mesocosms appearing to have pH levels much lower than the control and snail treatments, but no consistent trend.

During the monitoring period, the green algae, bluegreen algae, and diatoms did not have a discernable response to the treatments even though these groups make up the majority of the plankton group composition (Figure 18). The crayfish treatment had a higher proportion of cryptophytes and a lower proportion of yellow substance than the control or snail treatments. An increase in Cryptophyta could be attributed to their high efficiency in rapidly photo-regulating changes to variable light conditions, allowing them to thrive in a high light stress environment. Compared to diatoms, cryptophytes have a higher flexibility to grow in a range of environmental light levels (Mendes et al., 2023). With the decreased light transmission seen in the crayfish mesocosms, Cryptophyta may have had an advantage over other algal groups due to their light stress tolerance. Also, cryptophytes are mixotrophic, alternating between phototrophy

(production) and phagotrophy (consumption). During cell division, the endosymbiont green alga can be lost in one daughter that becomes phagotrophic. Once it obtains another green alga it changes its morphology converting back to phototrophy (Wiser, 2024). It is possible that cryptophytes in the phagotrophic state had access to resources allowing them to divide and increase their population size.

Changes in yellow substance are more complicated. Kalle (1966) defined yellow substance (gelbstoff) as the dissolved organic matter giving water a yellow color via selective absorption of blue wavelengths. More recently, this substance has been characterized as being humic and referred to as CDOM (Hojerslev & Aas, 2001). In the equipment we used, measurements of yellow substance are used as a correcting factor to improve readings for the other focal algal groups (Twiss, 2011).

In response to the nutrient pulse on Day 5 and 6, my treatments did not differ in their uptake rates of nutrients. The environmental factors DO and pH saw an increase in all treatments, with the smallest increase in the crayfish treatment. With an increase in dissolved nutrients from the nutrient pulse, algae may consume CO_2 for photosynthesis at a faster rate than what can be replaced by diffusion and respiration. This change in CO_2 levels leads to a shift in the carbonate equilibrium towards carbonic acid and bicarbonate leading to higher pH levels (Jakobsen et al., 2015). Neal et al. (2000) observed that pH levels increase with a decrease in CO_2 levels noting that these changes occurred in the spring in association with an algal bloom. In addition, crayfish predation on sediment periphyton could decrease the abundance of benthic primary producers and, therefore, the DO near the sediment. Lower light transmission associated with bioturbation could also inhibit benthic primary producers. The lower DO and pH levels seen

in the crayfish treatment during the nutrient pulse could be due to the lower levels of DO and pH we saw in the crayfish treatment before the nutrient pulse was added to the mesocosms.

My results suggest that the crayfish treatment altered the ecosystem dynamics, including how it responded to the nutrient pulse. Given that crayfish are well-established as ecosystem engineers (Albertson & Daniels, 2018; Emery-Butcher et al., 2020; Moore, 2006) it is not surprising they altered the mesocosms. It has been established in the literature that they can change the benthic habitat by altering the topography of riverbeds, reduce bank stabilization (Barbaresi et al., 2004; Guan, 1994; Johnson et al., 2010), and resuspending sediment via bioturbation (Creed & Reed, 2004; Harvey et al., 2014; Statzner et al., 2003). However, research on crayfish impacts have mainly focused on river systems with wetland studies being limited. This study contributes to our understanding of crayfish effects on wetlands and how they impact nutrient cycling.

Although the effect of invertebrates presence on nutrient uptake rates was not detected in this short-term experiment (6 days), changes to the systems' water chemistry could affect its ability to sequester nitrogen and phosphorus in the long term. Decreased pH levels can reduce ammonia volatilization rates (pH < 8) (Koottatep & Polprasert, 1997; Reddy & D'Angelo, 1997) and decrease nitrification rates (Shammas, 1986). However, high pH (pH > 9.6) can also decrease rates of nitrification (Hofman & Lees, 1953). pH levels in the mesocosms ranged from 7.32 - 10.88 over the full experimental period (Day 0 – 6) with crayfish having the lowest pH levels that did not exceed a pH of 9.26. Low levels of DO can also inhibit nitrification rates. DO levels less than 1.0 mg/L significantly inhibited decomposition by ammonia-oxidizing and nitrite oxidizing bacteria (Liu & Wang, 2015). DO levels in the mesocosms ranged from 2.85 – 24.51 mg/L over the full experimental period (Day 0 – 6). It is important to note that nitrification rates

are also dependent upon many other factors including temperature, inorganic carbon source, microbial population, and concentration of ammonium (Vymazal, 2007). However, these other factors either did not respond to my treatments or they were not measured.

For phosphorus, decreased pH can decrease precipitation rates (pH < 8) (Diaz et al., 1994). An increase in pH can also increase the rate of deposition of carbonate phosphate complexes and the long-term burial of phosphorus (Dodds, 2003). Low levels of DO can decrease rates of SRP (soluble reactive phosphorus) uptake (Grace et al., 2008) and increase phosphorus diffusion from the sediment to the water column and decrease deposition of metal phosphates (Dodds, 2003).

Understanding a wetlands' ability to sequester and process nutrients can help us improve the design of new wetlands and inform management strategies of existing wetlands with the goal of limiting nutrients from entering Lake Erie. This study shows that animals can have a role in wetland nutrient cycling and potentially impact its efficacy as a tool in managing HABs. For example, bioturbation activity of crayfish has been shown to be a driver of regime shifts in a system from a macrophyte-dominated clear water state to a phytoplankton-dominated turbid water state (Matsuzaki et al., 2009). In stream systems, crayfish have been shown to move gravel at the bed surface, increase the number of pits and mounds, thus increasing the abundance of macroinvertebrates (Albertson & Daniels, 2016). Though hydroperiod can limit which species of crayfish are present within a wetland, some species in fact prefer shorter or intermittent hydroperiods while other species prefer longer or permanent hydroperiods (Yarra & Magoulick, 2018). Therefore, crayfish can potentially cause problematic shifts in wetlands spanning a range of hydroperiods, increasing phytoplankton in the system. However, they may also have benefits in the system, especially in increasing prey for insectivorous wildlife. Further research needs to

be done to investigate the effects of invertebrates on wetland nutrient cycling and ecosystem functions.

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APPENDIX A. FIGURES

Figure 1: Oakwoods Nature Preserve (Findlay, Ohio USA). Wetland construction projects outlined in yellow and the pools contoured in light green. Mesocosms were installed in pools 40 and 26 highlighted in red.

Figure 2: Oakwoods Nature Preserve project pools 40 and 26. Pink dots are the locations of the six mesocosm triplicates installed.

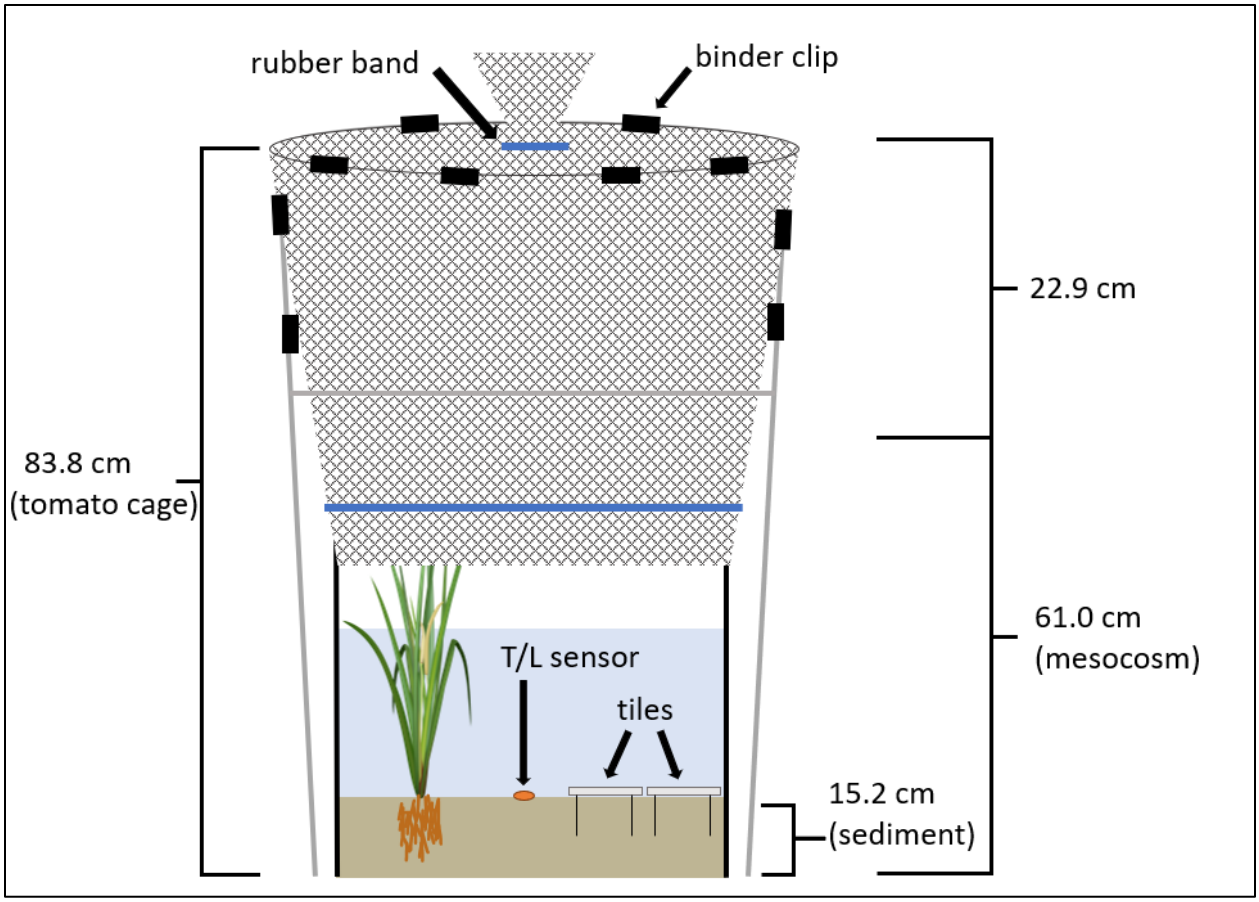

Figure 3: Outer mesocosm design and dimensions.

