# PHYTOREMEDIATION ASSESSMENT OF NATIVE HAWAIIAN PLANTS:

## NITROGEN UPTAKE BY ‘AHU‘AWA AND ‘AKA‘AKAI UNDER VARIOUS SALINITY CONDITIONS


Executive Summary:

Elevated nitrogen levels in coastal nearshore waters can lead to a decline in coral heath and bleaching. In Kahalu‘u Bay, Hawai‘i Island, high levels of nutrients create a chronic stress to the coral ecosystems. Plants have the capacity to remove pollutants in a process called phytoremediation. They have been used as part of wastewater and stormwater treatment processes to remove nutrients and other pollutants. This study aimed to fill a gap in research on the phytoremediation capabilities of native Hawaiian plants, especially in brackish water conditions. This is a novel phytoremediation project that was conducted in partnership between Roth Ecological Design Int. (REDI) and the Kohala Center (TKC) and was designed to bridge ancestral knowledge with modern science to create natural solutions to address today’s water quality issues.

An important outcome of this study is to increase community knowledge about the potential ecological services native Hawaiian and indigenous wetland plants provide. The purpose of this research was to determine if native Hawaiian plants improve water quality and could be selected to restore coastal ecosystems impaired by excess nitrogen. Nitrogen is a common pollutant in waterways from fertilizers, sewage, and nonpoint source pollution.

Importantly, prior research has been done on the phytoremediation capabilities of several native Hawaiian wetland plants. One study evaluated *Cyperus laevigatus* (makaloa) for its ability to uptake nitrogen and phosphorus from treated wastewater effluent (Van Dyke, 2001). A second study investigated three native Hawaiian wetland plants—*Cladium jamaicense* (‘uki), *Cyperus javanicus* (‘ahu‘awa) and *Cyperus polystachyos* (Unser, 2010)—for their abilities to accumulate nitrogen into their tissue from the water column and the water quality

improvements from a stream. Van Dyke suggests further research evaluating *Cyperus javanicus* (‘ahu‘awa) and *Schoenoplectus lacustris* (‘aka‘akai) as well.

REDI and TKC’s project also focused on evaluating two indigenous wetland plants for their potential in removing nitrogen. The research also included evaluating the adaptation of these plants to brackish/saltwater conditions and observing any changes to nitrogen uptake rates. Understanding salinity limitations of these plants also plays a key role in deciding suitable locations for outplanting these plants under various brackish water conditions. The outcomes of this research support restoration initiatives in Hawai‘i where water quality in coastal areas has been compromised as well as their potential function in green stormwater infrastructure and natural systems wastewater treatment.

Materials:

- Plant Hydroponics Systems - 33 (total initial) Hydrofarm Root Spa Deep Water Culture Bucket System (5-Gallon)
    - Total plants: 30
      Two species of plants:
        - 15 ‘ahu‘awa (Labeled “A” when sampling)
        - 15 ‘aka‘akai/bulrush (Labeled “B” when sampling)
    - Three control hydroponic systems that included growing media, but not any plants
    - Hydroponics systems were placed on outdoor tables at NELHA to simulate natural Kona coastal conditions (makai side of Gateway Building)
- Hydroton Clay Pebbles (Mother Earth Clay pebbles) for media
- Peter’s Professional (20-20-20) General Purpose Water Soluble Fertilizer with Micronutrients
- YSI ProDSS Multiparameter Digital Water Quality Meter
- 250 mL bottles for samples (provided and cleaned by NELHA WQ lab)
- Test strips
    - 0-50ppm Hach 2745425 Nitrate and Nitrite Test Strips or AquaChek 50 641426E Nitrate/Nitrite Test Strips Swimming Pool/Spa
    - Tetra EasyStrips 100 Count, Ammonia Test Strips For aquariums, Water Testing, Model Number: 19541

Methods:

