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# Crayfish (*Procambarus clarkii*) Effects on Lentic Lake Water Quality

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# Crayfish (*Procambarus clarkii*) Effects on Lentic Lake Water Quality



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A Major Qualifying Project Report

Submitted to the Faculty

of the

WORCESTER POLYTECHNIC INSTITUTE

in partial fulfillment of the requirements for the

Degree of Bachelor of Science

By

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Submitted to  
Professor Michael Buckholt  
Professor Lauren Mathews

Date:  
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## **ABSTRACT**

Salisbury Pond, a small lentic lake in Worcester, Massachusetts, is a habitat for the invasive crayfish species *Procambarus clarkii*. In order to study the changes *P. clarkii* have on the ecosystem, we studied the water chemistry of individual, semi-closed, aquatic microenvironments of 4 experimental groups. These included two treatment groups with crayfish that either contained or did not contain macrophyte and two control groups without crayfish that either contained or did not contain macrophyte. The presence of macrophyte was used to determine if the native plants would have an effect on the water parameters. Water quality tests included phosphorus, nitrate, nitrite, ammonia, dissolved oxygen (DO), turbidity, and pH. After 14 data collection weeks, the treatment and control tanks were compared. Due to crayfish activity such as turbation and burrowing, ammonia and absorbance concentrations were higher in treatment tanks while DO and pH were lower. Nitrite, nitrate, and phosphorus concentrations varied over time, but all had similar patterns of concentration change including a peak and then slow decrease. The results of this study will offer insight into the methods by which invasive species disturb aquatic systems as well as the ecology of the lentic ecosystem and the resident benthic organisms.

## **ACKNOWLEDGEMENTS**

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# 1. INTRODUCTION

Invasive species have garnered increasing attention as society becomes more aware of their ecological, social and economic impacts. In this study, the impact of the invasive red swamp crayfish in a small man-made Massachusetts pond is analyzed by measuring the changes they effect on ecologically important water parameters. The results of this study will not only offer some insight into the methods by which invasive species disturb aquatic systems, but also into the functioning of lentic ecosystems and the role of benthic organisms in them. More importantly, by analyzing the changes in the biotic communities and nutrient composition, we will be able to discover the extent of change the pond has experienced and make predictions for the future.

Lentic ecosystems are a general category of aquatic ecosystems which comprise slowly flowing open bodies of water (mainly freshwater) with clearly defined boundaries in ground depressions not in contact with the ocean (e.g. lakes, ponds, saline lakes, glacial ice dams, pools formed at edges of large lakes, damming by excessive plant growth) (Dodds, 2002). This definition excludes estuaries and other embayments that fall within the marine jurisdiction. Lakes are a type of lentic system. They are large, temporary bodies of standing or slowly moving water enclosed by defined boundaries with a river inflow and outflow. As part of many nutrient cycles, lake are dominated by biomass produced by phytoplankton (primary producers), which is consumed by zooplankton, and zooplankton consumed by heterotrophs (Dodds, 2002). This simple model of energy flux in lentic ecosystems is illustrated in Figure 1. Following this model, many ecological studies have assumed that benthic (bottom layer) primary production is not important. Although this may be true for large, deep lakes, benthic primary production may play a significant role in small, shallow lakes and ponds. Half or more of the total primary production in shallow lakes may be attributed solely to macrophytes (Wetzel, 1983). In other words, phytoplankton dominate the producer communities in large, deep lakes, while macrophytes are more dominate in shallow water bodies. Our study analyzed the interactions in a small, temperate pond in central Massachusetts. The following paragraphs will focus on the biotic communities, trophic interactions and abiotic processes that rule this kind of lentic environment.

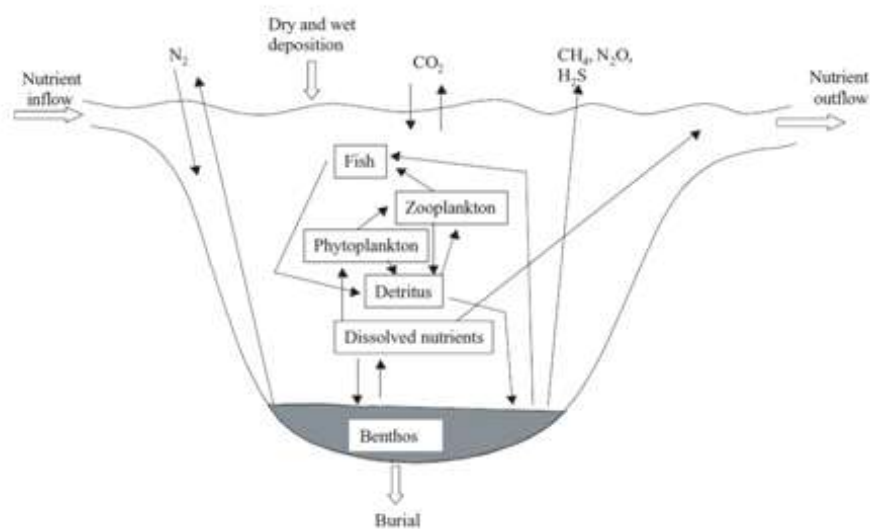


Figure 1: A simple diagram of energy and nutrient flux through a lake ecosystem (Dodds, 2002)

Biotic communities are formed from all living organisms in a given area. In aquatic and terrestrial communities, there are three main trophic levels: producers, consumers, and decomposers. Each specific type of organism falls into one or two trophic levels and gains energy from the trophic level below. Producers are autotrophic and make their own food by primary production via photosynthesis (Field et al., 1998). In order for primary production to occur, abiotic factors such as nutrients, light, water, and stable temperatures are required. The location, availability, and cycling of these resources limit primary and thus secondary production. Secondary production is the creation of biomass by consumers, heterotrophs who eat the biomass created by producers (Field et al., 1998). These complex ecosystem production hierarchies are not exclusive of their environments. Any change in an ecosystem, both natural (such as flooding) and manmade (such as habitat destruction), can inhibit the availability of required nutrients and the outputs of primary and secondary production. Our experiment studied the interactions between crayfish (both a consumer and a decomposer) and primary producers in a small pond, a semi-closed, freshwater lentic system.

The biotic communities of these ponds comprise a wide variety of bacteria, macrophytes, rotifers, annelids, crustaceans, insects, mollusks and fishes, many of which are unique to these ecosystems. The microflora of ponds is an integral part of the chemical limnology and trophic dynamics of these ecosystems. Bacteria are known to play a significant role in pond trophic dynamics and energy transfers and they are mediators in abiotic cycles (e.g. hydrogen, sulfur, carbon, iron, manganese and phosphorus) within these systems (Cole, 1983). The macroflora of ponds is largely formed by aquatic plants able to adapt to the large amount of water and sunlight, such as floating or emergent macrophytes found in the water column near land (Robinson, 2004). Macrophytes are an important primary producer in freshwater environments as they provide numerous resources for organisms, including oxygen, food and shelter, and transform inorganic chemicals, such as nitrate and ammonium nitrogen, into usable forms for other organisms. Moreover, the vast majority of lake organic matter is in the form of dead plant biomass. Rotifers, annelids, crustaceans, insects, mollusks and fishes are considered by limnologists to be the most important animal taxa in pond ecosystems (Cole, 1983). This does not mean, however, that other animals (e.g., waterfowl, amphibians and some mammals) are not present and are not of relevance. Macroinvertebrates, including crustaceans (e.g., crayfish), worms, mollusks, and insects, are consumers found in or near sediment. Besides controlling plant biomass, macroinvertebrates are bioturbators who move sediment and release nutrients back into the water column (Robinson, 2004).

The abiotic processes of pond ecosystems are dominated by three nutrient cycles: carbon, nitrogen, and phosphorus. Nitrogen and phosphorus are limiting, primary nutrients in freshwater environments. Nitrogen compounds are products of microbial digestion and the nitrogen cycle (Hall, 2004). Both plants and animals rely on nitrogen for tissue growth and protein synthesis (Minnesota, 2008). Nitrogen is found naturally in three forms: ammonia ( $\text{NO}_4$ ), nitrates ( $\text{NO}_3^-$ ), and nitrites ( $\text{NO}_2^-$ ). Atmospheric nitrogen ( $\text{N}_2$ ) gas can be converted into these forms by nitrogen fixation. In aquatic ecosystems, blue-green algae are the primary producers that do this. After fixation, ammonia, nitrates, and nitrites incorporate into soil or sediment. In this case, nitrogen settles into the water as well. Specific types of bacteria and fungi oxidize ammonia to nitrite and nitrite to nitrate for energy (U.S. EPA, 2000). Ammonia concentrations in freshwater can measure between 0.2 and 3 mg/L while nitrite concentrations average between 0.001 to 1 mg/L. Nitrate concentrations range from 0-5 mg/L in freshwater and higher concentrations indicate

pollution or eutrophication (Chapman & Kimstach, 1996). Nitrogen in any form returns back to the atmosphere by denitrifying bacteria.