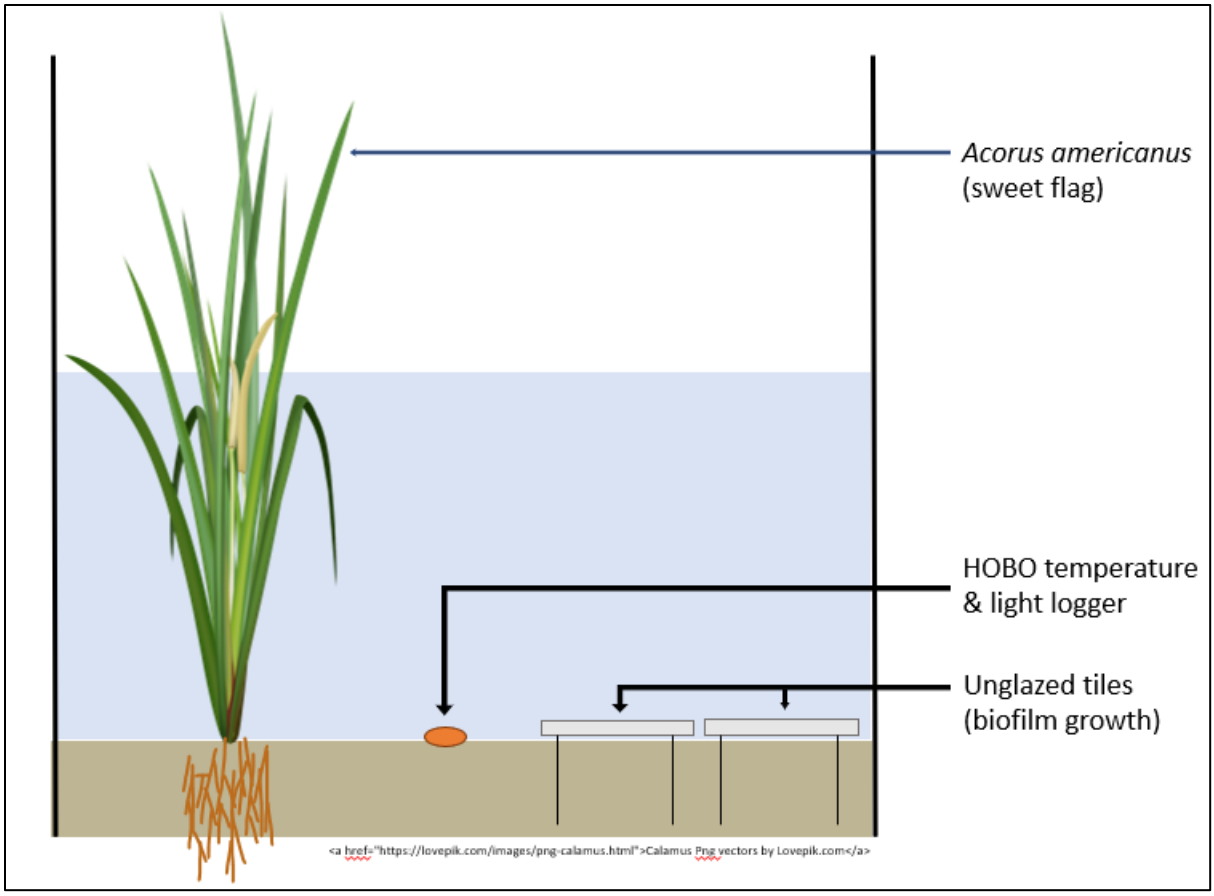

Figure 4: Inner mesocosm elements installed for experiment including Sweet flag (*Acorus americanus*), American Common Horehound (*Lycopus americanus*) which was removed before the experiment, HOBO temperature and light logger, and two unglazed tiles to measure biofilm growth.

Figure 5: Oakwoods Nature Preserve project pools 40 and 26. Individual dots represent one mesocosm, the color of the dot represents the treatment (blue = control, orange = crayfish, gray = snail). Number next to a set of three dots represents the triplicate number.

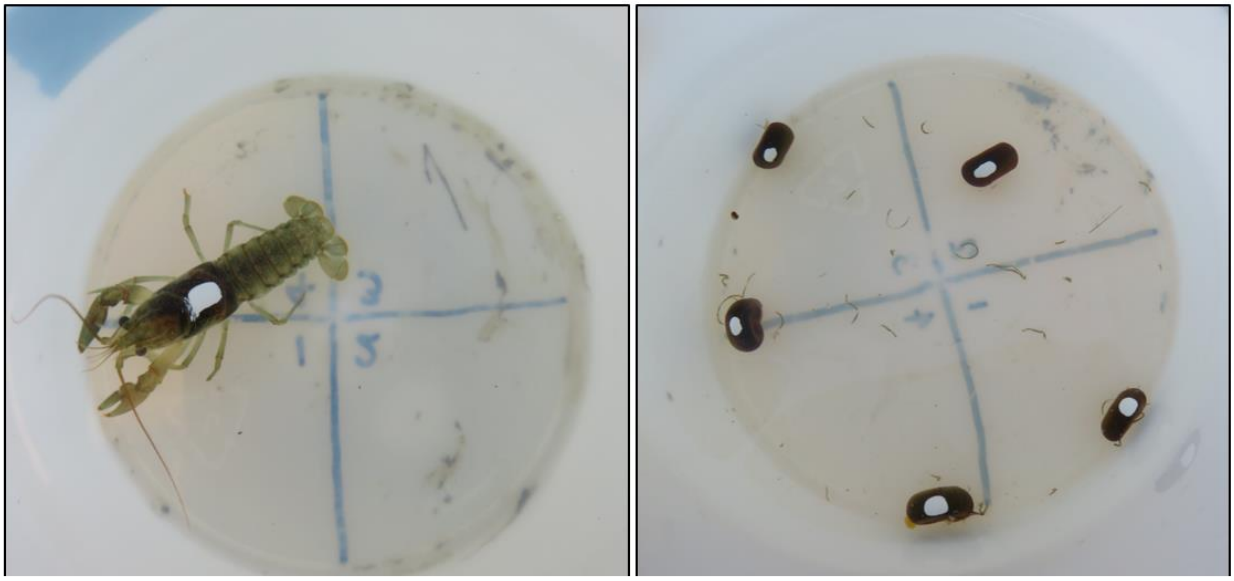

Figure 6: Rusty crayfish (*Orconectes rusticus*) and Rams-horn snails (*Helisoma spp.*) marked with white nail polish dot on their carapace and shell, respectively, for easier monitoring of the invertebrates.

Molar Masses

DAP = 132.06g/mol

PO_4 = 94.97g/mol

DAP = Diammonium Phosphate

0.132 mg/L PO_4 = 0.000132 g/L

$$.000132\frac{g}{L}PO_4 \times \frac{1\ mol}{94.97\ g}PO_4 \times \frac{132.06\ g}{1\ mol}DAP = 0.001748\frac{g}{L}DAP \approx 1.75\frac{mg}{L}DAP$$

1.75 mg/L DAP was rounded to 1.9 mg/L to give enough PO_4 if some small amount binds on impact to other factors

Figure 7: Calculations to determine target DAP concentration for nutrient pulse.

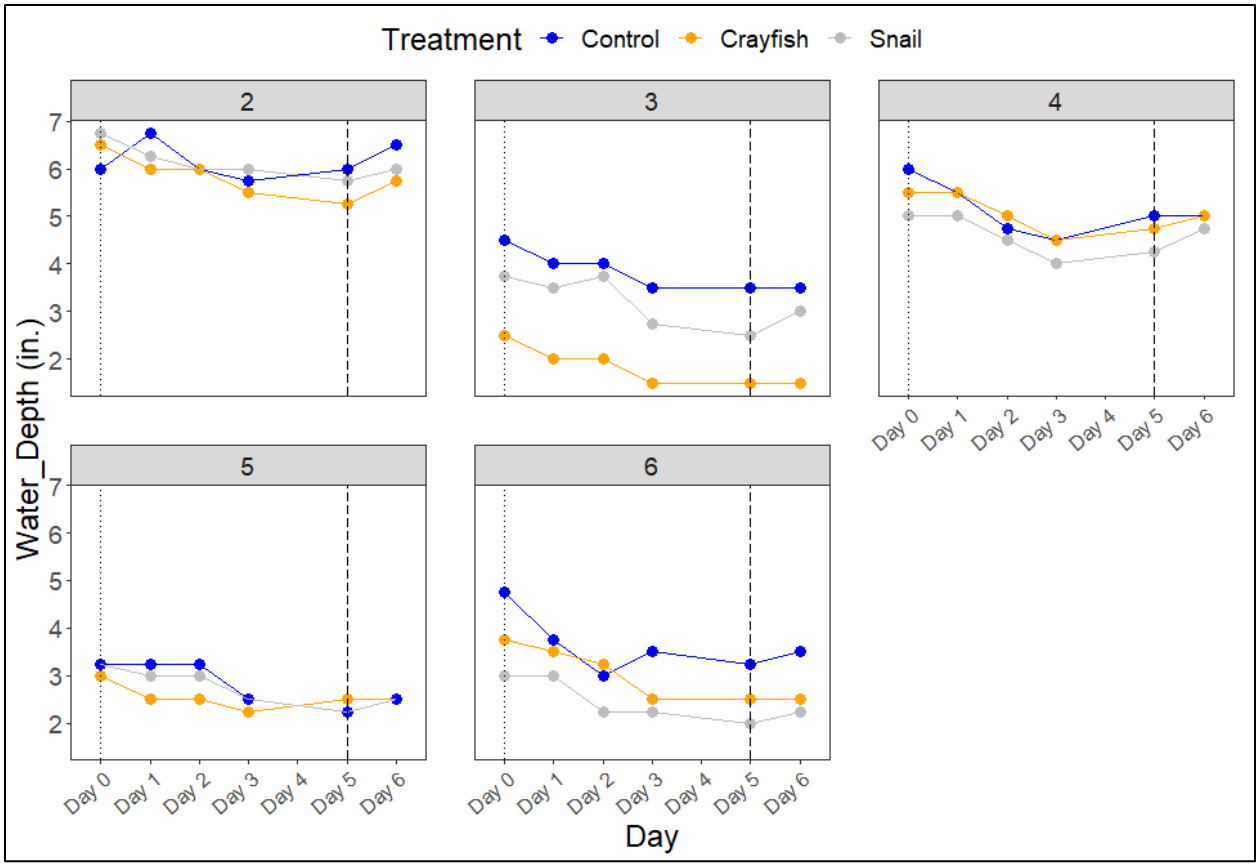

Figure 8: Water levels for mesocosm within triplicate 2-6. The small dashed line marks Day 0 of the experiment and the large dashed line marks Day 5 when the nutrient pulse was added.