REDI tested the ability of two indigenous wetland plants, ‘ahu‘awa and ‘aka‘akai, to acclimate to brackish and saltwater conditions while assessing and comparing nitrogen uptake between the species and to the control. Testing of the plants at various salinities was conducted to

observe impacts of nitrogen removal, stress and survival. The experiment was set up at the NELHA Gateway Center in an outdoor environment to simulate coastal climate conditions. The following methods were used:

- Plants were weighed before and after experiment to assess estimated growth during the experiment period and if there was any correlation between nutrient uptake and plant biomass. Plants were removed from their soil and rock media and placed on a digital scale to measure their weight in grams.
- Hydroponic systems were set up using the buckets and media and the wetland plants that were removed from the prior soil media were transplanted into hydroponic brackish water conditions.
    - Initial salinity was approximately 5-8 ppt; salinity was increased during the course of the experiment for select hydroponic buckets.
    - Brackish water was made from combining potable fresh water (from hose) and with a fraction of seawater (from spigot at the Gateway Center). To achieve a salinity within a range of 5-10 ppt, 13.5 L of freshwater was combined with 4 L of salt water. To achieve the higher salinity range of 10 - 15 ppt, 11.5 L of freshwater was combined with 6 L of salt water. This ratio was confirmed to match actual salinity using the YSI probe.
- Hydroponics systems were placed outdoors at NELHA to simulate natural Kona coastal conditions.
- Nutrients were added in the form of dissolved fertilizer crystals
    - Fertilizer was measured out (using measuring spoons) into freshwater to make a stock solution that was then dosed into each full bucket.
    - Initial total nitrogen (TN) concentrations varied from the beginning of the experiment to the end. Initial concentrations of approximately 30 ppm TN, used 1/8 of a tablespoon of fertilizer per bucket. Lower concentrations of nitrogen used 2/15 of a tablespoon of fertilizer to achieve approximately 8 ppm TN. The initial concentrations across all individual hydroponic systems were targeted to be the same and were within 10 ppm of each other.

Prior to the start of the experiment, there was a period of four weeks to allow for plant establishment in the hydroponic systems and to determine these starting concentrations of nitrogen for the experiment and methods of creating the salinity concentrations.

- Once the experiment was initiated, periodic samples were taken from each hydroponic system. To collect the sample, the lids of the buckets were partially removed to allow for sampling from the water.
    - In-situ measurements in each of the hydroponic systems were taken using the YSI probe and recorded into the log (pH, temperature, dissolved oxygen, salinity)
        - The probe sensors were calibrated every sample day prior to use.
        - After logging the YSI probe data, the probe was used to stir the water inside of the buckets to ensure a well-mixed nutrient sample.
    - The following forms of nitrogen were tested: Nitrate, Ammonium and Total Nitrogen. Samples were collected from water in the buckets and dropped off at a third-party water quality lab (NELHA Water Quality Lab).
        - Composite samples were made of each treatment using a 125 mL bottle (with tape marking a level to sample), and poured into a 1-liter bottle to mix and pour into sample bottle (A: ʻahuʻawa, , B: bulrush).
            - A 1-5 & B 1-5 had a lower salinity; A 6-13 & B 6-13 had higher salinity
            - C1: control was low salinity, no composite needed
            - C2: control was high salinity, no composite needed
        - All bottles were triple rinsed with freshwater prior to filling.
        - Samples were kept on ice in a cooler for transport.
        - Labeled with date (example: “A_0402_L” = ʻAhuʻawa, April 2, 2021, Low Salinity).
        - Filled out sample drop off form.
        - For each sampling day, photos were taken to document plant growth and overall visual health.

- Samples were taken weekly during the first two months and then every two weeks thereafter or until there were trace levels of nutrients detected in the water.
    - Plant health was monitored regularly.
    - Once the plants ran out of nitrogen, a new batch of fertilizer was mixed and placed in all of the buckets. This process included the following:
        - Emptying and rinsing the buckets, followed by adding new water and nutrients.
        - Samples were taken on day 1 of a new round to determine exact initial nutrient concentrations
        - Testing occurred over a period of six months (there was an additional two-week calibration phase of two before samples were sent to the water quality laboratory for analysis).