Phosphorus occurs in nature combined with oxygen as phosphate, a component of DNA, RNA, ATP, and cell membrane phospholipids (as phosphate  $\text{PO}_4^{-3}$ , also known as orthophosphate). Phosphorus comes from many terrestrial sources, including animal waste, erosion of rocks, plant matter, and fertilizers (U.S. EPA, 2000). When it enters a water system, dissolved phosphates are either used by photosynthetic organisms for cell proliferation, reproduction, and growth or become incorporated into sediment (Minnesota, 2008). Phosphate is found in sediment at very low levels, between 0.005-0.020 mg/L (Chapman & Kimstach, 1996). Disruption of sediments and bacterial decomposition releases phosphates back into the water system (Hall, 2004). As of 1987, average total phosphorus, or the total concentration of all forms of phosphorus, ranged from 0.013 - 0.017 mg/L in Salisbury Pond (Massachusetts, 2002). According to the U.S. Environmental Protection Agency (2001), surface water total phosphorus reference levels range between 0.006 and 0.048 mg/L.

Carbon is the element that drives trophic consumption and is used for primary production. Carbon is emitted by organisms during cellular respiration in the form of carbon dioxide ( $\text{CO}_2$ ). In lentic ecosystems, carbon dioxide dissolves in water and precipitates into many forms. While  $\text{CO}_2$  is produced during cellular respiration, dissolved oxygen is needed to complete this process. Freshwater dissolved oxygen (DO) is a measure of the amount of gaseous oxygen present in water. DO averages about 8 mg/L at 25°C (higher for lower temperatures) (Chapman & Kimstach, 1996). DO less than 5 mg/L inhibits survival of organisms (Chapman & Kimstach, 1996). Organisms, including plants and algae, use carbon dioxide for photosynthesis, the process that drives primary production. Many organisms consume plant matter, including decomposing bacteria, for their carbon source (U.S. EPA, 2000). The cycle continues with respiration into the atmosphere.

In lentic ecosystems, these nutrient cycles provide an insight to the vast differences between lakes, including difference in levels of productivity and water retention, pH, and oxygen availability (Dodds, 2002). Excesses of either phosphorus or nitrogen may cause eutrophication, because these nutrients are typically limiting. This phenomenon is known as nutrient loading which caused an increase in primary production. The accumulation of dead organic matter ultimately deoxygenizes the body of water (Zarski et al., 2010). These abiotic characteristics provide a framework for the organisms that will be able to colonize and reproduce successfully (Bronmark & Hansson, 2005, Southwood, 1988, Moss et al., 1994). In recent years, however, there has been an increasing interest in the role of interactions between organisms and their environment, resulting in a new focus on how interactions between biota and abiotic processes determine the dynamics of freshwater systems (Bronmark and Hansson 2005).

An increasing number of biological introductions threaten to disturb the interactions of an ecosystem's biotic and abiotic elements. In the past few years, biological invasions have increasingly garnered environmental concern due to their deep ecological implications. The threat of invasive species introductions becomes even more pressing in inland freshwaters where extensive unintentional (e.g. via ship ballast water, fouling) and intentional (e.g. stocking of invertebrates and fish) releases of organisms are frequent despite increasing regulatory efforts to preclude them (Ricciardi, 2001).

Freshwater aquatic invasives are introduced through several different vectors. Since the early 1900s, the main introduction vector for aquatic invasive species has been shipping related. Of the introduced nonnative species documented in North America, 40 to 50% have been



associated to shipping activities (ballast water and, to a lesser extent, hull fouling) (Fuller, 2003). Stocking organisms for food, sport, or forage also accounts for 44% of all introduced fish species (Fuller, 2003). A smaller percentage of aquatic invasives can be attributed to aquarium and fish farm release/escape and bait release.

Approximately 2-4% of known introduced species have significant, irreversible impacts on the native community (Lodge, 1993). Successful aquatic invaders often displace natives through competition, parasitism, or predation, are vectors of exotic pathogens, alter patterns of natural selection and gene flow, and alter ecosystem processes. In the Great Lakes, the high-profile zebra mussel (*Dreissena polymorpha*) was responsible for the alteration of both abiotic (water transparency, nutrient cycling, and benthic habitat structure) and biotic (food-web structure, bioaccumulation of contaminants, and diversity of congeners) factors (Strayer et al., 1999). Furthermore, the zebra mussel invasion led to the introduction of a roundworm parasite (*Bucephalus polymorphus*) that was responsible for dramatic impacts on cyprinid freshwater fish, the parasite's intermediate host (Crowl et al., 2008). In Wisconsin, the exotic crayfish *Orconectes rusticus* led to the local extinction of native congeners through competition for shelter and selective predation by fish, and reduced macrophyte and other invertebrate populations by as much as 100% (Lodge, 1993). These biological invasions are expected to continue as expanding global trade increases the volume of flora and fauna that is transported from one geographic range to another.

This study focuses on the invasive red swamp crayfish (*Procambarus clarkii*). This species is a freshwater crustacean species naturally found in marshes, slow flowing rivers, and reservoirs of northeast Mexico and south central United States (Hobbs, 1989). They were later introduced by humans in different parts of the United States, including Massachusetts, as well as South America, Europe, Africa, and Asia, making it an invasive species (Fishar, 2006).

Red swamp crayfish are known for their dark red color and can weigh as much as ~50 grams and have a carapace length of ~5.5 to 12 centimeters as adults. Juveniles may lack the dark red coloration and can be mistaken for other *Procambarus* species (Henttonen & Huner, 1999). Like other crayfish species, individuals of *P. clarkii* reproduce sexually and their sex can be determined externally: males have hooks on the ischia of at the 3rd and 4th pereopods and females lack the hooks and contain an opening on the abdomen (Henttonen & Huner, 1999).

*Procambarus clarkii* have a short lifespan and high fecundity. They reach full maturity at around 4.5 months and produce 250-300 eggs at any time year around. The number of eggs depends on the female size (the bigger the female, the more eggs produced). The incubation time of eggs can vary between 3 weeks - 6 months depending on the water temperature and pH. They can survive in temperature from 20-34°C and pH from 6.5-9.0, but the optimal temperature and pH for growth and reproduction is 22°C and 7.0-8.0 respectively. The newly hatched crayfish live with their mother for about 8 weeks before they can survive on their own (Akefors, 2000). As the crayfish grows, it goes through different stages of molting and eating. The crayfish mainly feed on benthic invertebrates, detritus, macrophytes, and algae (Gherardi, 2007).

Records of *P. clarkii* outside their native range have documented the species' impact on their new environments. Red swamp crayfish are known to alter both abiotic and biotic factors of the ecosystems to which they have been introduced. Previous studies show massive reductions in macrophyte diversity and mass. For instance, 20 years after the introduction of *P. clarkii* to the Doñana National Park in Spain, more than 80% of macrophyte biomass was lost (Gutierrez-Yurrita et al., 1998). Laboratory and enclosure experiments carried out by Gherardi and Acquistapace (2007) confirm this species' ability to reduce macrophyte biomass through intense

grazing and damage done by clipping and uprooting at both low and high densities. Enclosure experiments carried out by Rodriguez et al. (2003) provided further evidence of *P. clarkii*'s non-consumptive macrophyte reduction (up to 65% in 15 days). Likewise, Lodge and Lorman (1987) found that *O. rusticus* reduced total macrophyte biomass by 64% in low crayfish densities, and eliminated macrophytes completely in 12 weeks in high crayfish densities. Similarly, *P. clarkii* was demonstrated to have an impact on abiotic factors. Various enclosure experiments have shown increased total phosphorus and nitrogen (up to 7 times higher) (Angeler et al., 2001, Rodriguez et al., 2003). Angeler et al. (2001) found a total depletion of nitrates within the system after crayfish introduction, while Rodriguez et al. (2003) found an increase in ammonium and total suspended solid levels. Conductivity, alkalinity, and pH were also recorded but results were not reported.

In this experiment, we studied the changes in water characteristics of individual, semi-closed, aquatic microenvironments effected by *Procambarus clarkii* in order to examine the overall changes this invasive crayfish may have on the local Salisbury Pond ecosystem. Half of these tanks also included local macrophytes harvested from Salisbury Pond to explore the effect *P. clarkii* have on the local macrophyte population. Water quality tests included orthophosphate, nitrate, nitrite, ammonia, dissolved oxygen, turbidity, and pH. In order to assess crayfish impact on macrophytes, we collected all macrophytes in each tank and measured change in wet weight. Additionally, we recorded the carapace length and mass of each crayfish over time to determine if these correlated with water chemistry.