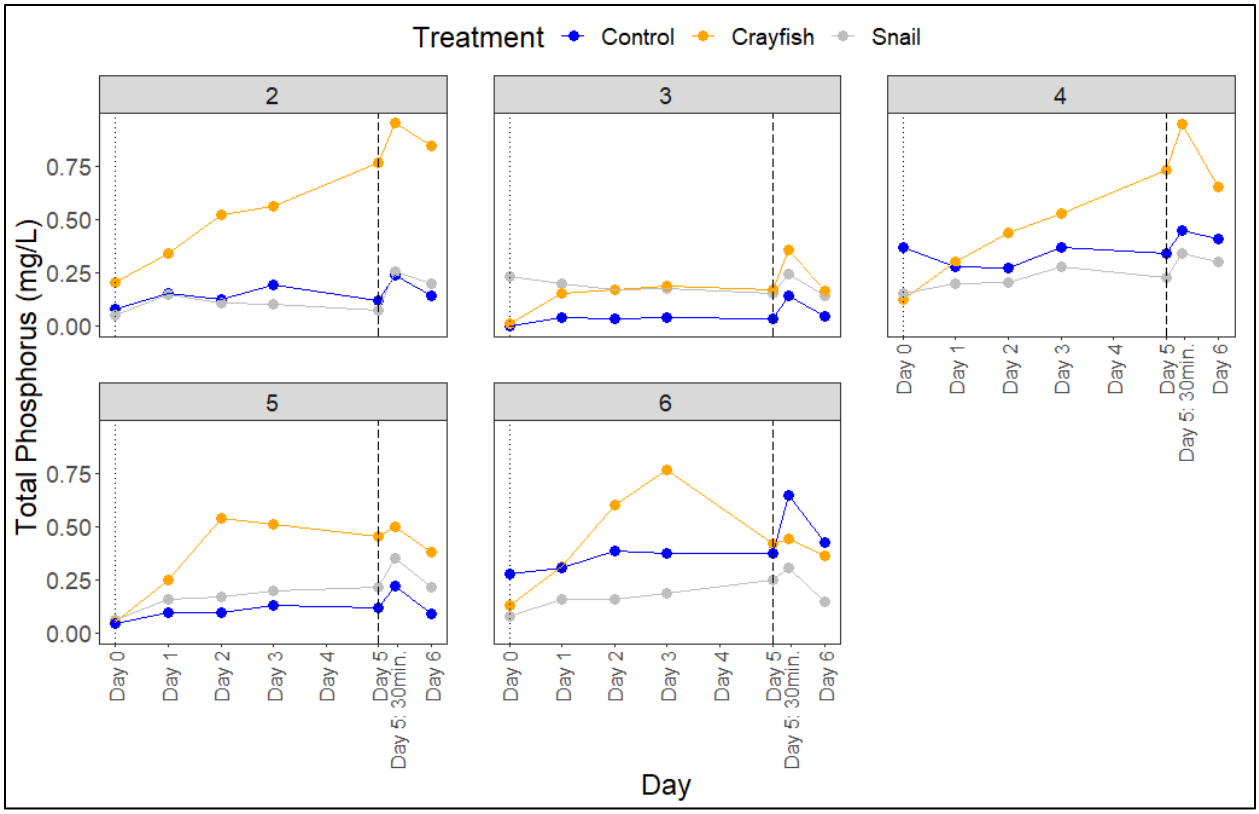

Figure 9: Water column total phosphorus levels for each mesocosm within triplicate 2-6. The small dashed line marks Day 0 of the experiment and the large dashed line marks Day 5 when the nutrient pulse was added.

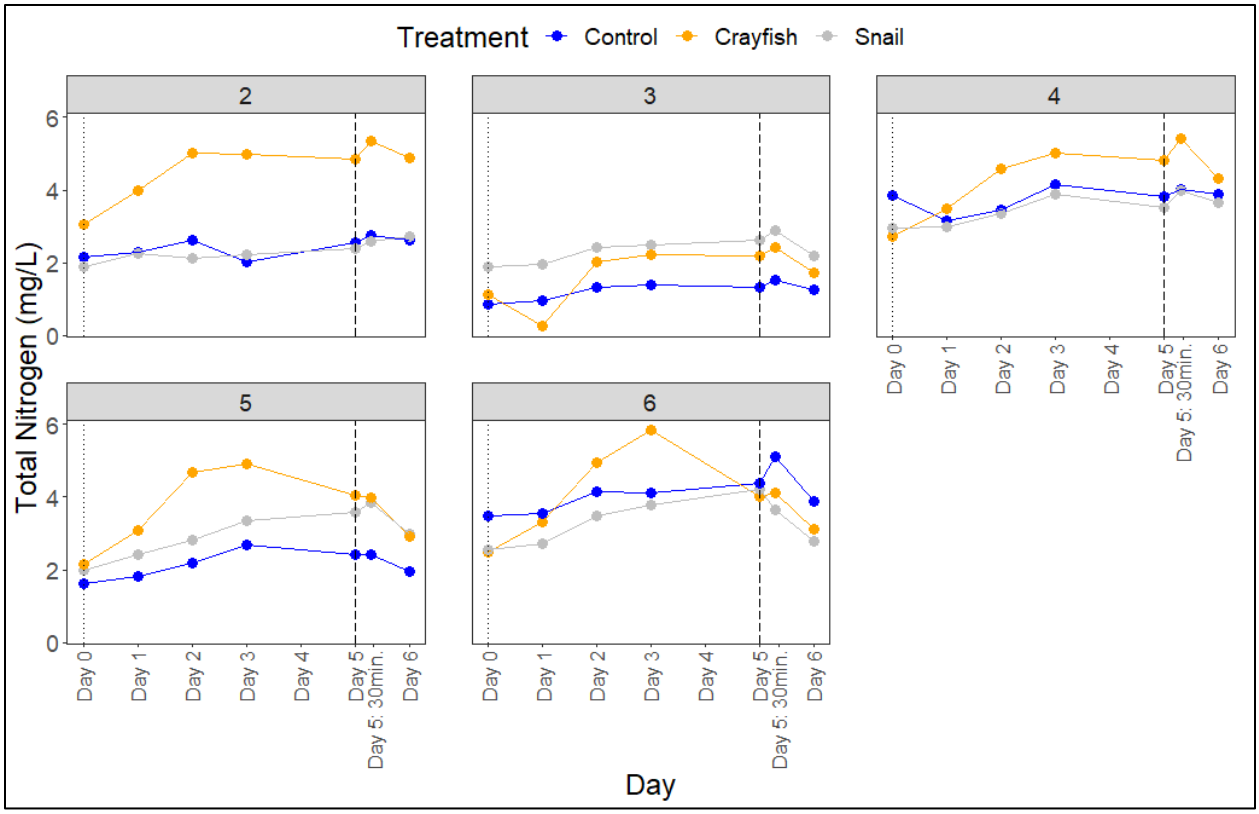

Figure 10: Water column total nitrogen levels for each mesocosm within triplicate 2-6. The small dashed line marks Day 0 of the experiment and the large dashed line marks Day 5 when the nutrient pulse was added.

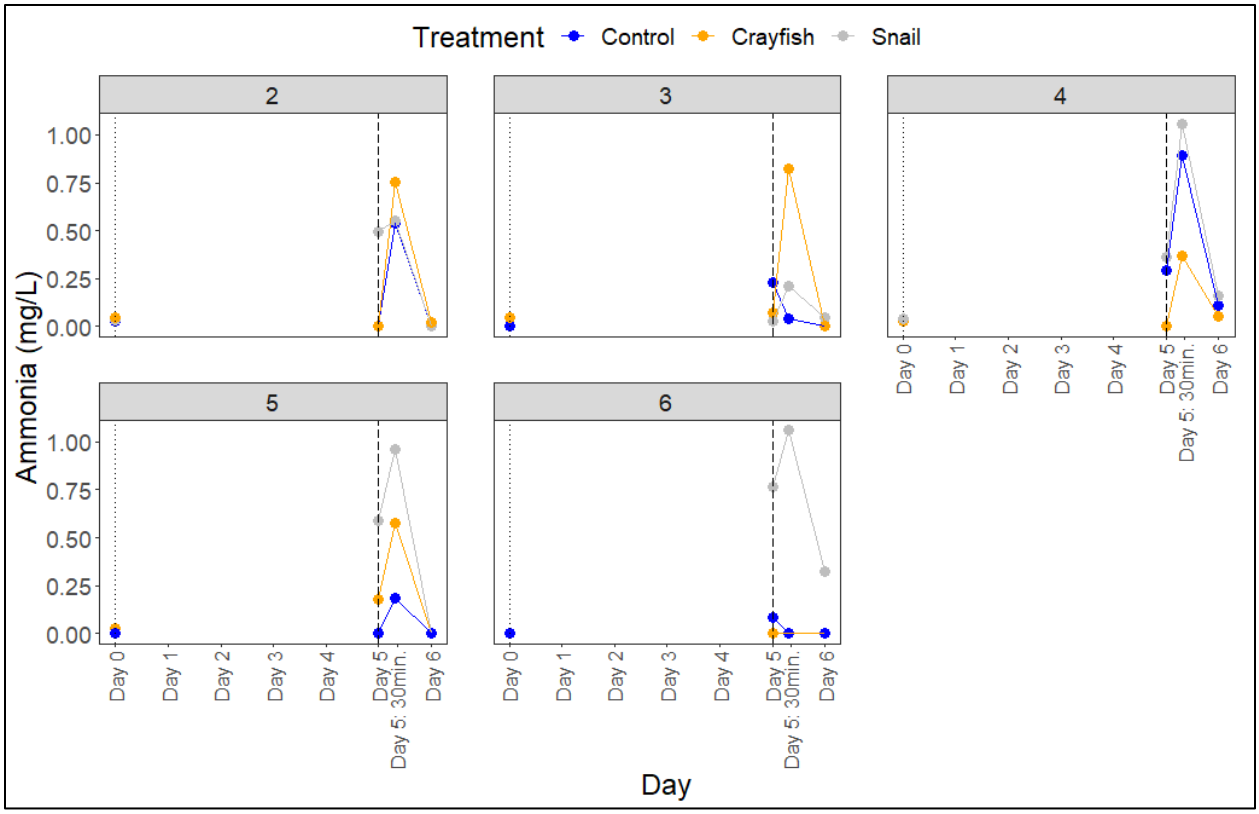

Figure 11: Water column dissolved ammonia levels for each mesocosm within triplicate 2-6. The small dashed line marks Day 0 of the experiment and the large dashed line marks Day 5 when the nutrient pulse was added.

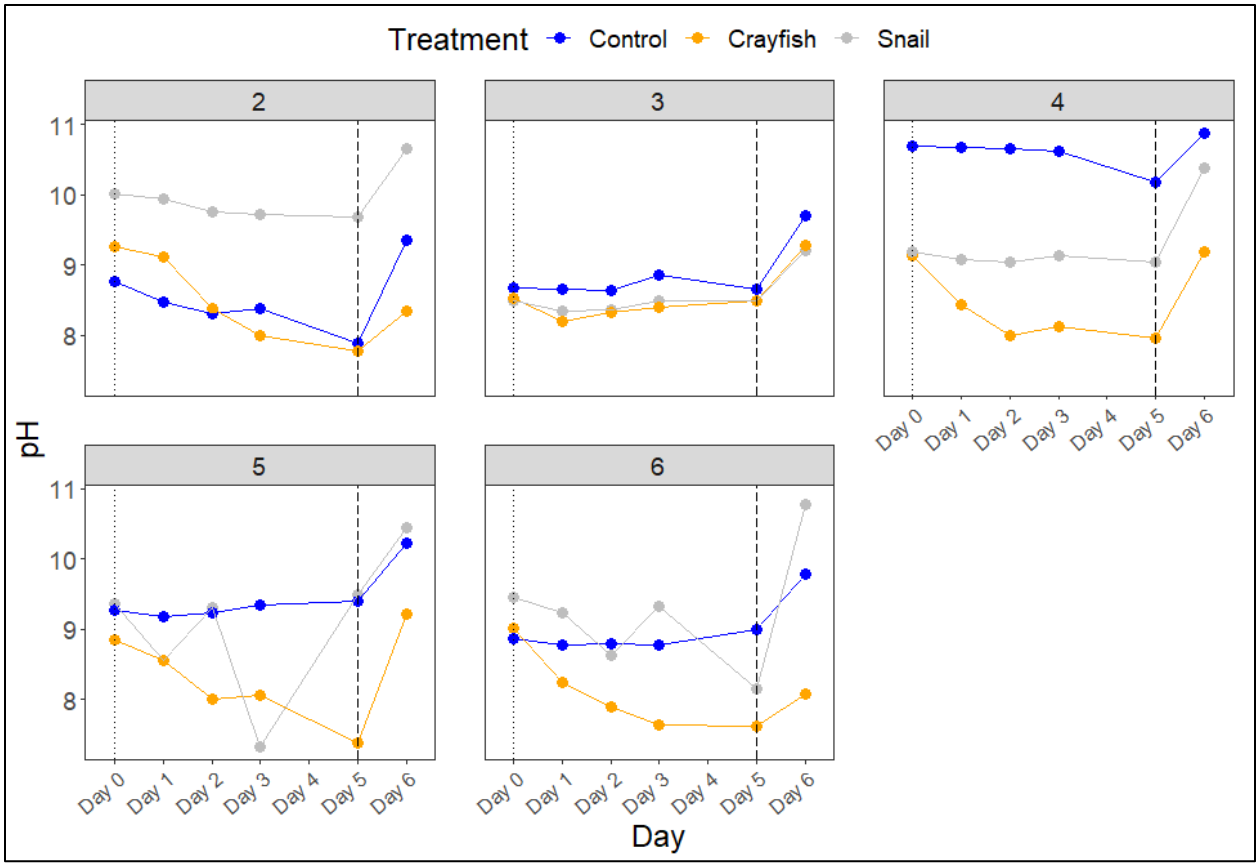

Figure 12: pH levels for each mesocosm within triplicate 2-6. The small dashed line marks Day 0 of the experiment and the large dashed line marks Day 5 when the nutrient pulse was added.

