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Too Little pH: How Freshwater Acidification Impacts the Abundance of Macrophytes Consumed by Rusty Crayfish

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Too Little pH: How Freshwater Acidification Impacts the Abundance of Macrophytes Consumed by Rusty Crayfish

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Abstract

Anthropogenic activities such as the burning of fossil fuels result in increased atmospheric carbon dioxide (CO_2) concentration. High levels of atmospheric CO_2 cause chemical shifts in the carbon cycle. Changes in the carbon cycle due to increased CO_2 levels lead to ocean and freshwater acidification. Freshwater acidification is problematic for species that synthesize their own shells as well as species that use olfaction for decision-making. Rusty crayfish (*Faxonius rusticus*) were subject to simulated freshwater acidification and fed two types of macrophyte, Chara (*Chara braunii*) and Myriopyllum (*Myriophyllum sibiricum*). A series of programming language of R (R Core Team, 2019) indicated that simulated freshwater acidification alters the abundance of macrophytes consumed by rusty crayfish. This study demonstrates that freshwater acidification due to atmospheric levels of CO_2 leads to foraging changes in crayfish.

Introduction

The atmospheric levels of CO_2 have been steadily increasing as the result of human fossil fuel usage coupled with deforestation. As of 2018, the global average amount of CO_2 was at a record high of 407.4 parts per million. Today, CO_2 levels are higher than any point in the past 800,000 years (IGCCP). Levels of CO_2 have been increasing steadily since the Industrial Revolution due to the increase in prevalence of human activities such as the burning of fossil fuels and deforestation (Bogan, 2009). Records show concentrations of atmospheric CO_2 have increased rapidly since the Industrial Revolution; scientific consensus today says CO_2 emissions are anthropogenic (Cerling, 2005). With the increase of CO_2 levels being continual, there are many extenuating impacts that need to be considered. Excess CO_2 in the atmosphere moves beyond the atmosphere and impacts both the biosphere and hydrosphere (Menon, 2007).

When fossil fuels are combusted, CO_2 is released into the atmosphere, which in turn causes the oceans, and other bodies of water, to uptake CO_2. This process can cause a phenomenon known as ocean acidification (Gattuso, 2011). The ocean plays a fundamental role in the carbon-cycle (Raven, 2005). Carbon is important, as carbon is a primary component of all forms of life on Earth. Carbon atoms are continually exchanged and recycled as CO_2 between the atmosphere and the Earth, forming the carbon-cycle (Menon, 2007). Regarding the ocean or bodies of freshwater, the exchange of carbon as CO_2 occurs between the Earth’s atmosphere and the surface of the body of water (Bolin, 1970). When the carbon molecules and the compounds present in the water interact with one another, chemical reactions take place.

Any CO_2 in the atmosphere is exchanged with the upper layer of any water surface through the process of adsorption (Tarbuck, 2006). As CO_2 enters water ionic bonds form with water producing HCO_3 (Stirling, 2010). Since HCO_3 is a weak acid, hydrogen associates and dissociates freely which forms the carbonate system (Zeebe, 2001). The carbonate system is a set of compounds, which include H_2CO_3, HCO_3^-, and CO_3^{2-} , produced by the carbonate equilibrium (Lower, 1999). Because the acidic components HCO_3^- are weak acids, hydrogen associates and dissociates from the compounds depending upon the pH of the environment and the concentration of both calcium (Ca) and CO_2 (Lundegard, 1989). As atmospheric concentrations of CO_2 change, the equilibrium shifts which creates the disturbances in the pH and chemistry of natural bodies of water. Once CO_2 has solvated into the ocean, carbonic acid (H_2CO_3) is formed (Adamczyk, 2009). This carbonic acid then dissociates into a hydrogen ion (H^+) and bicarbonate (HCO_3) (Likens, 1979). This results in a large concentration of H^+, which increases ocean acidity (Burger, 2020). Thus far, the ocean has experienced a 30% increase in acidity, which is equivalent to a 0.1 decrease in pH (Raven, 2005).