## 2. METHODOLOGY

Salisbury Pond, the location of this investigation, is an urban artificial lake located in northern Worcester, Massachusetts at 42 degrees 16'38" N, 71 degrees 48'22" W (Massachusetts DEP, 2002; Figure 2). The lake, which is created by the Grove Street dam in the Blackstone River watershed (Mill Brook sub-watershed), is approximately 6 hectares in area with an average depth of 1 foot. Being in an urban area, Salisbury Pond is inundated with water runoff from streets, paved areas, and a nearby park, which potentially carry chemical pollutants. The lake is also subjected to some contamination by sewage entering the watershed via structural damage to upstream sewer infrastructure (Massachusetts DEP, 2002).

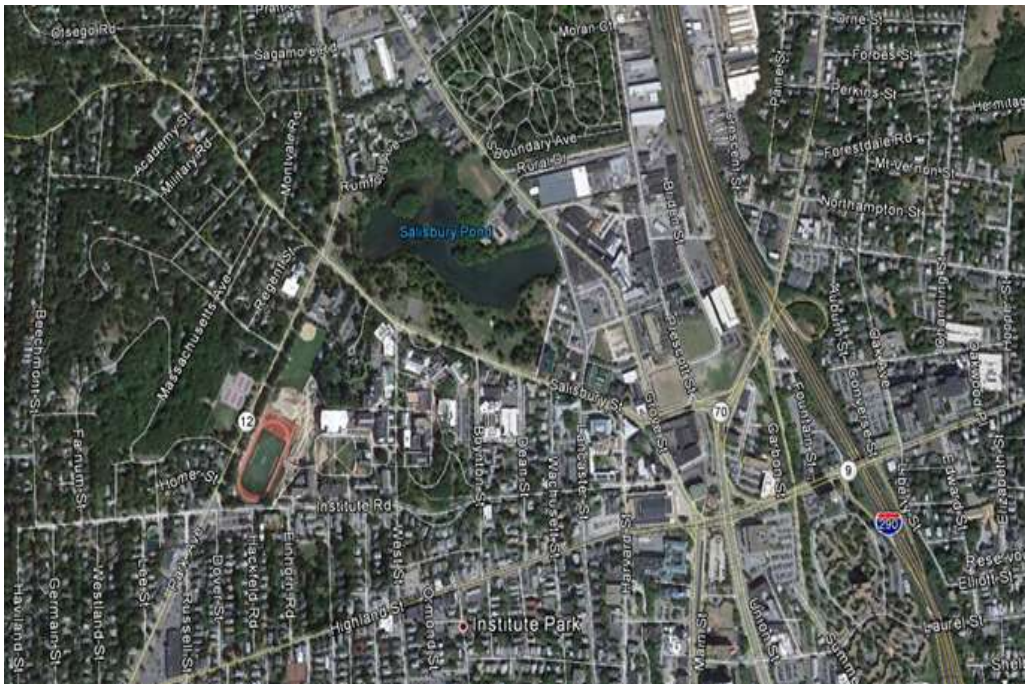


Figure 2: Salisbury Pond location in Worcester, MA (Google Maps, 2012)

Salisbury Pond is highly eutrophic, accelerated by the presence of algal and bacterial species and high phosphorus loading (Massachusetts DEP, 2002). Data from a state funded study in 1987 (Massachusetts DEP, 2002) and from our observations indicate dense macrophyte growth on the edge of the lake. The lake is relatively unshaded except near the banks. Invasive plant species such as Eurasian milfoil (*Myriophyllum spicatum*) have taken over the native plants and become detrimental to water quality and the overall health of the Salisbury Pond ecosystem (Indian Lake, 2007). Other invasive aquatic plants in the lake include duckweed (*Lemnaceae sp.*) and alligatorweed (*Alternanthera philoxeroides*).

Salisbury Pond is extremely polluted primarily because of historical industrial runoff, runoff from roads including the nearby Interstate 290, and an overall lack of management and upkeep. Poor water quality and high nutrient loading is a result of significant sediment buildup, increasing bacterial loads, high phosphorus levels, and noxious algal blooms (Massachusetts, 2002).

## 2.1 Collection of Crayfish

We collected crayfish between September and October 2012 in Salisbury Pond, Worcester, MA using six modified minnow traps distributed in four different locations along the pond (Figure 3). The traps were relocated when the number of crayfish caught per trap decreased, usually after 4-5 days.



Figure 3: Satellite image of trapping sites along Salisbury Pond (indicated by red markers) and site of water collection (indicated by yellow marker) (Google Maps, 2012)

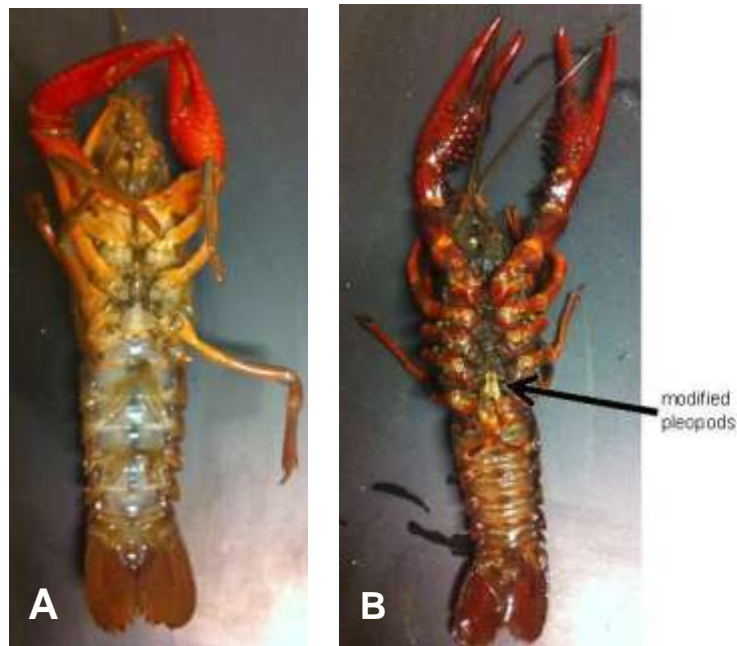
We used various baits to capture the crayfish. Chicken feet were the first choice of bait because they were inexpensive and easy to handle; however, canned sardines and fresh chicken gizzards resulted in a higher capture rate of crayfish. Each trap had two pieces of chicken gizzards or one sardine and the bait was changed once per week. The traps were checked each day (Figure 4 below) and captured crayfish were transported back to the laboratory in 20 liter buckets containing about 15 centimeters of water. As more crayfish were collected, we created a rubric to determine if the crayfish would be used in the experiment or returned to the pond. The crayfish were thrown back if they were not *P. clarkii*, if either of their chelipeds (pinchers) were misshapen or missing, if a female crayfish was carrying eggs, or if they weighed under 28 or over 55 grams. After the unused crayfish were returned to the pond, approximately 60 crayfish were retained in total.





*Figure 4: Minnow trap being checked for crayfish and bait being replaced*

Once back in the laboratory, we determined the sex of the crayfish by observing the pleopods, which are small appendages on the ventral side of the crayfish. The two most anterior pairs of pleopods indicate the sex of the crayfish. Female crayfish (Figure 5A) have soft and small swimmerets. Male crayfish (Figure 5B) have harder and longer swimmerets.



*Figure 5: *Procambarus clarkii* (A) female and Bb) male*

Each crayfish was placed in a labeled, individual temporary tank until the experiment was to begin. Each crayfish was fed one commercial shrimp pellet twice a week, but we withheld food from the 48 experimental crayfish for the week prior to the start of experimentation. We used 48 out of the 60 crayfish retained because of space constraints.

## 2.2 Tank Setup

In order to assess the impacts of *P. clarkii* on their environment, 88 Sterilite® polypropylene plastic tanks (l: 36cm, w: 24cm, h: 31cm) were each filled with 8 liters of room temperature pond water and 2 liters of sediment. Tanks were washed prior to use and we determined, from literature, that the plastic would not affect our experiment. The water and sediment was collected from Salisbury Pond between September and October. The water was collected in 20 liter buckets from one location (see Figure 3 above) and the sediment was collected from the uppermost layer above the anoxic, sulfurous layer at the same sites where the crayfish were collected. Coarse debris and any obvious living organisms were removed prior to filling the tanks.

During a two day period in mid-October, we employed a seine to collect representative samples of Eurasian milfoil (*Myriophyllum spicatum*), alligatorweed (*Alternanthera philoxeroides*), and duckweed (*Lemna sp.*) from Salisbury Pond from a location about 2 meters away from the shore. These macrophytes are invasive species found in abundance in Salisbury Pond (Wagner, 2004). As they are part of the crayfish habitat, it is important to account for their presence in the lake and effects on crayfish habitat. We were also interested in determining what effects the crayfish may have on these species. Before placing them in each tank, macrophytes were placed in buckets of tap water and washed in order to remove excess sediment and any visible organisms. We did not gather whole plants with retained roots, and thus the macrophyte samples included simple hand grabs of material.