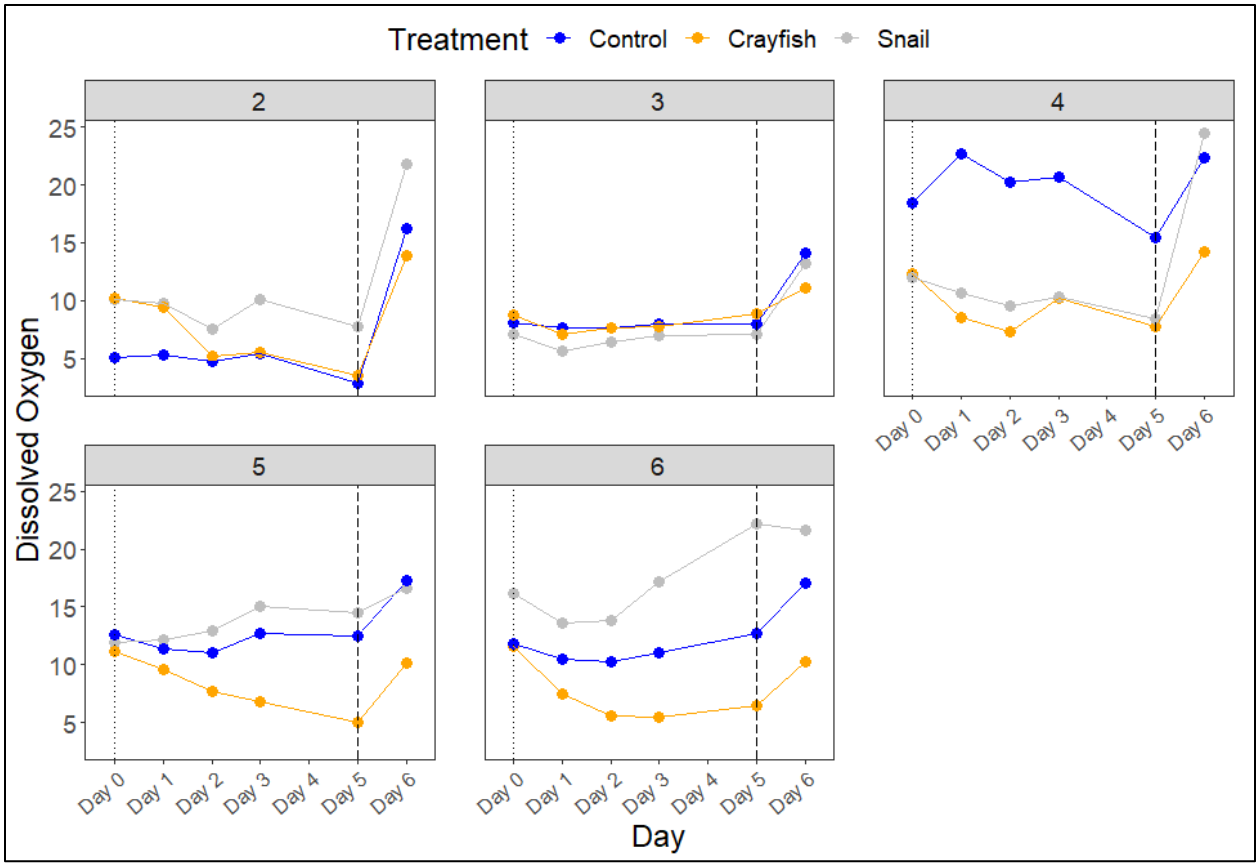

Figure 13: Dissolved oxygen levels for each mesocosm within triplicate 2-6. The small dashed line marks Day 0 of the experiment and the large dashed line marks Day 5 when the nutrient pulse was added.

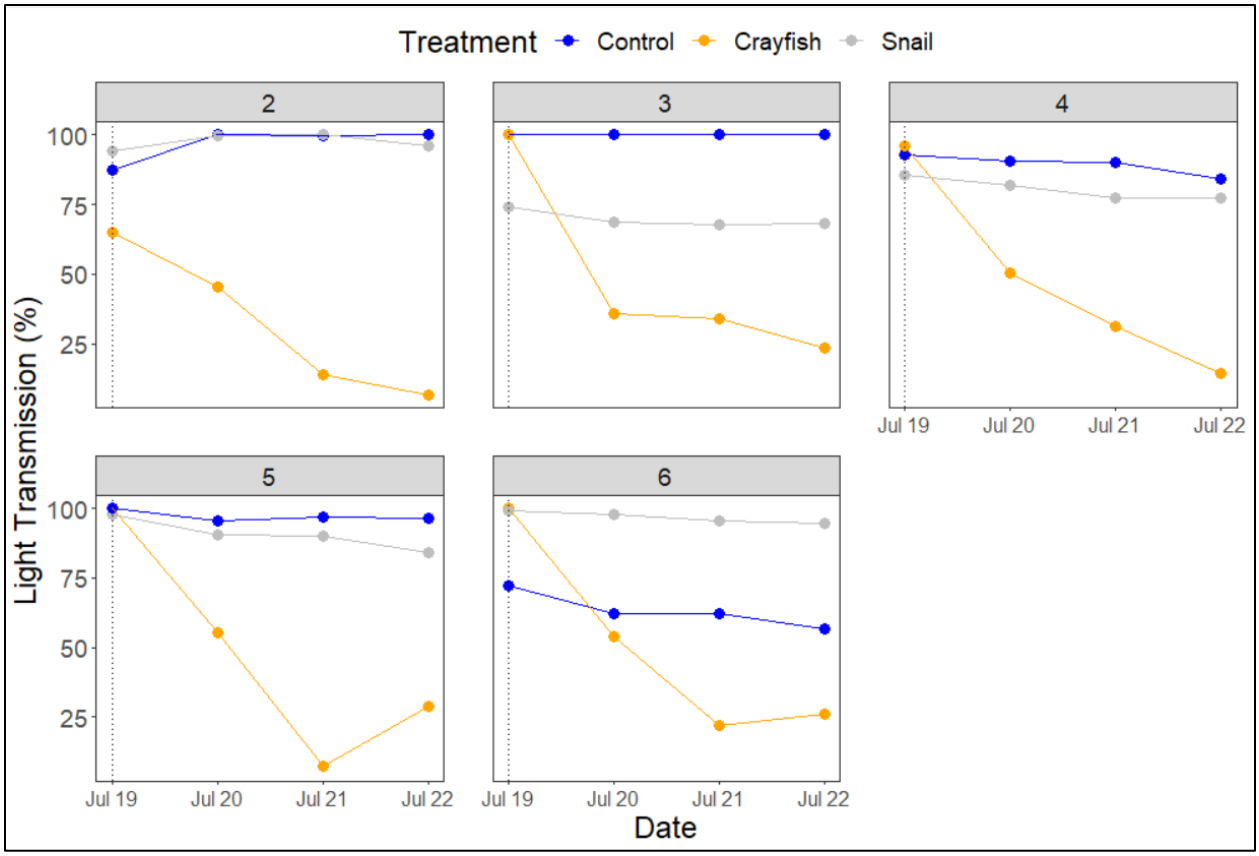

Figure 14: Water light transmission levels of each mesocosm within triplicate 2-6. The small dashed line marks Day 0 of the experiment. Data was collected only till Day 3 of the experimental period.

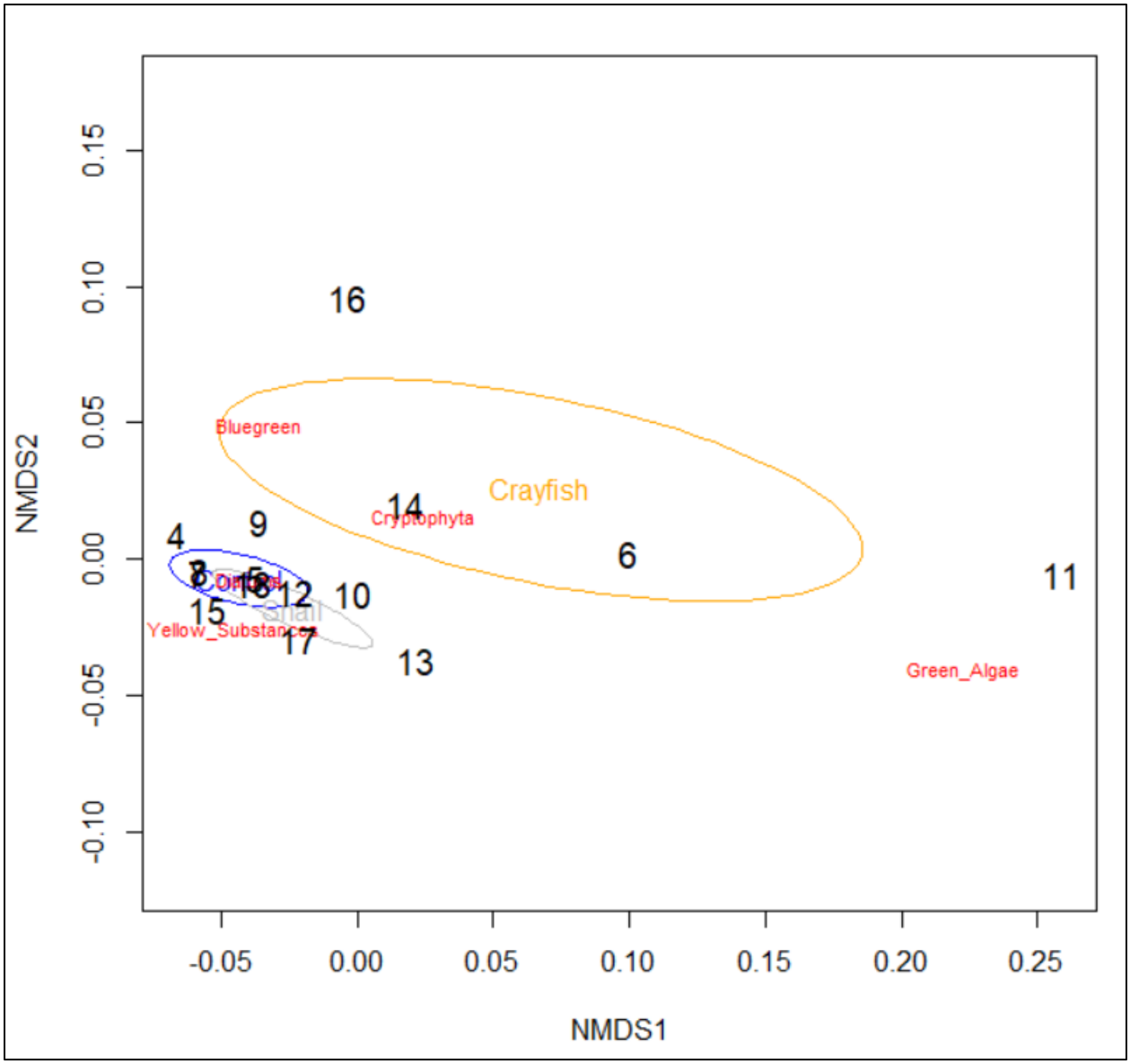

Figure 15: nMDS plotting the change in plankton group composition by treatment. The blue ellipse is the control treatment, the gray ellipse is the snail treatment and the orange ellipse is the crayfish treatment. Numbers represent individual mesocosms and red words represent the plankton groups.