A topic involving alterations in CO_2 and water chemistry is coral bleaching. Within coral reefs, coralline algal communities are fundamental to the production of carbon sediments and binding fragments of carbonate to the structure of the reefs (Mcleod, 2013). As pH in these underwater communities decreases, so does the rate of reef calcification due to the lower concentration of CO_3^{2-} (Andersson, 2009). While coral bleaching is driven primarily by ocean warming as opposed to ocean acidification, both are caused by anthropogenic CO_2 (Pörtner, 2008). In the case of ocean warming, higher concentrations of atmospheric CO_2 result in periods, lasting between weeks and months, of a locational increase in water temperature (Berkelmans, 2004). The combined impacts of ocean acidification and warming can be detrimental for coral reef systems, amongst others.

Freshwater acidification is also an issue, but freshwater acidification is not subject to as many studies or discussions as ocean acidification. Acidification of inland freshwaters via anthropogenic or ecological sources, such as acid rain, has the potential to be a major stressor for these ecosystems (Hasler, 2018). In areas with a low capacity to neutralize acid such as mountainous regions with high elevation, areas with underlying bedrock, and farmland, acid rain is a common issue (Lajewski, 2003). Acid rain occurs when the burning of fossil fuels emits gaseous sulfur dioxide (SO_2) and nitrogen oxides (NO_x) (Driscoll., 2001). Clouds containing particles of SO_2 and NO_x are formed where these particles are transported to via the wind (Parungo, 1987). Acid is then deposited as rain or snow onto the Earth’s surface, completing the process that is known as acid rain (Gattuso, 2011). In the United States, New York and Pennsylvania have been subject to acid rain the most (Stoddard, 1999). Western winds carry SO_2 and NO_x from states surrounding the Ohio Valley, where the concentration of these emissions is the highest, then they are converted into sulfuric (H_2SO_4) and nitric acids (HNO_3) (Eney, 1987). These acids are deposited over New York and Pennsylvania as acid rain (Likens, 1979). Acid rain results in acidification of aquatic ecosystems and poses several problems. One of these problems is the decreasing pH associated with ocean and freshwater acidification, which can be harmful, particularly to calcifying organisms (Orr, 2005).

As the pH within aquatic environments decreases, calcifying organisms, such as crayfish, coral, mussels, and others, face difficulty synthesizing their calcium carbonate ($CaCO_3$) shells (Orr, 2005). Within ocean environments, the CO_2 concentration is increasing, while the carbonate ion (CO_3^{2-}) concentration is decreasing (Andersson, 2005). This lower concentration of CO_3^{2-} poses a threat to calcifying marine creatures, as without CO_3^{2-}, these organisms face difficulty creating biogenic $CaCO_3$ (Feeley, 2004). This means these organisms will not be able to maintain a sustainable external skeleton. $CaCO_3$ shells are an important feature of these calcifying organisms, as they are a means of protection against predators (Hofmann, 2010). CO_2 emissions can result in limited nutrients within freshwater ecosystems, changes in temperature and water connectivity, and desiccation (Beaune, 2018). Changes in temperature are suspected to cause evapotranspiration, which is the transfer of water from the Earth's surface and the atmosphere (Duan, 2019). Evapotranspiration can cause issues with water connectivity as this process results in the excessive drying, or desiccation, and thus a reduction of water present in aquatic ecosystems (McKenney, 1993). Without enough water present, dry areas within streams and other bodies or freshwater can cause the ecosystem to have a disconnect (Sánchez-Carrillo, 2004). Disconnects within the ecosystem could also result in a limitation of nutrients (Sánchez-Carrillo, 2001). These changes within freshwater ecosystems affect the phenological and physiological of organisms living there, as well as migrations and extinctions (Weatherley, 1988). One example of a physiological change that is experienced in organisms subject to freshwater acidification is the loss of olfaction (Munday, 2009).

Given the lack of light in many aquatic environments, organisms tend to rely more heavily on the chemical senses to make ecological decisions (Dodson, 1994). Chemical signals guide aquatic organism's choices on foraging, mating, avoiding predation, and finding shelter (Hay, 2009). Yet, these behavioral actions are threatened by changes in the environment such as lowered pH. As previously stated, when the pH of freshwater ecosystems is lowered, the ecosystem's chemical composition is altered (Lundegard, 1989). An alteration of the chemicals present in an organism's ecosystem can deter their ability to respond to certain chemical signals, which can cause changes in their behavior (Leduc, 2013). Amino acids are present in neutral aqueous solutions as dipolar ions (Kirkwood, 1939). When acid is added the hydrogen ion (H^+)

is taken by the carboxyl group (Gu, 1994). The resulting undissociated carboxyl group is formed and when a base is added, a H^+ is released (Tierney, 1988). The H^+ ions released due to the lowering of pH impact the chemoreception of crayfish (Hay, 2010). Low pH levels cause complications within the binding of amino acids to the crayfishes' receptor sites, resulting in crayfish misreading chemical signals and experiencing behavioral changes, such as differences in preference to food stimuli (Allison, 1992).