Before the experiment began, the 88 tanks were numbered and subjected to a random number generator to assign them to treatments. We designated 24 tanks as “treatment without macrophyte” 24 as “treatment with macrophyte,” 21 as “control without macrophyte,” and 19 as “control with macrophyte”. We remeasured and reweighed all experimental crayfish chosen haphazardly from our crayfish collection. Treatment tanks (chosen by random number generator) received a single crayfish. Treatment and control tanks with macrophyte received 26 grams (wet weight) of macrophyte and a single crayfish (if applicable). The macrophyte was picked haphazardly from the mixed collection of three species, Eurasian milfoil (*Myriophyllum spicatum*), duckweed (*Lemnaceae sp.*), and alligatorweed (*Alternanthera philoxeroides*). Nothing was added to the control without macrophyte tanks. These tanks were placed uncovered approximately 20 cm below fluorescent strip lights 1.2 meters long containing 2- 40 watt fluorescent light bulbs, as shown below in Figure 6. The lights were timed to a 12 hr day: 12 hr night light cycle. Room temperature remained constant at 20-21°C.



*Figure 6: Tank setup in laboratory*

### **2.3 Testing Methods**

We sampled water parameters once a week for 6 consecutive weeks and then once every 3 weeks over another 6 week period using a variety of different methods. First, we measured dissolved oxygen and water temperature per tank using a YSI 550 DO Instrument. Before the first use, we replaced the membrane cap and calibrated the probe. For measurements, we inserted the probe into each tank and moved it back and forth until the reading stabilized. We then measured water pH using a glass electrode pH meter, calibrated using 7 and 10 pH buffer solutions. Next, we used a calibrated YSI Model 33 S-C-T electrical conductivity meter to measure water conductivity. Similar to dissolved oxygen, we placed both the pH and conductivity electrodes into each tank and recorded measurements after the readings stabilized.

We then measured phosphates, ammonia, nitrate, and nitrite. First, we used a low range Hach Phosphate Test Kit P0-23 to measure orthophosphate levels (mg/L  $\text{PO}_4$ ) (Hach, 1985b). Next, we measured ammonia nitrogen levels (mg/L N) using the Hach Ammonia Nitrogen 0-3 mg/L Test Kit (Hach, 1998). To calculate mg/L ammonia  $\text{NH}_3$ , original levels had to be multiplied by 1.2 (as directed by the test kit manual). Finally, using the Hach Nitrate-Nitrite Test Kit, we measured both nitrate and nitrite levels (Hach, 1985a). We had to then divide the nitrate nitrogen levels obtained by the kit by 4.4 to calculate nitrate (mg/L  $\text{NO}_3$ ) and divide the nitrite nitrogen levels obtained by the kit by 3.3 to calculate nitrite (mg/L  $\text{NO}_2$ ). Figure 7 shows 2 test kits we used. Finally, we measured turbidity, or absorbance at 540 nm of approximately 1.5 mL of sample per tank, using a Jenway UV spectrophotometer. We maintained water levels, marked by a line on the tank, weekly by adding deionized water.



*Figure 7: Hach testing kits for nitrate, nitrite, and phosphorus*

After all water testing was complete, crayfish were carefully removed, weighed and measured to determine if there were any changes in weight or length. We also recorded the presence of eggs or juveniles in tanks including female crayfish. Macrophytes were then collected with a fish net and a wet weight was obtained.

## **2.4 Data Analysis**

We used the software package SPSS for all data analyses. Before all analyses, we examined the data for adherence to a normal distribution. We opted to analyze the data with non-parametric tests, as many of our sample groups violated the assumption of normality for one or more parameters. We calculated the individual percent change in carapace length and mass from the initial week to the final week. A Pearson's correlation analysis was performed between the final week measurements of the covariates carapace length and mass of all crayfish in the treatment tanks. Pearson's correlations were also performed between the final week measurements of carapace length and each water parameter. We then performed Mann-Whitney U tests to compare the mean values of the water parameter data collected on week 14 between the treatment group and its respective control group. We also compared averaged parameter data with expected ranges that we identified through our literature review to determine the differences between our data and expected values. Finally, parameter data for all weeks were graphed to examine trends over time. All graphs and tables were created in Microsoft Excel. Assessment of statistical significance was set with alpha equal to 0.05 for exploratory analysis but, for comparison of parameter values between treatment and control groups, we used the Bonferroni correction for multiple comparisons to reduce the chance of Type 1 errors. For these comparisons, the final alpha equals 0.007.



### 3. RESULTS

Females and males were distributed randomly among the 88 tanks. Of the 88 tanks we began with, 7 tanks were eliminated due to crayfish molting, death, or disappearance. Of the 24 treatment tanks without macrophyte, 1 crayfish died, 2 molted, and 2 pregnancies occurred, which resulted in 19 recorded tanks. Of the 24 treatment tanks with macrophyte, 1 crayfish disappeared and 1 molted, which resulted in 22 recorded tanks. After elimination, 12 males and 7 females were in tanks with crayfish and 14 males and 8 females were in treatment tanks with macrophyte. Table 1 displays the initial and final mean crayfish length (CL) and mass for each treatment with crayfish (1 and 3) and each control without crayfish (2 and 4). The percentage of tanks that contained macrophyte was calculated after the 14 weeks, also shown in Table 1. All tanks without crayfish had macrophyte while all tanks with crayfish did not have macrophyte after the 14 weeks.

*Table 1: Summary data for treatment groups including initial and final sample size, mean mass, and mean length of crayfish and percentage of tanks that contained macrophyte on day 103.*

| Treatment | Condition                  | N Day 0 | N Day 103 <sup>1</sup> | Mean CL (SD) Day 0 | Mean mass (SD) Day 0 | Mean CL (SD) Day 103 | Mean mass (SD) Day 103 | % tanks with macrophyte Day 103 <sup>2</sup> |
|-----------|----------------------------|---------|------------------------|--------------------|----------------------|----------------------|------------------------|--|
| 1         | + Crayfish<br>- Macrophyte | 24      | 19                     | 34.421<br>(3.709)  | 13.993<br>(4.411)    | 34.103<br>(4.497)    | 14.271<br>(4.344)      | 0  |
| 2         | - Crayfish<br>- Macrophyte | 21      | 21                     | N/A                | N/A                  | N/A                  | N/A                    | 85.7   |
| 3         | + Crayfish<br>+ Macrophyte | 24      | 22                     | 37.091<br>(5.879)  | 17.395<br>(8.735)    | 37.409<br>(5.622)    | 18.265<br>(8.376)      | 0  |
| 4         | - Crayfish<br>+ Macrophyte | 19      | 19                     | N/A                | N/A                  | N/A                  | N/A                    | 100  |

<sup>1</sup>See text for explanation of tanks excluded from final dataset

<sup>2</sup>See text for explanation of how presence/absence of macrophyte was assessed

In order to ensure the results were not influenced by differences in variables for which we did not control, we performed a series of analyses to determine if any of these covariates had significant impacts on the experiment. To start, we calculated the percent change in carapace length and mass from the initial week to the final week using the standard error equation in order to eliminate the crayfish that varied in length or mass by 2% over the 14 weeks. There were too many crayfish whose mass or length had changed by more than 2% so we are considering size as a covariate. We then tested the correlation of crayfish mass and length to determine if they were different. The test returned a Pearson's  $r$  of 0.930 and 0.890 for initial and final mass and length values with a statistical significance of  $<0.001$  showing a strong correlation between these two variables (Figure 8H). Hence, we decide to only use length for other tests, as this variable is likely to take into account any influence mass could have.

We then considered whether there were any length differences between males and females by performing a Mann-Whitney U test. We found that female crayfish (N=15, SD=4.021) are statistically larger than males (Mann-Whitney U test, N=26, SD=5.903, U=121.00,  $p=0.450$ ). We had approximately equal sex ratios in treatments with crayfish present, and

therefore the difference in size between males and females is unlikely to result in different responses from treatment to treatment.

Finally, we performed a correlation test between length and the endpoint of every parameter tested for each tank. The results of the correlation tests were not significant (Table 2). Scatterplots of all parameter final measurement data and carapace length were also created, shown in Figure 8A-G. From these data, we assumed that the covariates length, mass, and sex had a negligible influence on the final results.

