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Article

The effect of *Anubias barteri* plant species on limiting freshwater acidification

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SUMMARY

As dependence on fossil fuel combustion solidifies, environmental impacts of carbon emissions are exasperated, including freshwater and saltwater acidification. These processes result in acidifying water ecosystems, negatively changing ecosystem conditions for organisms. Existing research identifies the potential of aquatic plants on limiting ocean acidification. However, research relating to freshwater acidification is minimal, so the impact of aquatic plants, *Anubias barteri var. congensis* and *Anubias barteri var. nana*, on minimizing changes in pH was explored in an ecosystem in Northern California. Creek water samples, with and without the aquatic plants, were exposed to dry ice to simulate carbon emissions and the pH was monitored over an eight-hour period. Water samples with aquatic plants were hypothesized to have a smaller change in pH than water samples without aquatic plants. The data was used to measure the ability of aquatic plants to serve as a buffer in limiting pH change. Statistically significant differences were identified between the water samples with and without plants using a t-test after 285 minutes of the experimental period. There was a 25% difference in the observed pH based on molar hydrogen ion concentration between the water samples with plants and those without plants, suggesting that aquatic plants have the potential to limit acidification to some extent. These findings can guide future research to explore the viable partial solution of aquatic plants in combating freshwater acidification.

INTRODUCTION

As the world becomes more reliant on fossil fuel combustion for energy and transportation, the impacts of carbon pollution on the environment will only continue to drive many environmental issues. Carbon emissions are on the rise as the Environmental Protection Agency (EPA) estimates an increase of 5.9% since 1990 (1). These emissions are trapped in the atmosphere, which allows bodies of water to absorb carbon dioxide pollution, fueling freshwater and saltwater acidification.

Acidification occurs when multiple chemical reactions take place between water and carbon dioxide, resulting in an increased concentration of hydrogen ions and a decreased pH level (2). Since the industrial revolution, the pH of the ocean has dropped from 8.2 to 8.1, representing a 25% increase in acidity based on converting these pH levels to molar hydrogen ion concentrations (3). Ocean acidification has many implications on saltwater ecosystems. Organisms require specific pH conditions to prosper, meaning they face a wide range of complications when these conditions are manipulated. These negative implications primarily affect calcifying organisms that rely on their calcium carbonate shells and skeletons for structure and protection as those carbonate molecules are used in chemical reactions with carbon dioxide instead of calcification (4). Growth in organisms is also impaired, as changes in acidity force them to use more energy for maintaining their body fluid at the expense of other bodily functions (5).

Research from 2016 suggests that specific aquatic plants like *Laminariales* and *Zostera* have the potential to effectively minimize the impacts of ocean acidification. A researcher examining the long-term impacts of ocean acidification at Cheeca Rocks in Florida, found a 0.38 difference in pH levels between inshore waters with much seagrass growth and the average Florida reefs, highlighting the potential of aquatic plants in combating ocean acidification (6).

While research exists in relation to ocean acidification, research pertaining to the effect of aquatic plants on freshwater acidification remains limited. Unlike saltwater, freshwater ecosystems are affected by many varying conditions that lead to freshwater acidification like acid rain, nutrient runoff, decomposing matter, and anthropogenic pollutants in addition to carbon dioxide emissions, meaning freshwater acidification varies from ecosystem to ecosystem (7, 8). Because of this lack of research, an investigation was prompted to see if a similar effect of aquatic plant life on acidification existed in a local freshwater ecosystem.

In the east bay of Northern California, the freshwater ecosystem is impacted mostly by the proximity to human developments like vehicles or buildings, suggesting the examined ecosystem may potentially be exposed to carbon dioxide from vehicle or building carbon emissions. Consequently, carbon dioxide emissions were mimicked using dry ice, since dry ice is composed solely of carbon dioxide. When the dry ice sublimates at room temperature, it releases gaseous carbon dioxide, similar to that of wide scale carbon dioxide emissions. *Anubias barteri var. congensis* and *Anubias barteri var. nana* plant species were selected to serve as aquatic plant life as they are locally accessible aquatic plants that are common in freshwater ecosystems (9).