Crayfish use olfaction to locate and identify environmental food resources, as well as for movement and homing (Kubec, 2019). Crayfish have a chemosensory organ, called a sensilla, located on their antennules (Horner, 2008). Within the olfactory organ, there are olfactory receptor neurons that send signals to the olfactory lobe located in the crayfish's central nervous system (CNS) when the crayfish senses a smell (Blaustein, 1988). Crayfish rely on their olfactory systems throughout their lifespan to find food (Willman, 1994). However, external stimuli can cause the olfactory systems of crayfish to be modified, which can have a negative impact on the ability to locate and identify their food sources (Hay, 2010). Crustaceans such as crayfish are stimulated to perform particular behaviors by sequences of chemical signals (Hay, 2009). When these sequences of chemical signals are disrupted due to an influx of compounds such as CO_2, crayfish are unable to perform the same behaviors they would otherwise be able to complete (Tierney, 1986). A decrease in pH because of the increase of CO_2 alters the ability of crayfish to olfaction, and thus has the potential to alter their food preferences (Allison, 1992). When considering these impacts, visible differences in food preference of other aquatic creatures is not unforeseeable.

This experiment focuses on how increasingly lower pH levels in freshwater will impact the abundance of macrophytes eaten by rusty crayfish. Aquatic environments where crayfish are present rely on them for biomass turnover, as they consume the ecosystem's vegetation, animal waste, and corpses (Momot, 1978). As noted, the lowering of pH as a result of CO_2 can impact crayfish beyond shell formation. The goal of this experiment is to determine whether lowering the pH of a crayfish's ecosystem will cause the crayfish to hold a preference toward one macrophyte over another. Two species of macrophyte, Chara (*Chara braunii*) and Myriophyllum (*Myriophyllum sibiricum*), were used in this experiment. Chara is a species of aquatic plant known for their ability to control the clarity and quality of water (Amirnia, 2019). Myriophyllum is a species of aquatic plant known for its roles as a stabilizer of sediment and a potential detoxifier (Hanson, 2001). If crayfish favor one macrophyte, the other species of macrophytes could overgrow and cause a chain reaction within the ecosystem. The results of this experiment will help to determine how the abundance of different macrophytes within an environment will differ as ocean acidification occurs.

Materials and Methods

Collection and Housing of Animals and Plants

Sixteen form two (non-reproductive) rusty crayfish (*Faxonius rusticus*, *Cambaridae*) were captured from Maple Bay of Burt Lake in Cheboygan County, Michigan (45.4873ºN, 84.7065ºW) during the summer of 2019. Animals were transported to the laboratory at Bowling Green State University and housed in a flow through system where they were isolated physically and visually, but not chemically, from other animals. During the trials, crayfish were held in the experimental or control aquaria, both containing peat gravel, the macrophytes, and water. Only healthy individuals with all appendages intact were used in the experiments.

Macrophytes, Chara (*Chara braunii*) and Myriophyllum (*Myriophyllum sibiricum*), were kept in two 40-liter aquaria. Plant health was augmented by providing a source of additional CO_2 and full spectrum lights. Additional CO_2 was created by a by-product of yeast fermentation in a system with one 590-milliliter bottle and a 390-milliliter bottle. The first bottle, containing the yeast and fermentation source (pure sugar), was connected via small diameter tigon tubing to the second bottle, which served as collecting spot for yeast and liquid that came through the tubing. The second bottle had an additional tube that provided CO_2 to the holding tanks for the macrophytes.

Acidification Impact on Crayfish's Food Preference

Experimental Design

To test the effects of freshwater acidification on foraging preferences and consumption of macrophytes, sixteen crayfish were placed within a testing aquarium and provided known quantities of macrophytes to consume over a 5-day period. All crayfish and macrophytes were placed in the test aquaria starting at a pH of 7. To simulate changes in acidification, the pH of the test aquarium was lower by 1 pH unit every day to a final level of pH 4 on day 4. The control aquarium remained at a pH of 7 throughout the experimental period.