Table 2: Pearson's correlation between carapace length and week 14 average parameter data for crayfish treatments 1 (N=19) and 3 (N=22)

| Parameter  | Treatment 1 |         | Treatment 3 |         |
|------------|-------------|---------|-------------|---------|
|            | Pearson's r | P value | Pearson's r | P value |
| Nitrate    | -0.086      | 0.725   | 0.220       | 0.325   |
| Nitrite    | -0.086      | 0.725   | -0.055      | 0.807   |
| Ammonia    | 0.249       | 0.304   | -0.013      | 0.953   |
| Phosphorus | -0.272      | 0.261   | -0.175      | 0.436   |
| DO         | -0.080      | 0.743   | -0.188      | 0.401   |
| pH         | -0.094      | 0.703   | 0.011       | 0.962   |
| Absorbance | 0.264       | 0.275   | -0.443      | 0.039   |

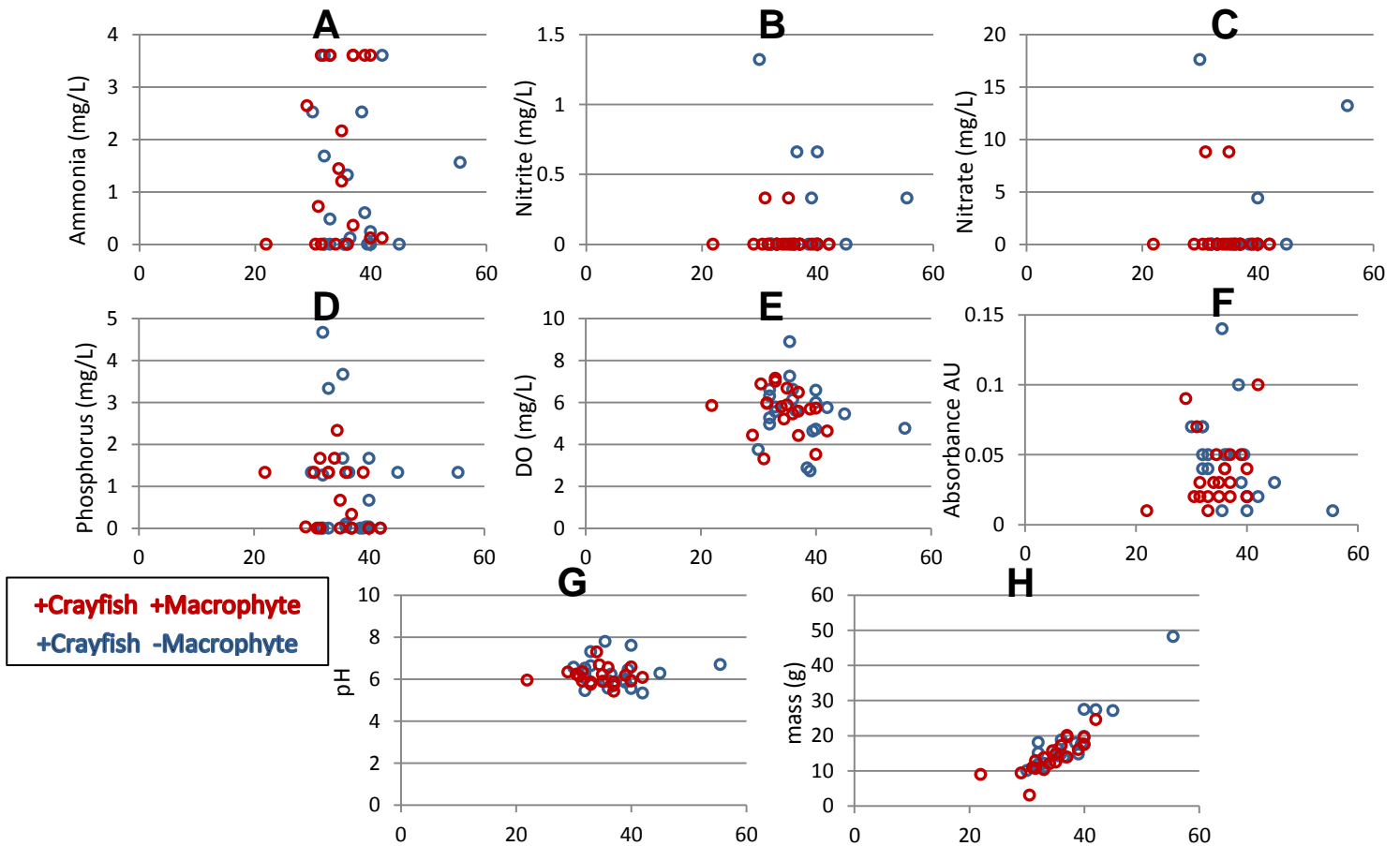


Figure 8: Scatter plots of parameters (A) ammonia, (B) nitrite, (C) nitrate, (D) phosphorus, (E) dissolved oxygen, (F) absorbance, (G) pH, and (H) mass correlated with length (x axis) for treatment groups 1 (N=19) and 3 (N=22).

Figure 9 shows the means and standard errors for each parameter as measured during week 14, the final testing data. Treatment groups 1 and 3 displayed significantly higher levels of ammonia, nitrite, nitrate, and phosphorus (Figure 9A-D). Additionally, groups 3 and 4 (with macrophyte) showed higher levels of nitrate and phosphorus (Figure 9C, 9D). Treatment groups 1 and 2 (without macrophyte) displayed higher levels of nitrate and phosphorus as well, but not as variable as nitrite and ammonia (Figure 9B, 9C). Absorbance was also higher for crayfish tanks with and without macrophytes, but the difference between crayfish and non-crayfish tanks was not prominent (Figure 9E). Dissolved oxygen and pH were slightly higher in control groups 2 and 4 (Figure 9F, 9G).

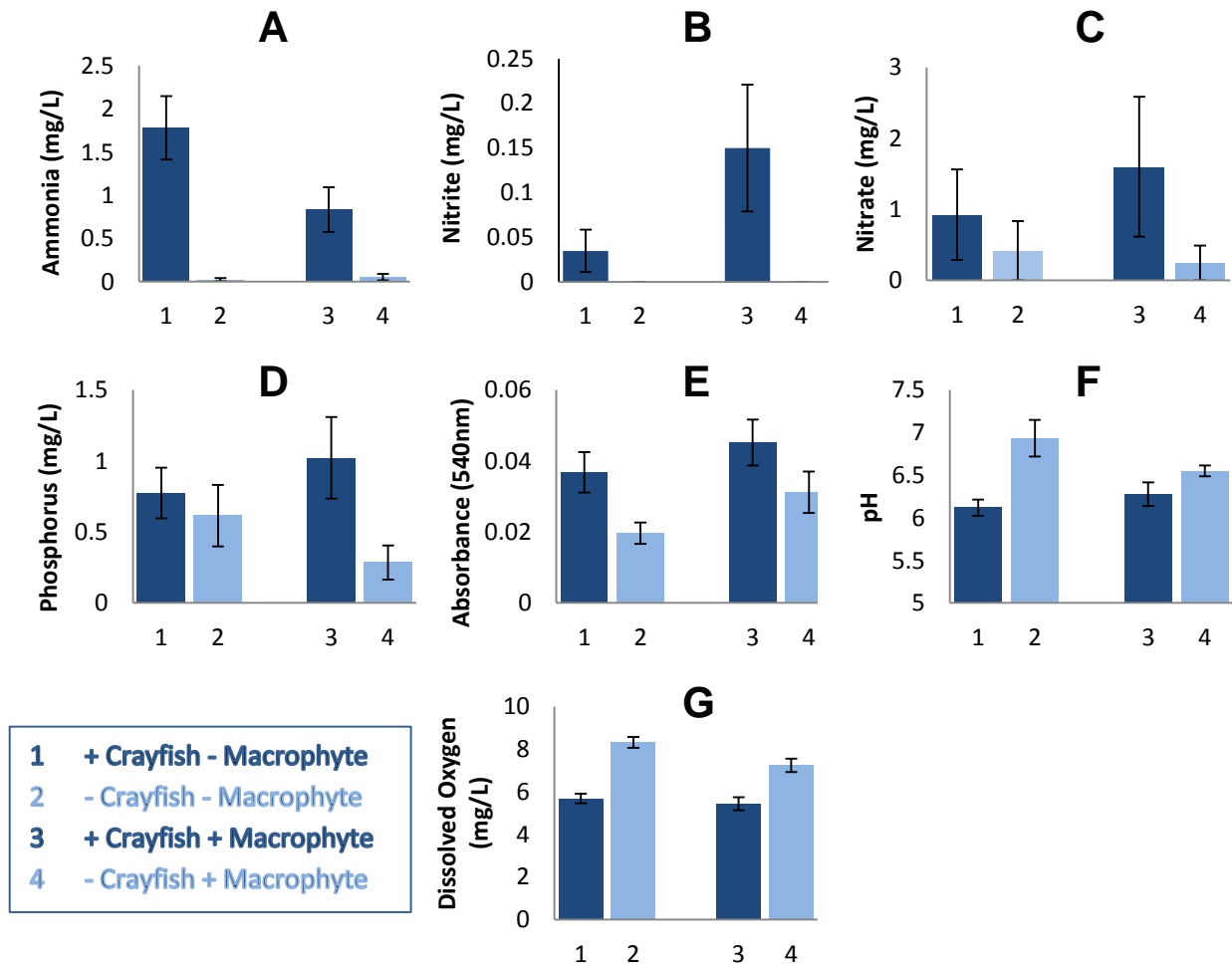


Figure 9: Mean values at end of experiment for each treatment group for all parameters. (A) ammonia, (B) nitrite, (C) nitrate, (D) phosphorus, (E) absorbance, (F) pH, and (G) dissolved oxygen over time. Numbers over bars indicate sample size and error bars represent standard error. Refer to Table 1 for sample size.