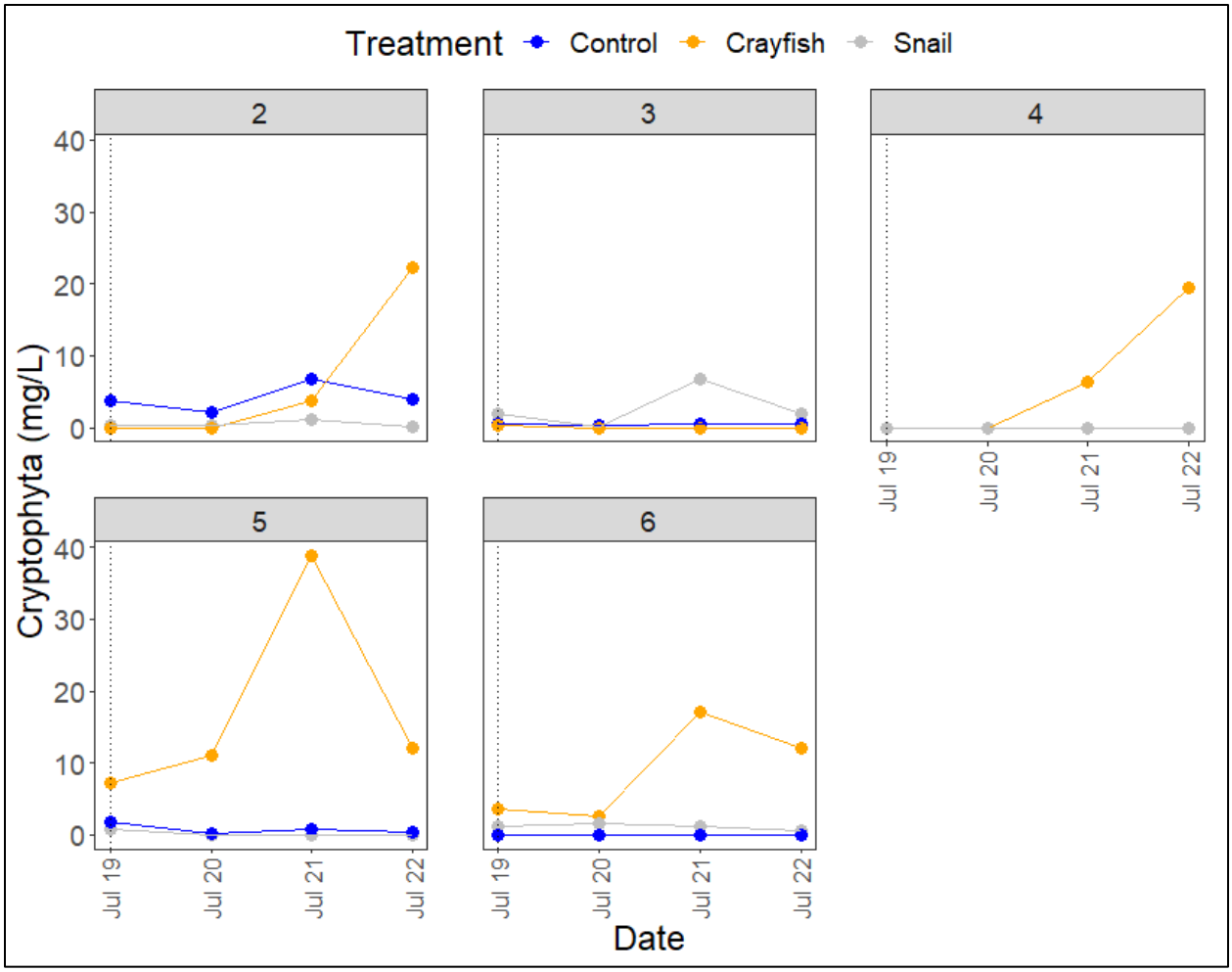

Figure 16: Cryptophyta levels of each mesocosm within triplicate 2-6. The small dashed line marks Day 0 of the experiment. Data was collected only till Day 3 of the experimental period.

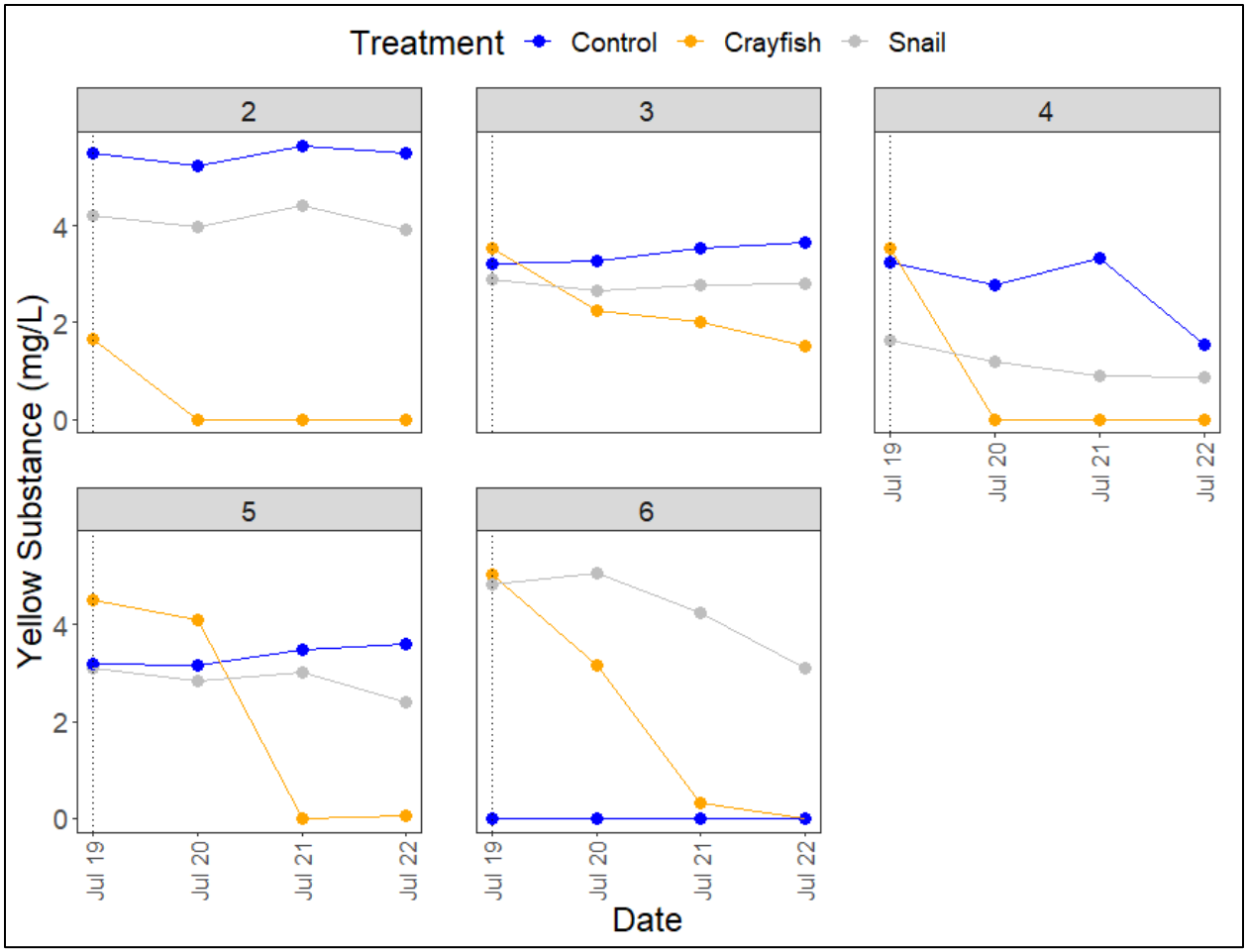

Figure 17: Yellow substance (CDOM) levels of each mesocosm within triplicate 2-6. The small dashed line marks Day 0 of the experiment. Data was collected only till Day 3 of the experimental period.

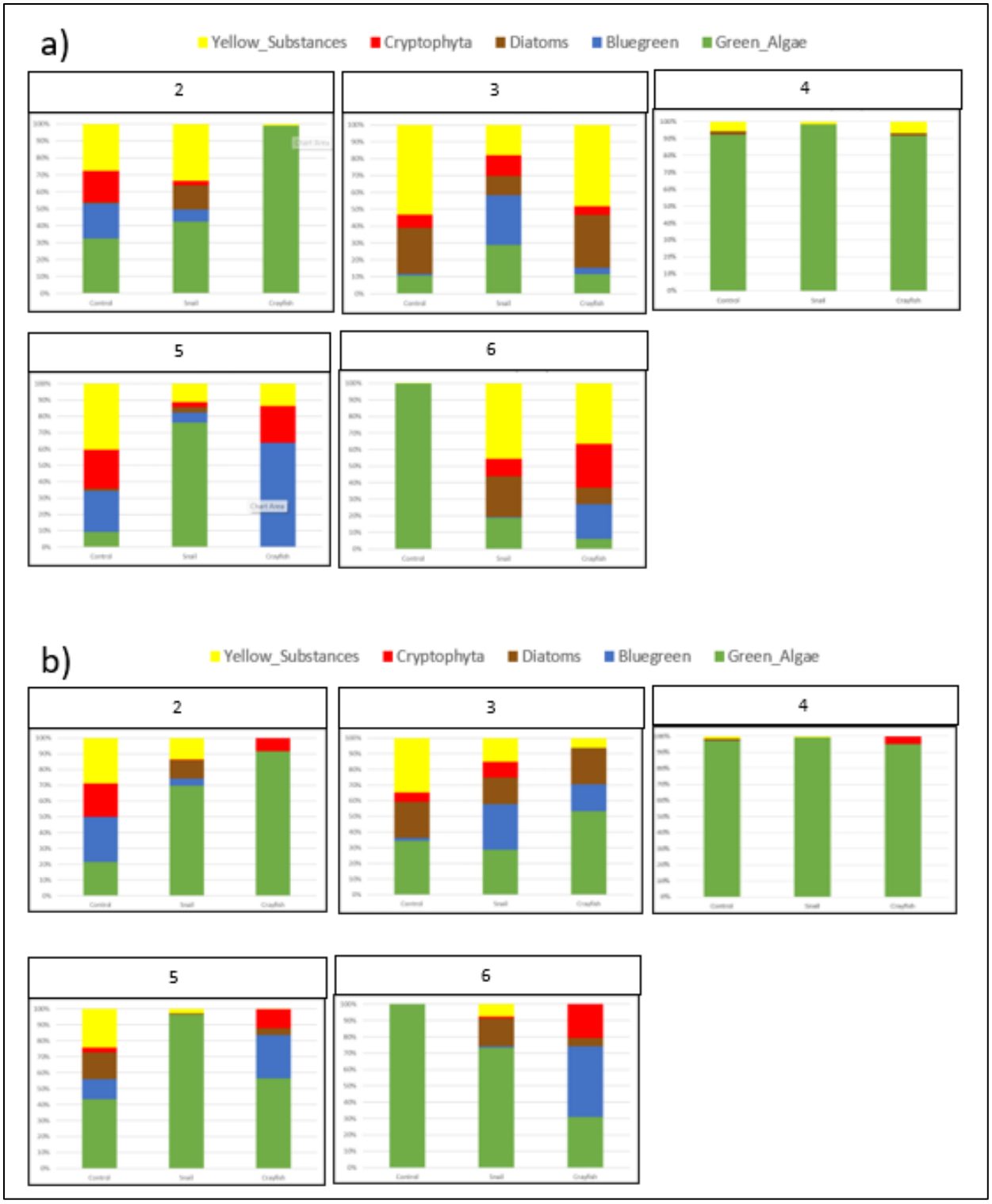

Figure 18: Plankton Group Composition stacked bar charts for a) Day 0 and b) Day 3.