To begin the feeding trials, plant samples were then selected from their respective aquaria, dried via salad spinner (Farberware Basics, Item No. 5158683), then weighed to the nearest 0.001 g. These macrophytes were then attached to glass stirring rods (255 x 6 mm: l x OD) with 26-gauge green painted floral wire. To ensure the plants would be kept in place for the entire trial, the rods were placed into three hardware cloth brackets (24 x 19 cm: l x w). The arrangement of the macrophytes on the brackets were rotated across trials, as to prevent bias based on location within the aquaria.

Protocol of Experiment

The experiment began October 2, 2020 and was concluded on December 7, 2020 after completing 8 of the 5-day trials. On day 1, the macrophytes in both experimental and control aquaria were dried, weighed and the mass of the macrophytes would be recorded. The pH in both aquaria were measured and altered until the water in the aquaria reached a unit of 7. The pH of the experimental aquarium, on day 2, would be lowered to 6 via pH down. pH down, composed of phosphoric acid, is produced by General Hydroponics as an acidic buffering solution for pH stability. Before the pH in the experimental aquarium was lowered to 6, the macrophytes in both aquaria were dried, weighed, and their masses were recorded. The macrophyte stems were dried, weighed, attached to the rods, and connected to the bracket in the same position as before, and placed into the experimental and control aquaria. The macrophytes were removed from both aquaria, dried, weighed, and had their masses recorded on day 3. The pH was lowered in the experimental aquarium to 5 after new, dried, and weighed macrophyte stems were added. On day 4, the pH was lowered to the final unit of 4 in the experimental aquarium after removing, drying, and weighing the macrophyte stems in the aquaria, and adding new stems to the glass rods and brackets. Day 5 was the last day of the trial, and after the macrophyte stems were dried and weighed, results would be collected and analyzed.

Data Analysis

All data analysis took place within the programming language of R (R Core Team, 2019). The beginning steps of the data conditioning follow those typically done in mixed model analysis (Zuur, 2009). The first step in the analysis was to produce histograms, qqplots, and normality tests of all the behavioral variables. All data included in the analysis were normally distributed.

All statistical models were performed using generalized linear mixed models (Zuur et al., 2009). All models run in R used the lmer function from the lmerTest package in R (Kuznetsova et al. 2017, R Core Team 2019). Following model construction, the outputs were extracted using the anova function from the car package in R (Fox et al., 2012). For the models, the amount of macrophyte consumed as the dependent variable and the treatment (lowered or constant pH), day (1-4), pH, and macrophyte served as categorical independent measures. Finally, the trial number was used as a random factor within each model.

Results

Macrophyte Consumption

The overall impact of differing pH on the macrophyte consumption of crayfish was found to be significant ($F_{[3,111,0.05]} = 12.220$, $p < 0.0007$). The linear mixed model for the daily macrophyte consumption of crayfish shows that the consumption of Myriophyllum is affected more by altering pH than the consumption of Chara. When comparing the biomass changes within macrophyte species across treatments, the crayfish consumed much less Myriophyllum than Chara. The amount of Myriophyllum consumed decreased from day 1 to day 3, and then increased on day 4 for both the crayfish receiving treatment and the crayfish in the control group. For the Chara, both groups experienced some variance, but not to the same extent as the Myriophyllum.

These graphs display the distribution of macrophytes consumed by crayfish throughout this experiment. The first graph shows the distribution of Chara consumed, and the second graph shows the distribution of Myriophyllum consumed. The control group experienced an increase in consumption of Chara from day 1 to day 4, with the net increase being approximately 0.02g. The group receiving treatment experienced a greater decrease in Chara consumption from day 1 to day 4, as the difference between these days is approximately 0.08g. For Myriophyllum, the control group and the group receiving treatment experienced decrease in consumption from day 1 to day 3, ending with an increase in consumption from day 3 to day 4. However, the group receiving treatment experienced a much greater increase in the consumption of Myriphyllum than the control group between days 3 and 4. The control group displayed a net decrease of approximately 0.05g in the consumption of Myriophyllum. The group receiving treatment experienced net increase of approximately 0.03g in Myriophyllum consumption. The largest difference in the distribution of macrophytes eaten by crayfish is experienced on day 4. Overall, the crayfish consumed a larger amount of Chara than Myriophyllum, regardless of being under control, or receiving treatment.