In order to determine if treatment groups differed significantly in their mean values for each water parameter, we performed Mann-Whitney U tests between the treatment and control groups for all parameters tested. We found that differences in ammonia and dissolved oxygen were significant in treatments 3 and 4 (with plants) and differences in ammonia, dissolved oxygen, pH, and absorbance are significant in treatments 1 and 2 (without plants) (Table 3).

Table 3: Mann-Whitney U test for all parameters for treatment groups 1 and 2 (without macrophyte) and 3 and 4 (with macrophyte). Degrees of freedom = 38 for all parameters.

| Parameter          | Treatments 1 and 2 |                  | Treatments 3 and 4 |                  |
|--------------------|--------------------|------------------|--------------------|------------------|
|                    | Mann-Whitney U     | P value          | Mann-Whitney U     | P value          |
| Nitrate            | 181.0              | 0.375            | 188.0              | 0.495            |
| Nitrite            | 153.0              | 0.033            | 178.5              | 0.132            |
| <b>Ammonia*</b>    | <b>107.0</b>       | <b>0.004</b>     | <b>59.5</b>        | <b>&lt;0.001</b> |
| Phosphorus         | 141.5              | 0.113            | 165.0              | 0.327            |
| <b>DO*</b>         | <b>67.5</b>        | <b>&lt;0.001</b> | <b>9.0</b>         | <b>&lt;0.001</b> |
| <b>pH*</b>         | 110.5              | 0.017            | <b>68.0</b>        | <b>&lt;0.001</b> |
| <b>Absorbance*</b> | 126.5              | 0.052            | <b>90.0</b>        | <b>0.003</b>     |

\* Significant parameters

Next we compared average parameter values over time per treatment with the suggested expected range compiled from literature. The significant parameters nitrite and ammonia were all within the expected range while dissolved oxygen and pH values for tanks with crayfish were lower than the expected range (Table 4). There was no comparable range for absorbance.

Table 4: Expected parameter values and observed means and standard deviations for treatment groups and their respective controls

|                 | Expected Range           | Crayfish + Macrophyte - | Crayfish + Macrophyte + | Crayfish - Macrophyte - | Crayfish - Macrophyte + |
|-----------------|--------------------------|-------------------------|-------------------------|-------------------------|-------------------------|
| Nitrite mg/L    | 0.001-1 <sup>1</sup>     | 0.117 (0.036)           | 0.227 (0.050)           | 0.274 (0.099)           | 0.199 (0.069)           |
| Nitrate mg/L    | 0-5 <sup>1</sup>         | 2.182 (0.825)           | 3.172 (0.937)           | 3.096 (0.880)           | 3.219 (1.062)           |
| Ammonia mg/L    | 0.2-3 <sup>1</sup>       | 2.601 (0.249)           | 2.049 (0.213)           | 0.560 (0.106)           | 0.819 (0.195)           |
| Phosphorus mg/L | 0.006-0.048 <sup>2</sup> | <b>0.550 (0.211)*</b>   | <b>0.489 (0.142)*</b>   | <b>0.527 (0.184)*</b>   | <b>0.571 (0.231)*</b>   |
| pH              | 6.5-9 <sup>3</sup>       | <b>6.310 (0.055)*</b>   | <b>6.412 (0.067)*</b>   | 6.779 (0.092)           | 6.724 (0.076)           |
| DO mg/L         | 5-8 <sup>1</sup>         | <b>4.425 (0.252)*</b>   | <b>3.639 (0.273)*</b>   | 6.766 (0.240)           | 6.122 (0.344)           |
| Absorbance AU   | n/a                      | 0.045 (0.007)           | 0.045 (0.006)           | 0.022 (0.003)           | 0.022 (0.004)           |

\*Out of range

<sup>1</sup> Chapman & Kimstach, 1996; <sup>2</sup>U.S. EPA, 2001; <sup>3</sup>Akefors, 2000

In order to analyze the changes in parameter concentration during the experimental period, we plotted the average recording for each parameter per week for all groups and compared data between treatments 1 and 2 (without macrophyte) and between treatments 3 and 4 (with macrophyte). Parameter data for all tanks in treatments 1 and 2 (without macrophyte) is shown in Figure 10. Ammonia concentrations during the initial week were relatively similar but over time, ammonia in tanks without crayfish, treatment 2, decreased to almost 0 mg/L (Figure 10A). In tanks with crayfish, treatment 1, ammonia increased and peaked at week 3 and then decreased steadily. Except for weeks 3 and 4, ammonia concentration in the treatment tanks were within normal range (black heavy bars) while after week 3, control tanks had less ammonia than the optimal range. Nitrite concentrations showed a dramatic increase during the first 2 weeks and then a similar dramatic decline in both treatment and control tanks (Figure 10B). Overall, control tanks had the greatest variability in concentrations among weeks but all tanks without macrophyte were within the normal range. Nitrate levels were extremely variable between treatments and controls and from week to week (Figure 10C). Between weeks 0 to 2 tanks without crayfish had higher concentrations but from weeks 3 to 14, tanks with crayfish had higher concentrations. Overall, nitrate decreased over time and most weeks were within normal range. Phosphorus levels in tanks without macrophyte remained above the expected ranges, with a clear peak in concentration during week 6 (Figure 10D). Dissolved oxygen remained consistent and generally increased slightly over time (Figure 10E). Control tanks had significantly higher levels of DO and remained above the normal range for the duration of the experiment. Treatment tanks for most weeks had concentrations of DO within normal range. A similar pattern occurred for pH over time, except control tanks were within range while treatment tanks had lower levels than expected (Figure 10G). Finally, tanks with crayfish were more turbid than tanks without crayfish (Figure 10F). Absorbance levels generally remained constant over time.

Parameter data for treatments 3 and 4 (with macrophyte) is shown in Figure 11. Ammonia concentrations on the initial week were similar but over time, ammonia in tanks without crayfish, treatment 4, decreased significantly to <0.5 mg/L (Figure 11A). In tanks with crayfish, treatment 3, ammonia peaked at week 3 and then decreased slowly. Except for weeks 3, treatment tank ammonia concentrations were within normal range (black heavy bars). After week 5, control tanks changed concentrations between expected range and below range. Nitrite concentrations increased during the first 2 to 3 weeks, with control tanks having more nitrite. At week 3, treatment tanks had a very large concentration of nitrite while control tank concentrations decreased significantly (Figure 11B). Over the next 10 weeks, all tanks had a decline in nitrite concentrations. All tanks were within the normal range. Between treatments and controls over time, nitrate levels were variable (Figure 11C). Between weeks 0 to 4 tanks without crayfish had slightly higher concentrations; at week 2, nitrate peaked at nearly 12 mg/L, a large increase from previous concentrations. Tanks with crayfish had higher concentrations after week 4 and all tanks steadily declined until week 14. Except for weeks 2 and 5, concentrations were within the expected range. Phosphorus levels were significantly higher than the normal range (Figure 11D). Generally, the amount of phosphorus was similar in all tanks. At week 6,

concentrations for all tanks peaked, with the highest phosphorus level in tanks without crayfish. Dissolved oxygen increased slightly over time with a clear difference between treatment and control tanks (Figure 11E). Control tanks had significantly higher DO levels and after week 2 remained above the normal range. DO in treatment tanks for weeks 1-10 were within normal range. Tanks with crayfish were more turbid than tanks without crayfish but absorbance levels remained constant over time (Figure 11F). Finally, pH was the most consistent parameter (Figure 11G). The initial pH was low at around 6 but both treatment and control pH increased to around 7, the minimum threshold pH.