APPENDIX B. TABLES

Table 1: Names of nutrient pools sampled and description.

Nutrient Pool	Description of nutrient pool
Water Column	Water contained within the mesocosm system above the sediment
Macrophyte	Sweet flag (*Acorus americanus*), an emergent wetland plant, planted within the mesocosms
Microbial	Photoautotrophic and heterotrophic microbes that grow on surfaces and suspended in the water column
Tile Biofilm	Photoautotrophic and heterotrophic microbes that grow on the surface of unglazed ceramic tiles
Mesocosm Wall Biofilm	Photoautotrophic and heterotrophic microbes that grow on the surface of the mesocosm walls (plexiglass acrylic)
Epiphytic Plant Biofilm	Photoautotrophic and heterotrophic microbes that grow on the surface of aquatic plants
Floating Algal Mat	Photoautotrophic and heterotrophic microbes that grow in mats on the surface of the water column
Suspended Plankton	Photoautotrophic and heterotrophic microbes that grow suspended within the water column
Aquatic Macroinvertebrate	Rams-horn snails (*Helisoma spp.*) and Rusty crayfish (*Orconectes rusticus*) introduced to assigned mesocosms

Table 2: Water depth of mesocosms at time of installation (June 21-23, 2022)

Mesocosm	Triplicate	Treatment	Water Depth (cm)
1	1	Crayfish	7.62
2	1	Control	10.80
3	1	Snail	12.70
4	2	Control	20.32
5	2	Snail	20.32
6	2	Crayfish	21.59
7	3	Snail	15.24
8	3	Control	13.97
9	3	Crayfish	11.43
10	4	Control	19.05
11	4	Crayfish	17.78
12	4	Snail	17.78
13	5	Snail	12.70
14	5	Crayfish	13.97
15	5	Control	17.78
16	6	Crayfish	15.24
17	6	Snail	15.24
18	6	Control	17.78

Table 3: Diammonium phosphate (DAP) volumes added to mesocosm for nutrient pulse according to the volume of water within each mesocosm.

Height (in.)	Volume (L)	Amount of DAP (mL)
0.64	0.32	0.013
1.27	0.64	0.027
1.91	0.97	0.040
2.54	1.29	0.053
3.18	1.61	0.066
3.81	1.93	0.080
4.45	2.25	0.093
5.08	2.57	0.106
5.72	2.90	0.120
6.35	3.22	0.133
6.99	3.54	0.146
7.62	3.86	0.159
8.26	4.18	0.173
8.89	4.50	0.186
9.53	4.83	0.199
10.16	5.15	0.213
10.80	5.47	0.226
11.43	5.79	0.239
12.07	6.11	0.253
12.70	6.43	0.266
13.34	6.76	0.279
13.97	7.08	0.292
14.61	7.40	0.306
15.24	7.72	0.319
15.88	8.04	0.332
16.51	8.37	0.346
17.15	8.69	0.359
17.78	9.01	0.372
18.42	9.33	0.385
19.05	9.65	0.399
19.69	9.97	0.412
20.32	10.30	0.425
20.96	10.62	0.439
21.59	10.94	0.452
22.23	11.26	0.465
22.86	11.58	0.478
23.50	11.90	0.492
24.13	12.23	0.505
24.77	12.55	0.518
25.40	12.87	0.532
26.04	13.19	0.545
26.67	13.51	0.558
27.31	13.84	0.571
27.94	14.16	0.585

Table 4: Methods used by the Midden Lab to analyze water samples for dissolved and total nutrients. Brackets indicate the source of the methods from either the U.S. Environmental Protection Agency [EPA] or Patton and Kryskalla [USGS].

Constituent	NWIS parameter code	Method and NWIS method code	Reporting limit, mg/L
Dissoved Ammonia, as nitrogen (NH_3)	00608	Colorimetry, alkaline phenol and hypochlorite (auto phenate), EPA-103-A, EPA 350.1 [EPA], CL015	0.05
Dissolved nitrite plus nitrate nitrogen (NO_x)	00631	Colorimetry, cadmium reduction, EPA-114-A, EPA 353.2 [EPA], CDR06	0.31
Dissolved reactive phosphorus (DRP)	00671	Colorimetry, acidic molybdate, EPA-118-A, EPA 365.3, [EPA], 0019	0.013
Total nitrogen (TN)	62855	Colorimetry, after alkaline persulfate digestion [USGS, EPA], AKPO1	0.31
Total phosphorus (TN)	00665	Colorimetry by discrete analyzer, after alkaline-persulfate digestion, [USGS, EPA], PSF03	0.01

Table 5: Summary of analyses used for each data type and period

Data Period & Type	Analysis
Monitoring Period	
Time Series	RMANOVA nested by triplicate with Greenhouse-Geiser correction
Single Value	ANOVA, Welch's ANOVA, Kruskal-Wallis test nested by triplicate with post-hoc Tukey or Games-Howell
Nutrient Pulse Period	
Time Series	RMANOVA nested by triplicate with Greenhouse-Geiser correction
Single Value	ANOVA, Welch's ANOVA, Kruskal-Wallis test nested by triplicate with post-hoc Tukey or Games-Howell
Plankton Group Composition	PERMANOVA with post-hoc ANOVA

Table 6: Analysis used for each variable in each experimental period.

Variable	**Monitoring**	**Monitoring (No Triplicate 1)**	**Nutrient Pulse**	**Nutrient Pulse (No Triplicate 1)**
pH	RMANOVA	RMANOVA	ANOVA	ANOVA
Temperature	RMANOVA	RMANOVA	ANOVA	ANOVA
Conductivity	RMANOVA	RMANOVA	ANOVA	ANOVA
Dissolved Oxygen	RMANOVA	RMANOVA	ANOVA	ANOVA
Light Transmission	ANOVA	ANOVA	-	-
Total Algae & Microbial Growth Dried Mass	ANOVA	ANOVA	-	-
Total Macrophyte Dried Mass	Kruskal-Wallis	ANOVA	-	-
Water Column TP	RMANOVA + ANOVA	ANOVA	Welch's ANOVA	Welch's ANOVA
Water Column TN	ANOVA	RMANOVA + ANOVA	Welch's ANOVA	Welch's ANOVA
Water Col DRP	Welch's ANOVA	Kruskal-Wallis	ANOVA	Kruskal-Wallis
Water Col NH3	Welch's ANOVA	Welch's ANOVA	ANOVA	ANOVA
Water Col NO3NO2	ANOVA	Kruskal-Wallis	Welch's ANOVA	Welch's ANOVA
Algae Group Composition	PERMANOVA + ANOVA	PERMANOVA + ANOVA	-	-

Table 7: Summary table of non-significant data results.

Variable	Test	Statistic
Monitoring Period		
Dissolved Reactive Phosphate (DRP)	Kruskal-Wallis T-test	(X^2 = 1.68, df = 2, p = 0.432)
Nitrate-Nitrite (NO3NO2)	Kruskal-Wallis T-test	(X^2 = 3.39, df = 2, p = 0.183)
Temperature	RMANOVA	(F = 0.958, df = 2, 8, p = 0.424)
Conductivity	RMANOVA	(F = 0.718, df = 2, 8, p = 0.517)
Total Microbial Growth Dried Mass	ANOVA	(F = 3.934, df = 2, 11, p = 0.051)
Total Macrophyte Dried Mass	ANOVA	(F = 0.589, df = 2, 11, p = 0.571)
Nutrient Pulse Period		
Total Phosphorus	Welch's ANOVA	(F = 0.831, df = 2, 7.647, p = 0.471)
Total Nitrogen	Welch's ANOVA	(F = 1.160, df = 2, 5.666, p = 0.378)
Ammonia (NH3)	ANOVA	(F = 0.268, df = 2, 8, p = 0.771)
Nitrate-Nitrite (NO3NO2)	Welch's ANOVA	(F = 0.446, df = 2, 1.414, p = 0.708)
Dissolved Reactive Phosphate (DRP)	Kruskal-Wallis T-test	(X^2 = 2.84, df = 2, p = 0.242)
Temperature	ANOVA	(F = 0.019, df = 2, 26, p = 0.981)
Conductivity	ANOVA	(F = 2.009, df = 2, 26, p = 0.154)
Plankton Group Composition		
Day 0	PERMANOVA	(F = 0.267, df = 2, 12, p = 0.966)
Day 3	PERMANOVA	(F = 0.992, df = 2, 12, p = 0.327)
Green Algae	ANOVA	(F = 1.733, df = 2, 12, p = 0.218)
Bluegreen	ANOVA	(F = 2.384, df = 2, 12, p = 0.134)
Diatoms	ANOVA	(F = 0.604, df = 2, 12, p = 0.562)

APPENDIX C: RESULTS ANALYSIS INCLUDING TRIPLICATE 1

6. RESULTS

6.1 EXPERIMENT PERIOD (DAY 0-5)

6.1.1 Nutrients

The crayfish treatment saw higher levels of total phosphorus in the water column (RMANOVA, F = 4.470, df = 2, 10, p = 0.041; Figure 19) than the control and snail treatments. Treatment effects were not detected for total nitrogen, and the dissolved nutrients: DRP, NH_3, and NO_3 and NO_2.

6.1.2 Water Chemistry

All treatments saw a decrease in pH (RMANOVA, F = 7.521, df = 4, 40, p = 0.004; Figure 20) and dissolved oxygen (F = 4.024, df = 4, 40, p = 0.036; Figure 21) as the experimental period progressed.

We found the crayfish treatment had lower light transmission levels (One-way ANOVA, F = 56.184, df = 2, 14, p = <0.001; Figure 22).

I did not detect an effect of the treatment for temperature or conductivity.