Based on background information, the potential of aquatic plants

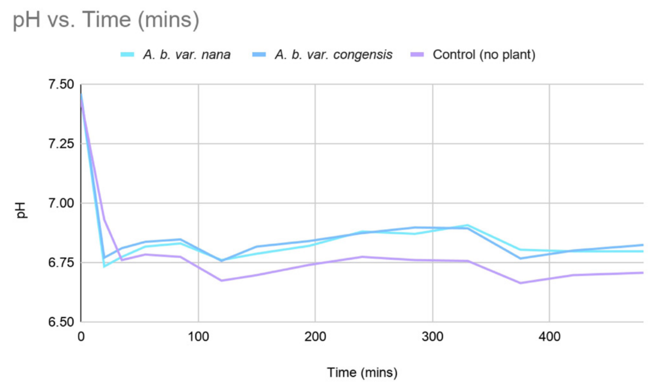

Figure 1. Mean pH of three samples per plant throughout an eight-hour period with zero minutes representing pH before the addition of dry ice. In general, the control pH was lower than the *A. b. var. nana* and *A. b. var. congensis* pH levels, which were both comparable to each other.

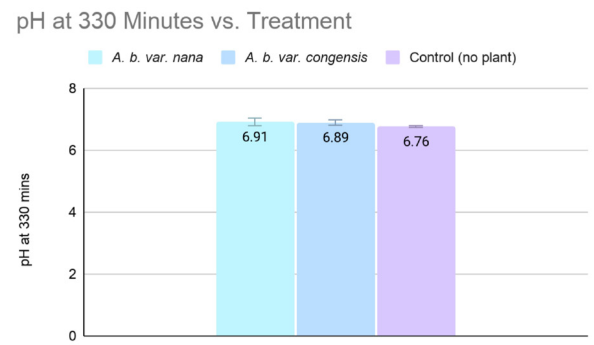

Figure 2. Comparison of mean pH of three samples per selected plants at 330 minutes based on treatment. At 330 minutes, a *p*-value of 0.022177 was obtained using a t-test of the *A. b. var. nana* and *A. b. var. congensis* vs the control.

serving as a buffer in limiting acidification of freshwater ecosystems was explored. Given that aquatic plants have the capability of limiting acidification in marine ecosystems, the pH of the water samples with aquatic plants were hypothesized to have a smaller change in pH than water samples without aquatic plants, because the plants were expected to absorb some of the carbon dioxide.

RESULTS

To examine the impact of aquatic plants on freshwater acidification, the effects of carbon emissions on a freshwater ecosystem were simulated in a controlled setting. Freshwater samples with plants of the *A. barteri* species and samples without plants were exposed to dry ice while the pH levels were recorded over a period of eight hours to identify any prolonged variation between trials with and without aquatic plants. The recorded pH levels overtime would determine the extent to which the *A. barteri* plant species could limit freshwater acidification.

Throughout the eight-hour period, all trials, with and without plants, experienced an overall decrease in pH (**Figure 1**). Within 30 minutes of the exposure to dry ice, the samples all sustained a significant decline in pH. After 50 minutes, the samples experienced varying marginal changes in pH, consisting of both increasing and decreasing phases. This trend was reflected in all trials to some extent (**Figure 1**).

Generally, the mean pH levels were higher throughout the trial time for both the samples with *A. b. var. nana* and *A. b. var. congensis* aquatic plants in comparison to those without plants. The pH of the samples with plants was higher by 0.11 pH units than the pH of the samples without plants after 120 minutes on average, which converts to a difference of 3.98×10^{-8} M in molar hydrogen ion concentration. At 330 minutes, the average pH levels of the *A. b. var. nana* and *A. b. var. congensis* plant trials were comparable while the control pH level is about 0.14 pH units lower than plant trial pH levels (**Figure 2**).

Given *p*-values of 0.05 or below for pH values after 285 minutes, the data obtained from this experiment is statistically significant, especially given that only three trials were performed per each plant type (**Table 1**). Based on these *p*-values, there was a 95.1% to 97.8% probability that the results were not obtained by random chance, indicating that there was a significant difference in pH levels between water samples with plants and without plants.