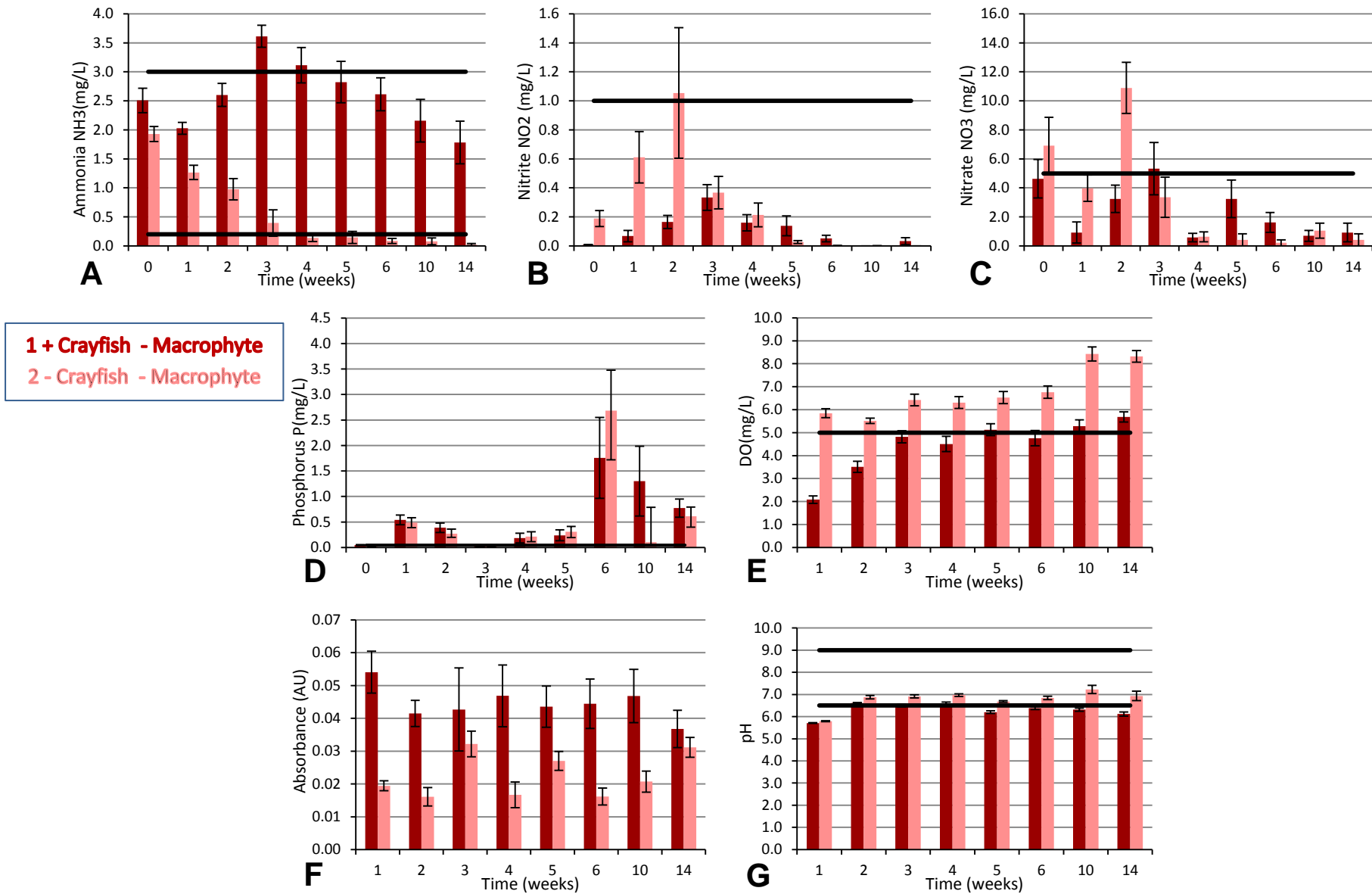


Figure 10: Mean values over time for all measured parameters for treatment groups 1 ( N=19) and 2 ( N=21) (without macrophyte). (A) ammonia, (B) nitrite, (C) nitrate, (D) phosphorus, (E) dissolved oxygen, (F) absorbance, and (G) pH. Error bars indicate standard error. Horizontal black bars represent maximum and/or minimum concentration within normal range.

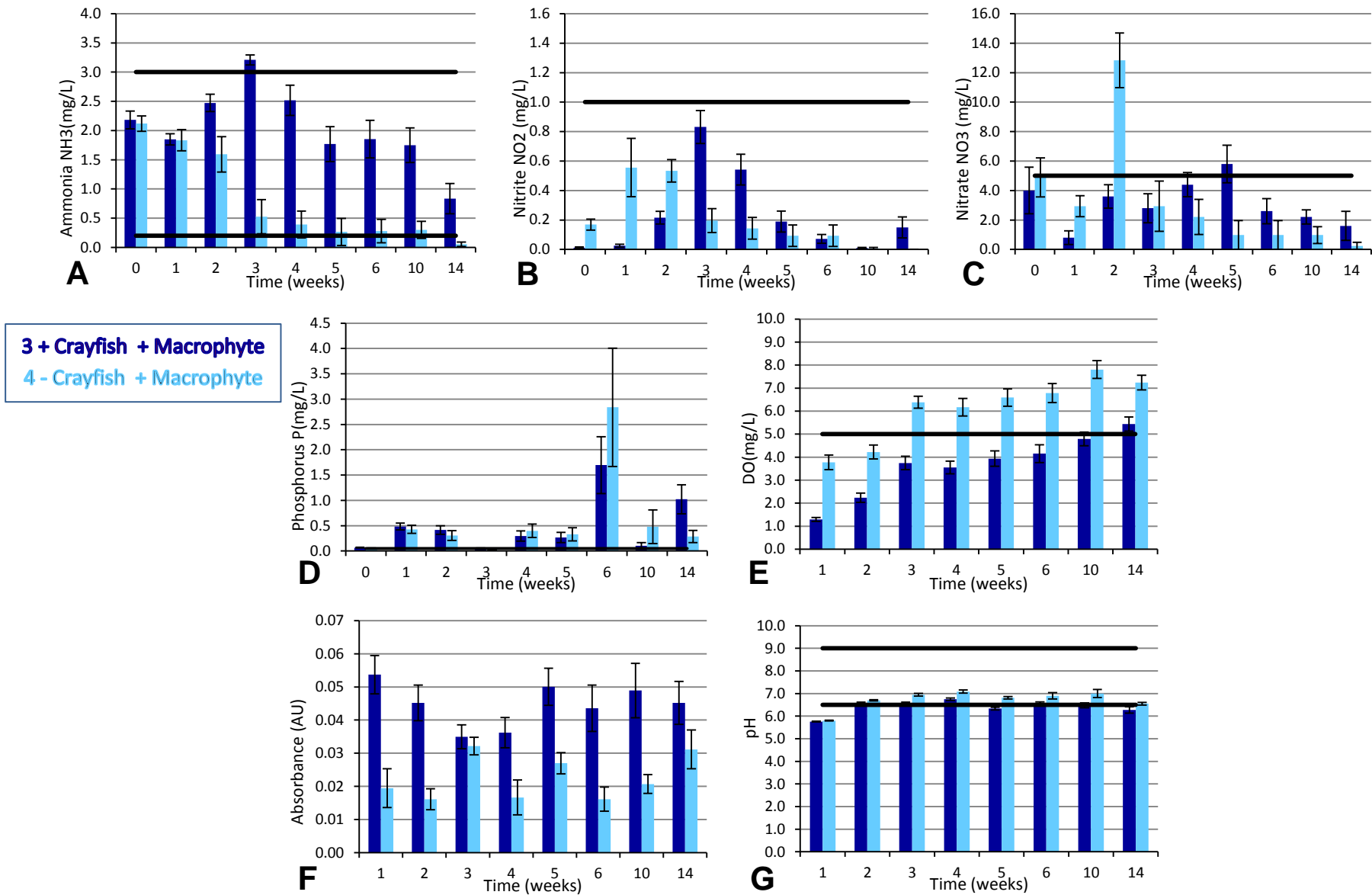


Figure 11: Mean values over time for all measured parameters for treatment groups 3 (N=22) and 4 (N=19) (with macrophyte). (A) ammonia, (B) nitrite, (C) nitrate, (D) phosphorus, (E) dissolved oxygen, (F) absorbance, and (G) pH. Error bars indicate standard error. Horizontal black bars represent maximum and/or minimum concentration within normal range.



#### 4. DISCUSSION

This study aimed to understand the impact invasive red swamp crayfish have in lentic freshwater environments by analyzing the changes associated with the presence of these crayfish in a Massachusetts man-made lake. To analyze these changes we quantified the levels of seven different parameters of water condition: nitrite, nitrate, ammonia, dissolved oxygen, phosphorus, pH, and turbidity. Using information from literature regarding lentic freshwater environments such as Salisbury Pond, we identified the normal parameter ranges for these environments and explored how the presence of crayfish would affect them. In total we had 4 different tank groups being tested: controls and treatments with and without macrophyte. During the experimentation period, readings for each parameter were taken and then compared between groups and observed across time. For all water parameter analyses, we used a Bonferroni corrected alpha value of 0.007 to assess statistical significance. The Bonferroni correction accounts for the multiple comparisons we performed on our data set. While using a lower alpha to minimize the number of Type I errors (false positives), the Bonferroni correction is thought by some to be conservative (Ryman & Jorde, 2001; Garcia, 2004; Narum, 2006). The correction can diminish the ability to differentiate among treatment groups and can increase the risk of committing Type II errors (Garamszegi, 2006; Narum, 2006). In this case, we used a Bonferroni adjusted alpha because we had 7 tested parameters that were analyzed repeatedly (Cabin & Mitchell, 2000).