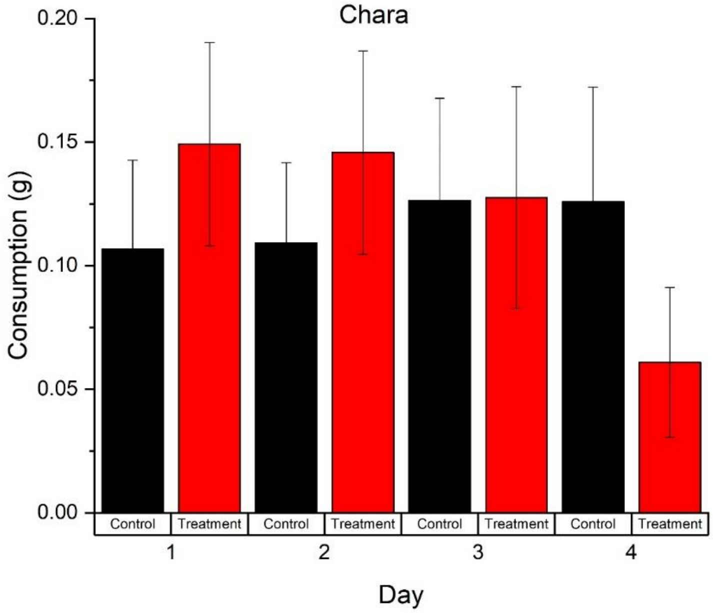

Figure 1. Distribution of Chara Biomass Consumed by Crayfish.

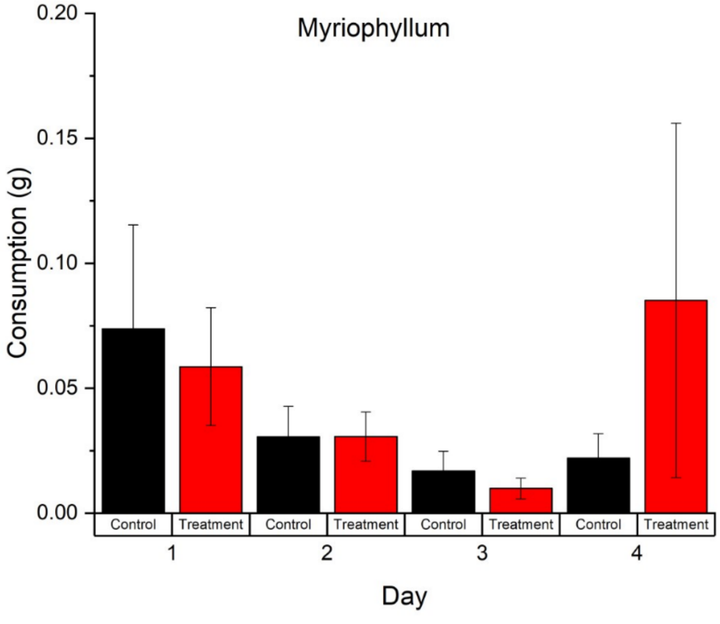

Figure 2. Distribution of Myriophyllum Biomass Consumed by Crayfish.

Discussion

The results of this experiment show that the consumption of Myriophyllum by crayfish is impacted more than the consumption of Chara. When analyzing all treatments, the crayfish in this study consumed much less Myriophyllum than Chara. The control group experienced a net increase of approximately 0.02 g in Chara consumption and a net decrease of approximately 0.05 g in Myriophyllum consumption. The group receiving treatment experienced a net decrease of approximately 0.08 g in Chara consumption and a net increase of approximately 0.03 g in Myriophyllum consumption. Regardless of the conditions, the crayfish consumed a larger amount of Chara than Myriophyllum. The changes in macrophyte consumption by crayfish are due to the simulation of freshwater acidification performed in this experiment.