Figure 1: Photos of ʻahuʻawa plants (left) and ʻakaʻakai plants (right) in February (beginning of experiment).

Results:

**Nitrogen Removal**

Table 1 shows results of the water lab analysis of nitrogen removal during each of the sample periods. The results show both plants removing nitrogen over the course of the experiment while the control had no observable removal of the nutrients. Both plants were able to successfully remove 80-95% of the total starting nitrogen within five weeks. There was not a significant difference in nitrogen removal between the two species. Initial nitrogen concentrations were approximately 30-40 ppm total nitrogen for most rounds (see table 1), with some variation due to limited precision in equipment. The targeted initial nitrogen concentration in round 2 was lower (average of approximately 8 ppm) in order to see how plants behave in lower nutrient concentrations, similar to those observed in coastal areas in Kona. Some stress was observed after a few weeks when total nitrogen was around 1 ppm, so a new round was started. An increase in total nitrogen values was observed between week 3 and 5 on average for the ʻakaʻakai/bulrush plants and for the low salinity ʻahuʻawa plants. This may suggest that ʻahuʻawa continues to phytoremediate even at lower nutrient levels and in higher salinity.

In the first round, plants were slower to start taking up nitrogen. This is likely due to an establishment period. Figures 2 through 5 show nitrogen concentrations over the duration of the experiment for all treatments (A: ‘ahu‘awa, low and high salinity; B: bulrush, low and high salinity; and C: control, low and high salinity).

Dissolved oxygen (DO) influences the nitrogen cycle and conversion between the forms of nitrogen. The DO in the plant buckets ranged from 0.2-2.9 ppm compared to the average DO of the Control (2.0-7.4 ppm). As the plants grew, less DO was observed in the solution. This could be caused by the plants shedding organic matter and anoxic conditions may have had an effect on the nitrogen removal rates. See Figure 8 for average DO measurements for the control and the plant solutions throughout the experiment time frame.