DISCUSSION

An interpretation that accounts for the variation in pH between water samples with and without aquatic plants is attributing the difference to the consumption of carbon dioxide during photosynthesis in the aquatic plants. When water samples are exposed to carbon dioxide, their reaction forms carbonic acid that decomposes into bicarbonate and carbonate, which releases a proton in the process, lowering the pH of the water as a result. However, if the plants absorb

Table 1. *p*-values of plants vs no plant samples for each time using a t-test from 0 minutes to 480 minutes.

time (mins)	*p*-value of plant vs no plant conditions
0	0.220094
120	0.084806
150	0.058886
195	0.087518
240	0.066264
285	0.040548
330	0.022177
375	0.037208
420	0.048678
480	0.044657

Note: 0 minutes is indicative of the pH levels recorded before exposure to carbon dioxide. N=3 without plants, N=6 plants (3 A. b. var. nana*, 3* A. b. var. congensis*). Red indicates a* p*-value greater than 0.10, yellow indicates a* p*-value greater than 0.05 but less than 0.10, and green indicates a* p*-value less than 0.05. A* p*-value less than 0.05 is considered statistically significant and a* p*-value greater than 0.05 is considered not statistically significant.*

some of the carbon dioxide that would typically be utilized in that chemical reaction, the plant can serve as a buffer in preventing a decrease in pH to the typical extent. Aquatic plants require carbon dioxide to perform photosynthesis to produce glucose and oxygen, meaning the water samples have a lower supply of carbon dioxide that could possibly be used in reactions that would decrease the pH level of the sample. Plants can process 44.14 parts per million (ppm) of carbon dioxide gas every minute for every gram of leaf surface during photosynthesis (10). Therefore, it is reasonable to explain the difference in pH levels by plants absorbing the carbon dioxide based on fundamental chemical reactions.

An important aspect of the experiment is identifying the influence of the water quality of each water sample on the experiment conducted. Since all samples consisted of water collected from the same location, the water quality likely has a similar influence on the results. In general, nitrates, nitrites, total chlorine, total hardness levels, and temperature have minimal impacts on changes in pH when exposed to carbon dioxide. Total alkalinity and pH are strongly impacted by each other as alkalinity is a measure of the water's ability to resist a change in pH based on the ability of bicarbonates, carbonates, and hydroxides to neutralize acids. Specifically, the water from the sample site had a total alkalinity measurement of 240 ppm, which is demonstrative of a somewhat high alkalinity level as the average total alkalinity for freshwater ecosystems ranges from 20 ppm to 200 ppm (11). A high total alkalinity indicates that the water samples had a strong ability to stabilize the pH level of the water and were resistant to changes in pH such as exposing the sample to carbon dioxide. Due to a higher-than-average total alkalinity level, the examined freshwater ecosystem may not be reflective of all freshwater ecosystems. Therefore, water samples of other freshwater ecosystems could possibly have more drastic changes in pH when exposed to carbon dioxide if they had lower total alkalinity levels.

Based on the increased mean hydrogen ion concentration of 3.98×10^{-8} M or 0.11 pH units in water samples with aquatic plants, plants have the potential to minimize freshwater acidification. Comparably, similar research conducted with seagrass meadows in coral reefs in the Indo-Pacific Ocean and Halimeda Cuneata alga in marine coastal ecosystems in Brazil found variations in pH up to 0.38 pH units and 0.18 ± 0.08 pH units, respectively, for limiting saltwater acidification (12, 13). In this experiment, the aquatic plants were able to prevent an increase in molar hydrogen ion concentration by 3.98×10^{-8} M based on the difference in pH between samples with and without plants. However, there was still a significant change in pH from before the carbon dioxide exposure (0 minutes) to the end of the period (480 minutes) for samples with aquatic plants as indicated by the 1.198×10^{-7} M increase in mean hydrogen ion concentration. Therefore, the plants were able to prevent approximately 25% of the increase in the observed molar hydrogen ion concentration for water samples with those plants. All samples began with a pH level around 7.45, but none of the mean samples were able to return to that original pH level throughout the experiment after their carbon dioxide exposure (**Figure 1**). Therefore, the aquatic plants were not able to consume all carbon dioxide from their exposure, suggesting that aquatic plants can only minimize acidification rather than impede acidification. Based on their proven potential, aquatic plants are a viable buffer for limiting the rates of acidification in freshwater ecosystems driven by carbon emissions to a minimal extent.