6.1.3 Dried Mass

Total biolfim dried mass was higher for the crayfish treatment (One-way ANOVA, F = 3.984, df = 2, 14, p = 0.0427; Figure 23). I did not detect an effect of the treatment for the total macrophyte dried mass.

6.2 NUTRIENT PULSE (DAY 5-6)

6.2.1 Nutrients

Treatment effects were not detected for nutrients in the water columns including total phosphorus, total nitrogen, dissolved NH_3, dissolved NO_3 and NO_2, and DRP.

6.2.2 Water Chemistry

We found lower pH levels in the crayfish treatment than the control (Tukey's $p = 0.014$) and snail treatments (Tukey's $p = 0.009$; One-way ANOVA, $F = 6.415$, $df = 2, 32$, $p = 0.005$; Figure 20). Dissolved oxygen levels were lower in the crayfish treatment than the snail treatment (Tukey's $p = 0.050$; One-way ANOVA, $F = 3.288$, $df = 2, 32$, $p = 0.050$; Figure 21) but was not lower than the control treatment. Conductivity was found to be lower in triplicate one then the remaining triplicates two through six (One-way ANOVA, $F = 11.245$, $df = 1, 32$, $p = 0.00206$; Figure 24).

6.3 PLANKTON GROUP COMPOSITION (DAY 0-3)

When comparing the change in plankton group composition from Day 0 and Day 3, visually the crayfish treatment appeared to be different than the control and snail treatments (Figure 25) but was not significant (PERMANOVA, $F = 1.3469$, $df = 2, 15$, $p = 0.091$).

APPENDIX D: FIGURES FOR APPENDIX C

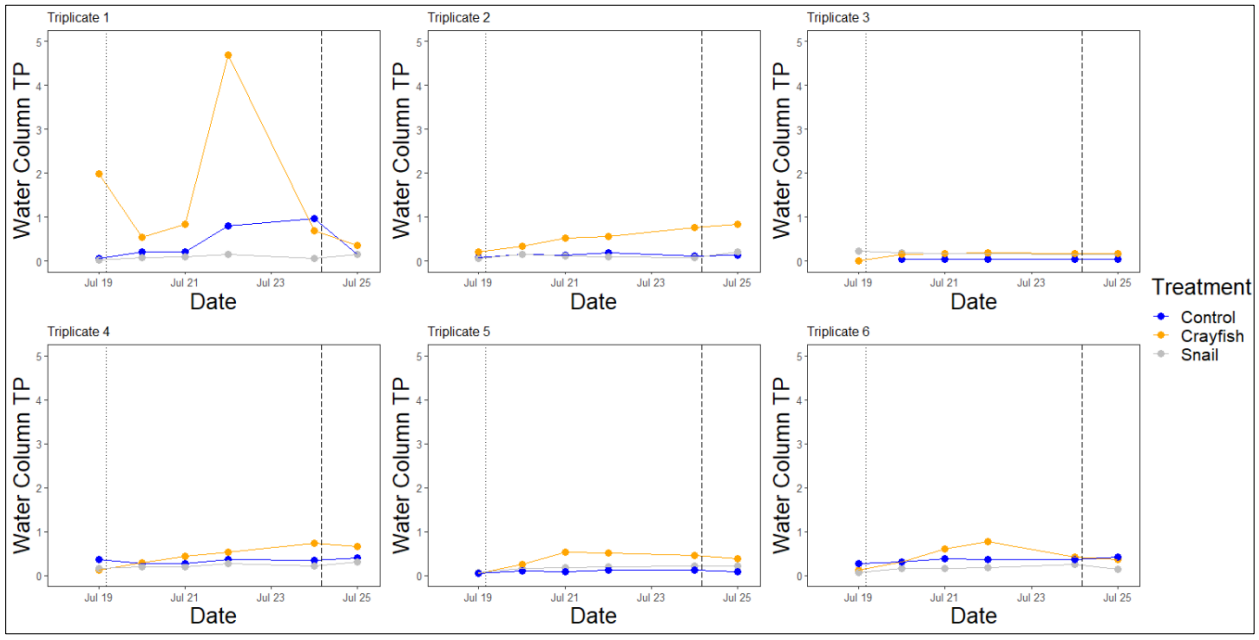

Figure 19: Water column total phosphorus levels for each mesocosm within each triplicate. The small dashed line marks Day 0 of the experiment and the large dashed line marks Day 5 when the nutrient pulse was added.

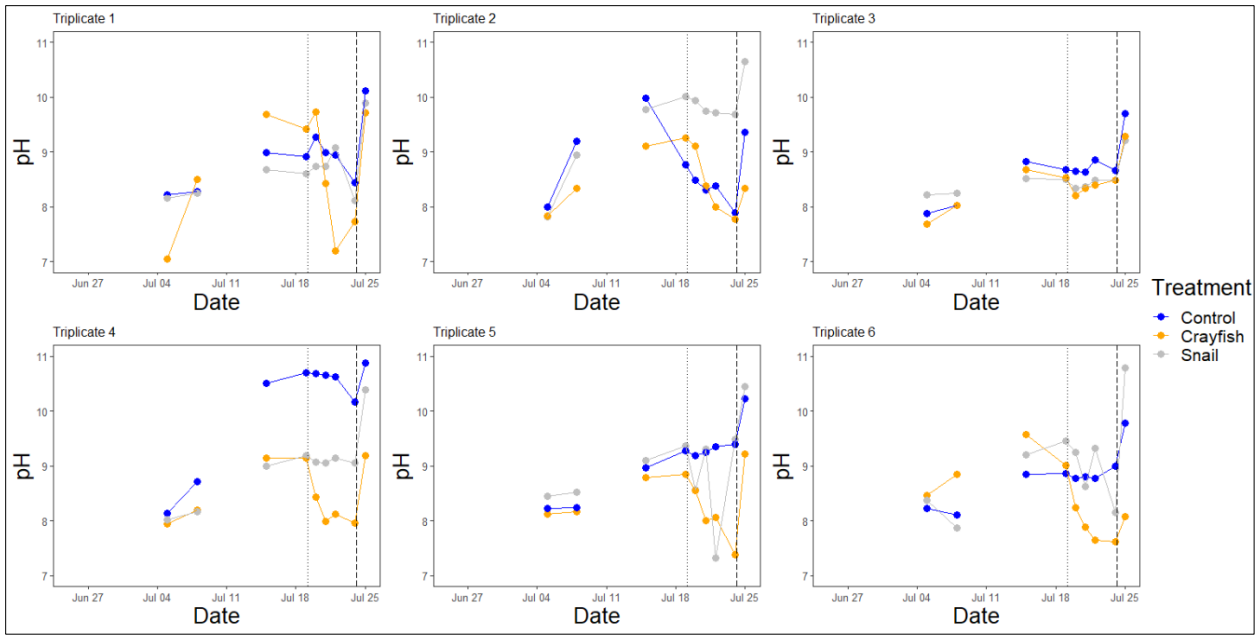

Figure 20: pH levels for each mesocosm within each triplicate. The small dashed line marks Day 0 of the experiment and the large dashed line marks Day 5 when the nutrient pulse was added.

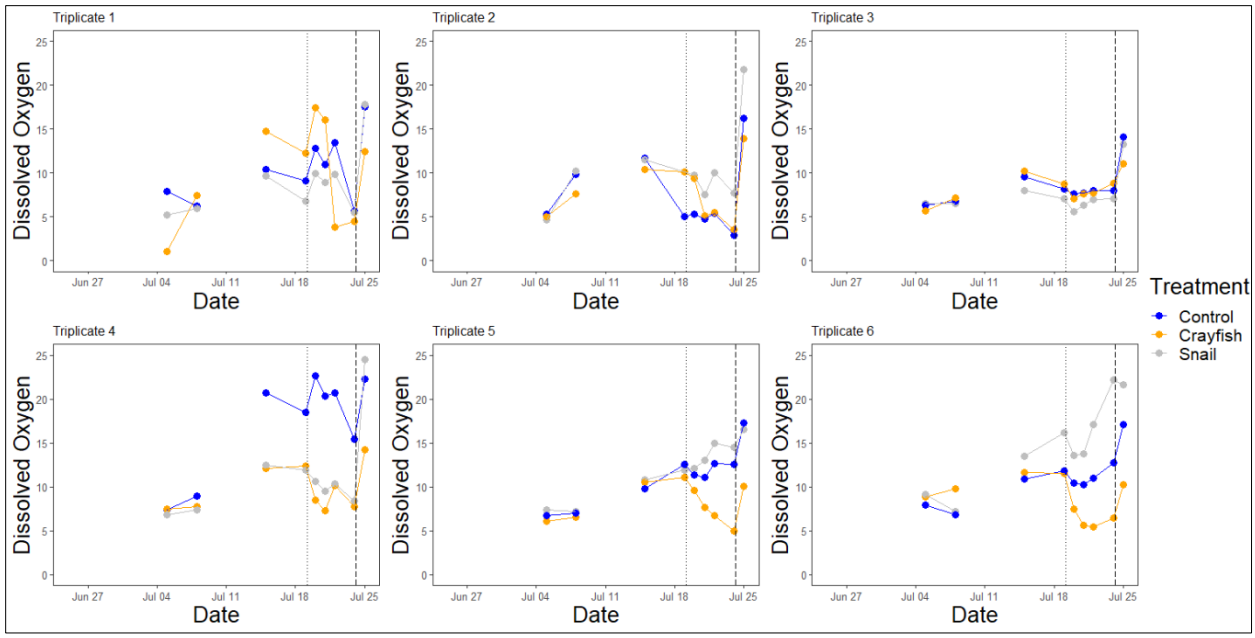

Figure 21: Dissolved oxygen levels for each mesocosm within each triplicate. The small dashed line marks Day 0 of the experiment and the large dashed line marks Day 5 when the nutrient pulse was added.

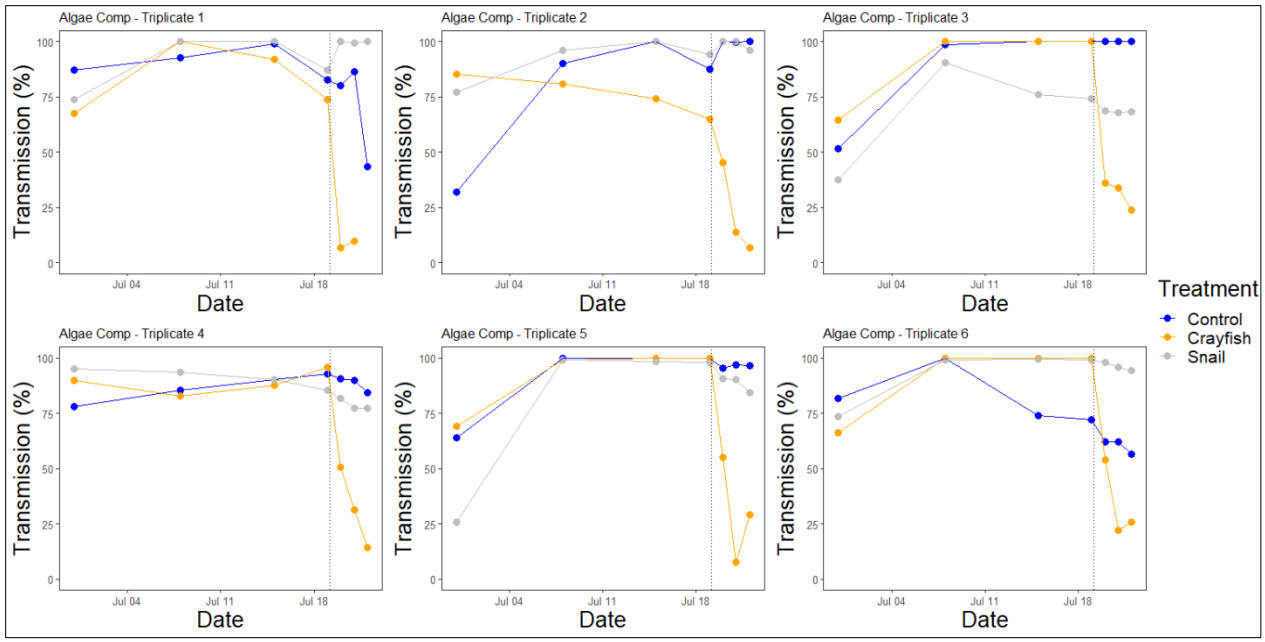

Figure 22: Water light transmission levels of each mesocosm. The small dashed line marks Day 0 of the experiment. Data was collected only till Day 4 of the experimental period.