Freshwater acidification results in changes within natural processes taking place in the environment. One of these disturbances is a loss of olfaction within crayfish (Munday, 2009). Freshwater acidification causes changes in the perception of chemical signals by crayfish (Hay, 2010). Crayfish use olfactory senses to differentiate between various chemical signals within their environment to make decisions on foraging, mating, and homing (Kubec, 2019). Crayfish are stimulated to perform these behaviors when they receive a sequence of chemical cues or signals (Hay, 2009). To differentiate between chemical signals, crayfish use the sensilla located on their antennules as a chemosensory organ (Horner, 2008). When crayfish sense a smell, the olfactory receptor neurons within their sensilla send a signal to the olfactory lobe in the crayfish's central nervous system (Blaustein, 1988). External stimuli such as freshwater acidification cause modifications within the crayfish's olfactory system due to changes in chemical signals, leading to alterations in the crayfishes' behavior (Leduc, 2013). Low pH levels due to an increasing concentration of CO_2 results in changes in the olfactory systems of crayfish, which can cause their food preference to change (Allison, 1992). When crayfish experience the effects of freshwater acidification their preference in macrophytes can alter biomass turnover within their habitat (Momot, 1978).

Changes in macrophyte consumption by crayfish can cause differences in biomass turnover than previously experienced in their habitat (Momot, 1978). If one macrophyte is favored over another within an ecosystem relative to their consumption by other species, the less favored macrophyte could overgrow. One macrophyte being preferred over another could cause a chain reaction within their habitat, as each macrophyte species plays a specific role (Rejmankova, 2011). Macrophytes serve many purposes within their habitats, such as influencing the movement of water and providing sustenance as well as shelter for other organisms (Dvořak, 1982). These functions of macrophytes within freshwater ecosystems have influence upon the nutrient cycling and food web interactions that occur (Ziegler, 2015). Changes in the concentration of macrophytes within a habitat can impact the availability for nutrients needed by other organisms, thus altering food web interaction (Warfe, 2006). Differences in these interactions could put stress upon the environment in the form of lower nutrient availability and lower rates of productivity (Cronin, 2003). Lower nutrient availably and productivity alter the processes that would normally occur, especially if the species has a large influence within their ecosystem (Small, 2011). Species with disproportional influences on their environment relative to abundance are known as ecosystem engineers or keystone species (Power, 1996).

Crayfish are commonly referred to as ecosystem engineers or keystone species because they have a disproportionately large effect on their environment relative to their abundance (Creed, 2004). Crayfish are considered keystone species or ecosystem engineers due to their ability to withstand a broad range in temperature, indicate water quality, and dictate foodweb interactions (Reynolds, 2013). As keystone species, changes in crayfish behavior can result in widespread consequences for other species interactions in their ecosystem (Hart, 1992). Crayfish consume a large amount of macrophytes and other ecosystem waste within their habitat (Momot, 1978). In addition to having an impact on the concentration of macrophytes within their habitat, crayfish can also influence the presence of other species due to the availability of particular macrophytes (Nyström 2002, Reynolds and Souty-Grosset 2012). The change in macrophyte consumption shown by crayfish who have experienced freshwater acidification can impose widespread consequences within their ecosystem.

While this experiment studied the impact of freshwater acidification on the macrophyte consumption of crayfish, other species interactions are altered as well. The crayfish used in this study consumed less Myriophyllum than Chara across all treatments. For Chara consumption, the control group experienced a net increase while the group receiving treatment experienced a net decrease. The opposite occurred for the consumption of Myriophyllum as the control group experienced a net decrease while the group receiving treatment experienced a net increase. These changes in macrophyte consumption can be attributed to the impact of simulated freshwater acidification on the olfactory systems of crayfish. The chemical signals received by the crayfish's olfactory systems are altered due to freshwater acidification (Tierney, 1988). Without being able to receive the proper chemical signals crayfish experience behavioral changes such as differences in their eating habits (Allison, 1992). Differences in macrophyte consumption by crayfish can cause problems within their habitat as macrophytes play roles within the environment in terms of nutrient cycling and availability, along with food web interaction (Warfe, 2006). If changes in macrophyte consumption by crayfish occur, ecosystems can experience lower rates or nutrient production and availability, putting stress upon the ecosystem (Cronin, 2003). This issue is particularly troublesome as crayfish are a keystone species, meaning crayfish are an organism which have a large impact on their environment disproportional to their abundance (Reynolds, 2013). This study found the reduction of pH as a result of simulated freshwater acidification has an impact on the abundance of macrophytes crayfish consume. Crayfish and the macrophytes they consume play a large role in their ecosystems' nutrient availability and species diversity (Reynolds, 2011). Studies on freshwater acidification are important for environmental sustainability.

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