To further examine the potential of aquatic plants limiting acidification, long term and wide scale research is required, which could possibly have substantial outcomes in combating acidification. Since the aim of this research was to mimic the effects of carbon emissions, any changes in pH from carbon emissions would occur over a long period of time. Therefore, conducting research that monitors pH levels over a period of years would be important for understanding the role aquatic plants play in relation to long-term freshwater acidification. Exploring the effect of carbon emissions on multiple samples from different freshwater ecosystems will provide more clarity about variation in pH changes between ecosystems. A limitation of the experiment was the lack of multiple trials, meaning having a larger sample size would increase the meaningfulness of the statistical tests in determining if a difference between water samples with plants and without plants exists. With further research, aquatic plants may be a viable partial solution for acidification by minimizing drastic changes in pH levels in freshwater ecosystems. Therefore, as rates of anthropogenic carbon emissions surge, identifying methods, including aquatic plants, to mitigate the implications of climate change is vital for sustaining the planet.

METHODS

The effect of aquatic plants on acidification was examined by exposing water samples with and without plants to carbon dioxide and monitoring the pH levels over an 8-hour period. Water was collected from the Arroyo Valle creek in Livermore, California and tested for water quality. This water was obtained using plastic bottle containers at the edge of the creek near the Arroyo Del Valle regional trail. The water quality test included testing the water's nitrate, nitrite, total hardness, total chlorine, total alkalinity, TDS, temperature levels and pH using Tetra Easystrips (nutrient test strips), a Lxuemlu Professional TDS and temperature meter, and a Vantakool pH meter. Aquatic plants *A. b. var. nana* and *A. b. var. congensis* species were selected for the experiment because of convenience. Plants of the *Anubias* genus are commonly found in freshwater ecosystems primarily in western Africa and require minimal light and nutrients for growth (9). All aquatic plants were purchased from a local aquarium store. Plants and water samples were transported to the experiment location and stored in an area of moderate sunlight prior to use.

The experiment was set up using the collected water samples and the selected plants. The plants were exposed to direct sunlight in the outdoors for approximately an hour on the morning of the experiment to ensure photosynthesis was occurring. Measurements including mass of plants and volume of water samples were recorded using a Weigh Gram Digital Pocket scale and Pyrex glass 500mL measuring cup, respectively. Qualitative observations of each sample and plant were taken. Each preselected plant was placed into a sample container, while select samples remained without plants to serve as the controls (**Figure 3**). 460-480 mL of water were measured and poured into each sample container so that the water was filled to the top of the sample containers. The water quality test was repeated for each sample. Plants rose to the top of the water

Figure 3. Experimental setup. Experimental containers with freshwater creek samples and preselected treatment (*A. b. var. nana*, *A. b. var. congensis*, and no plant) prior to dry ice exposure.

in the containers and remained floating throughout the experiment (**Figure 3**). The temperature of the water was recorded to ensure the water temperature was optimal for the aquatic plants selected. Using the pH meter, each water sample's pH was recorded.

Approximately 0.75 grams of dry ice were measured and were sprinkled onto the top of the water in each container. Dry ice was obtained from a local grocery store. The cap of each container was screwed on partially immediately after addition to the container to allow partial ventilation to address safety concerns. The pH of each sample was recorded every 15 to 20 minutes for the first hour by sampling water from the trials using the cap of the container and placing the pH meter in the cap. In the following hours, the pH of each water sample was measured every 30 to 40 minutes using this same process. Plants and water were properly disposed of after the experiment had run for 8 hours. A total of 9 trials were performed simultaneously, with 3 containing *A. b. var. nana* plants, 3 containing *A. b. var. congensis* aquatic plants, and 3 serving as controls without any plants.

Using a t-test from GraphPad software, *p*-values were obtained that compared trials with plants and trials without plants at each recorded time to determine the statistical significance between the two groups. A t-test was selected for the data analysis rather than an ANOVA test due to limitations in sample size, meaning both *A. b. var. nana* and *A. b. var. congensis* plants were grouped together as the plant group. To quantify how much aquatic plants could limit acidification, the recorded pH values were used to calculate the increase in molar hydrogen ion concentration for trials with plants and without plants.

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