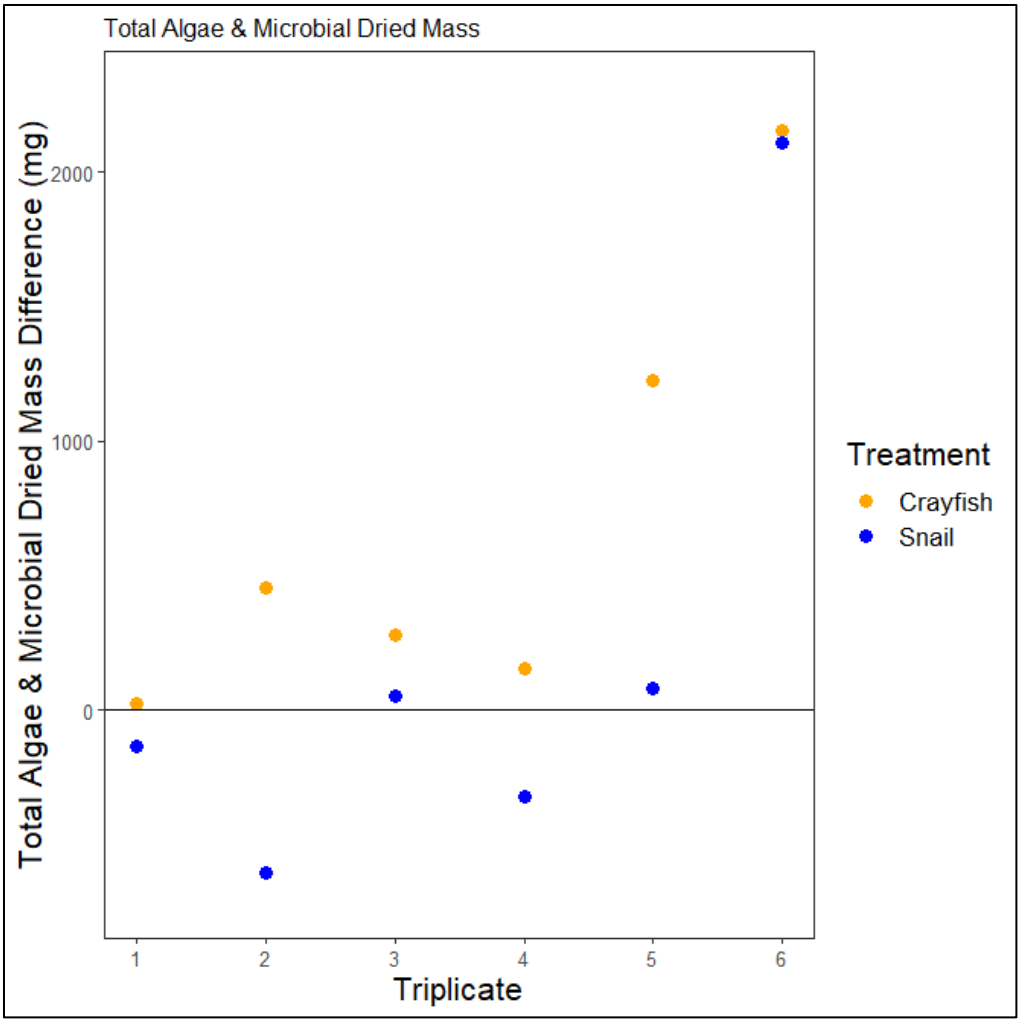

Figure 23: Total Dried Mass. Graphed values show the difference in mass of each treatment mesocosm to the control mass within the same triplicate.

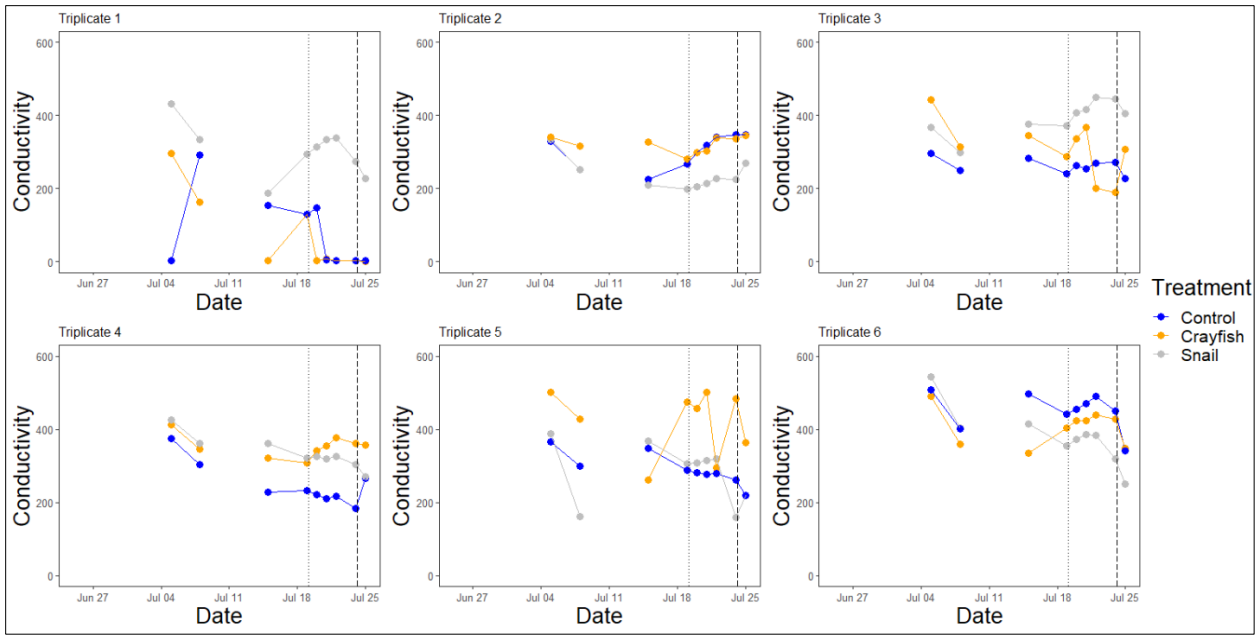

Figure 24: Conductivity levels for each mesocosm within each triplicate. The small dashed line marks Day 0 of the experiment and the large dashed line marks Day 5 when the nutrient pulse was added.

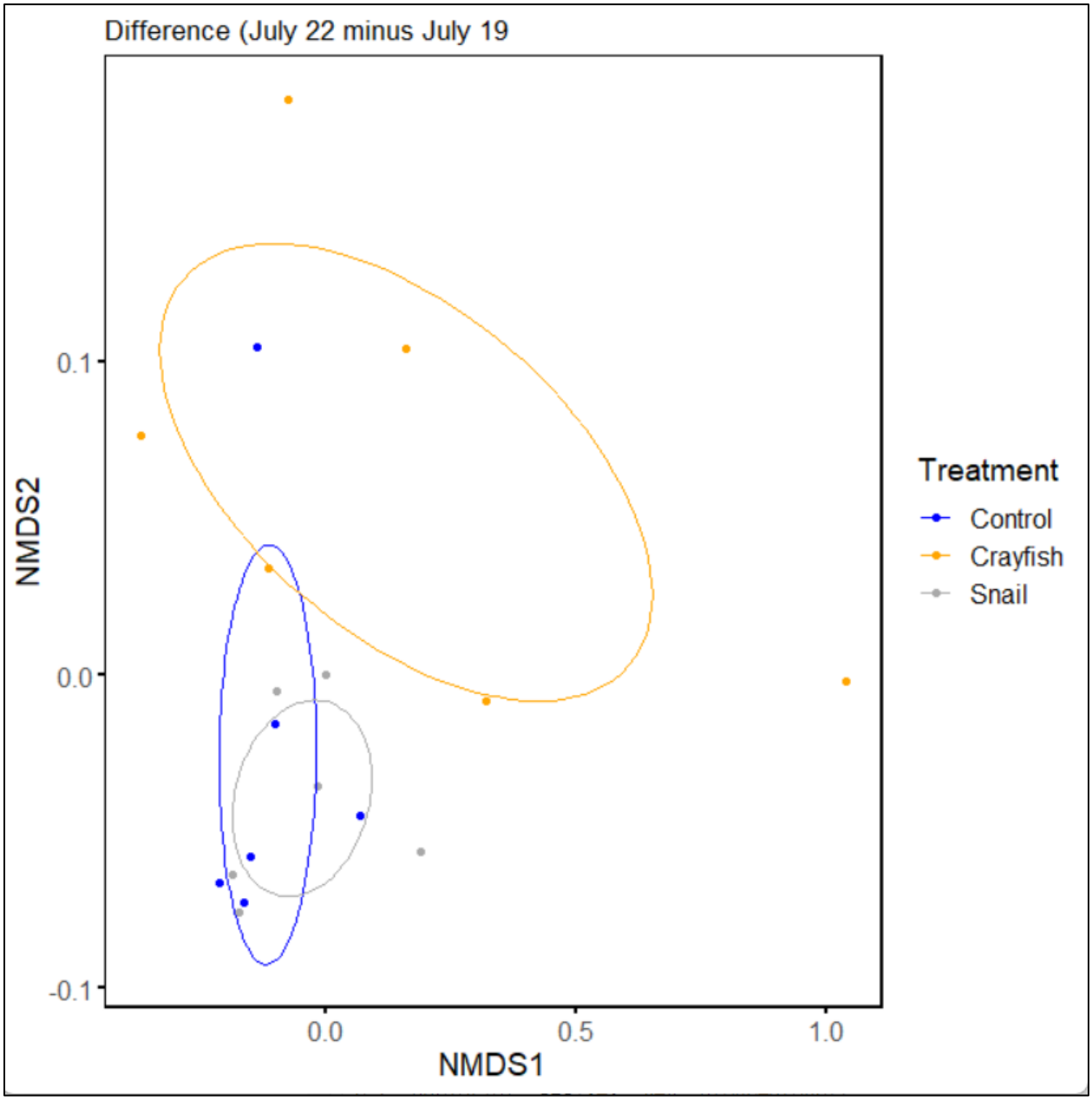

Figure 25: NMDS plotting the change in plankton group composition from Day 0 to Day 3 by treatment.