Our results showed that by week 14, crayfish tanks had significantly higher levels of nitrite, nitrate, ammonia and phosphorus than control tanks, suggesting that crayfish have an influence on water and sediment nutrient composition. These differences can be explained by analyzing the role of benthic macroinvertebrates on nutrient recycling and turnover in lentic environments. There are two possible causes for crayfish-driven nutrient recycling: detritivory and bioturbation.

Benthic macroinvertebrates, such as crayfish, have an impact on size distribution, standing stocks, deposition rates, and transport rates of sediment particles via consumption and egestion (Vanni, 2002). Consequently, crayfish have an impact on the movement of nutrients attached to these sediment particles. As detritivores, crayfish consume organic matter by ingesting pieces of sediment. The feeding process is not always complete, however. “Sloppy feeding,” whereby particles are broken up into smaller pieces but not ingested, allows crayfish to convert large particles to smaller particles that can be transported both passively (i.e. water flow) or actively (i.e. by lake in fauna) much more easily (Vanni, 2002). Additionally, detritivorous partial consumption of large particulate matter by crayfish plays a role in decay rates of organic material.

In order to investigate the possibility of “sloppy feeding” being an explanation for the observed results, we looked into previous studies on freshwater macrobenthos and the effects of detritivory on the biogeochemical and physical characteristics of their environment. We found that in Puerto Rico, an experimental exclusion of freshwater shrimp resulted in decreased leaf decay rates and an increased accumulation of organic matter, particulate carbon and particulate nitrogen (Pringle et al., 1999; March et al., 2001). In another study, shrimp of the genus *Xiphocaris* caused increases in leaf decay rates, downstream transport of suspended particulate organic matter, and concentrations of dissolved organic carbon and nitrogen (Crowl et al., 2001). A New Zealand mesocosm study on the New Zealand freshwater crayfish (*Paranephrops planifrons*) found that crayfish processing of leaf litter results in the production of fine

particulate organic matter (FPOM) (Parkyn et al., 1997). Parkyn et al. (1997) argued that without crayfish production of FPOM, much of the unprocessed sediment may have been lost from the stream during autumn rainstorms. These studies provided strong evidence for us to consider and look further into the partial consumption of sediment particles by benthic macroinvertebrates and its implications in the environment, this time in the context of the red swamp crayfish in our study.

It has been shown that crayfish ingest higher amounts of detritus than other invertebrates. A study in 2 Missouri streams showed that 56 % of the detritus consumed by the invertebrate community was ingested by 2 species of crayfish (*Orconectes sp.*) (Raben et al., 1995). Hence, it seems reasonable to infer that the considerable difference in nitrite, nitrate, ammonia, and phosphorus concentration between our crayfish and control tanks is due to the breakdown of detritus and decaying macrophyte into smaller nutrient-carrying particles that then accumulate or are released into the water column. As expected, crayfish tanks with macrophyte displayed higher concentrations of nitrite, nitrate, and phosphorus than crayfish tanks without macrophyte, since the former have a higher amount of organic matter. However, because we did not observe feeding behavior of the crayfish in our tanks throughout our experiment, we are not certain that the crayfish in our tanks consumed organic matter. We suggest that crayfish gut contents and organic matter content of sediment should be analyzed in further studies to investigate further the link between our observed results and crayfish detritivory.

Bioturbation by macroinvertebrates also has the potential to change the nutrient composition of lentic environments. The importance of bioturbation by marine organisms has long been recognized (Fager 1964; Heinzemann & Wallisch 1991), but not until recently has the effect of bioturbation in freshwater systems been explored. The physical changes caused to the environment by bioturbation behaviors (e.g., foraging behavior, construction of burrows and water pumping) have a considerable impact on nutrient composition by changing the circulation rate and distribution of nutrients across the sediment-water interface and regulating the supply of resources to other species, primarily bacteria (Matisoff et al., 1985; Parkyn et al., 1997; Svensson et al., 1999).

Bioturbation activities result in the displacement of sediment particles. Sediments have a key role in the biogeochemical cycling of materials in these environments, as they act as both a source and a sink for biologically important materials such as phosphorus, carbon, nitrogen, sulfur and silicon (Matisoff et al., 1985). The movement of sediment also plays a role in the quality of the aquatic light field, as increased amounts of suspended solids decreases the ability of light to pass through the water column, increasing turbidity. Benthic macroinvertebrates displace these particles and effect sediment and interstitial water through burrowing, feeding, locomotive, respiratory and excremental activities, promoting material exchange. Throughout the experimental period, we observed our crayfish engage in bioturbation activities such as burrowing, covering their bodies with sediment, flapping intensively and walking. In addition to higher levels of nitrite, nitrate, ammonia and phosphorus, we found that both treatment tank groups (with and without macrophyte) displayed higher levels of turbidity than their respective control groups. Consistent with our findings, a study on bivalves showed that bivalve bioturbation affected flux rates of solutes across the sediment-water interface, with unionids enhancing the release of nitrate and chloride, and inhibiting the release of calcium carbonate from the sediments (Matisoff et al., 1985). Furthermore, New Zealand freshwater crayfish (*P. planifrons*), which were observed to excavate under rocks for cover and to lift small stones with their walking legs when foraging for food, significantly decreased amounts of both surficial silt

cover and interstitial fine sediment accumulation compared to control channels (Parkyn et al., 1997). It has been suggested that particle transport per se is not what controls the diffusion of solutes, but the movement of materials from depth to the sediment-water interface (Aller, 1978). This in turn enhances the reactivity of the sediment surface. Due to their relatively large size and high mobility, we assumed that the relocation and release of materials is a plausible explanation for increased levels of nitrite, nitrate, ammonia, phosphorus and turbidity.

Macroinvertebrate burrowing results in a deeper oxygenation of sediments, thereby extending the aerobic habitat of the sediment. The result is higher bacterial activity and a more complete remineralization of organic matter and flux of nutrients to the overlying water (Goedkoop et al. 1997). For instance, bioturbation activity of macroinvertebrates has been shown to increase the rate of bacterial nitrogen transformation processes of nitrification and denitrification in aquatic sediments. In Svensson's (1997) laboratory studies, 2000 individuals of *Chironomus plumosus* enhanced denitrification by 2.5–5.6 times compared to sediment without chironomids. An indication of possibly increased denitrification in Lake Ringsjön is the finding that annual nitrogen retention was significantly greater after the chironomid number increase. These studies were carried out in a similar lentic environment than that of Salisbury Pond and lead us to believe that these results might be translatable to our findings.

These effects may be particularly important in the context of the regeneration of specific nutrients which limit pelagic primary production. Increased bacterial processes might not only be an explanation for higher concentrations of nitrite, nitrate, ammonia and phosphate in crayfish tanks, but also for the observed decline of dissolved oxygen levels in these tanks. Our results suggest that higher demand for oxygen by respiring bacteria and other ecological processes, coupled with a considerable reduction or absence (in tanks without macrophyte) of macrophyte, and thereby in photosynthetic production of O<sub>2</sub>, are the causes for lower dissolved oxygen levels. Consistent with our findings, Dorn and Wodjak (2004) observed that crayfish ponds had lower peak dissolved oxygen levels and argued that this could have been caused by decreased light, consumption of primary producers, and/or increased respiration of decomposers. To explore these interacting effects more thoroughly, we suggest that further experimentation of bacterial activity in these tanks must be performed.

Finally, there was a sharp difference in final macrophyte mass between the treatment and control tanks. Both treatment tank groups showed a complete absence of macrophyte. However, both control group either maintained or increased their macrophyte mass. Even tanks in the no-macrophyte control group displayed macrophyte growth. According to Parkyn et al. (1997), the significantly increased breakdown of wineberry leaves in the presence of crayfish may have resulted directly from consumption of leaf material and/or indirectly from an improvement in microbial processing conditions. Since we did not observe any grazing activity from the crayfish in our experiment and did not perform any kind of gut content analysis, we believe macrophyte decrease in crayfish tanks is due to a change in nutrient levels of limiting materials.

The results of this study suggest that the invasive red swamp crayfish has an impact on the biogeochemistry of its surroundings and its macrophyte populations. We observed an increase in levels of nitrite, nitrate, ammonia, phosphorus, and turbidity in the environment and a decrease in levels of dissolved oxygen. We inferred that these changes in the nutrient composition of the water column were a result of partial consumption of sediment and bioturbation activities. Partial consumption of sediment results in the breakdown of large particles of sediment, allowing an easier active and passive displacement of particles. In addition, bioturbation activities (e.g., burrowing, flapping, walking) lead to the movement of sediment

across the sediment-water interface and into the water column, thereby changing their nutrient composition, and increase the aerobic environment of the sediment. These changes are potentially detrimental for other organisms in the ecosystem. For instance, we observed an absence of macrophyte growth in crayfish tanks that originally contained macrophyte, suggesting crayfish might inhibit macrophyte growth by changing the levels of limiting nutrients. We suggest analyses of gut content, organic matter composition of sediment, and bacterial activity in order to further investigate the relationship between crayfish activity and water composition.

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