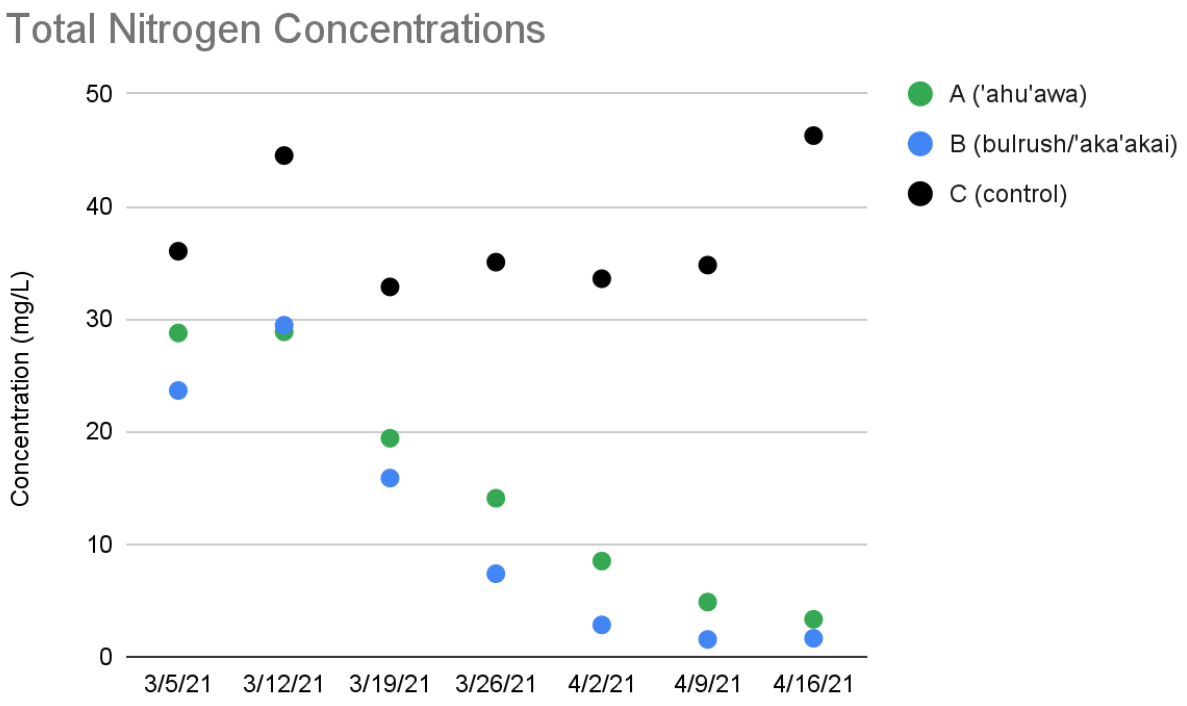


Figure 2: Fertilizer added on March 5th, initial salinities approximately 7 ppt

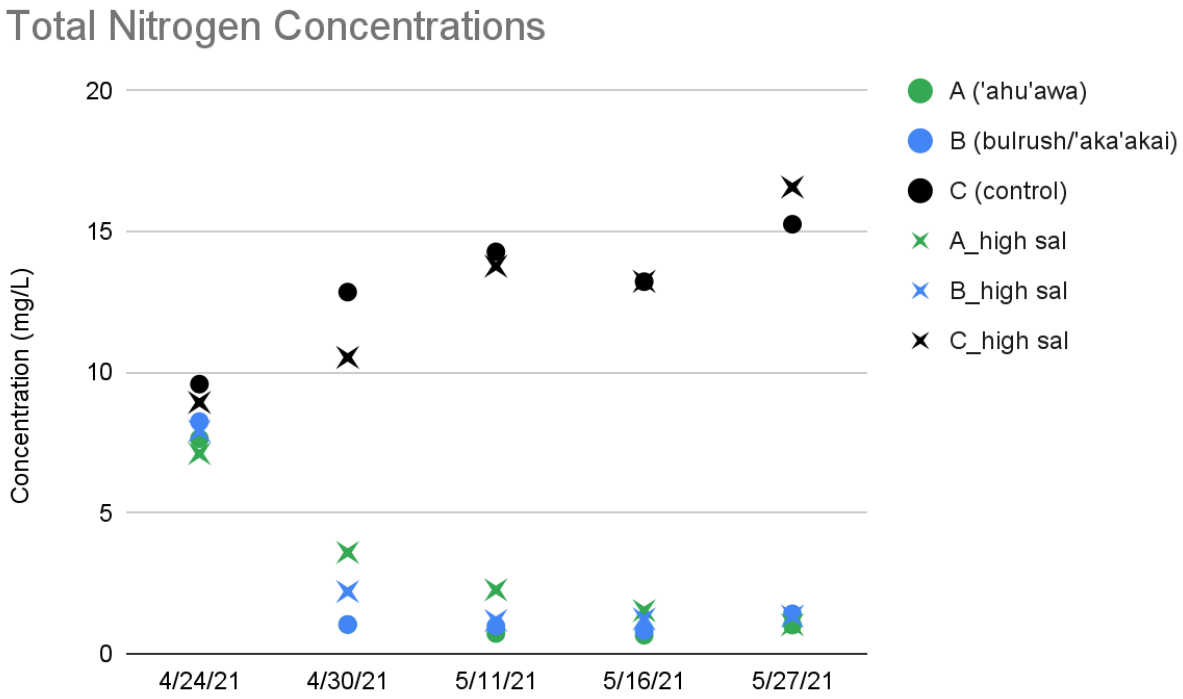


Figure 3: Fertilizer added on April 24th, initial salinities approximately 9 ppt and 13 ppt (high salinity treatment)

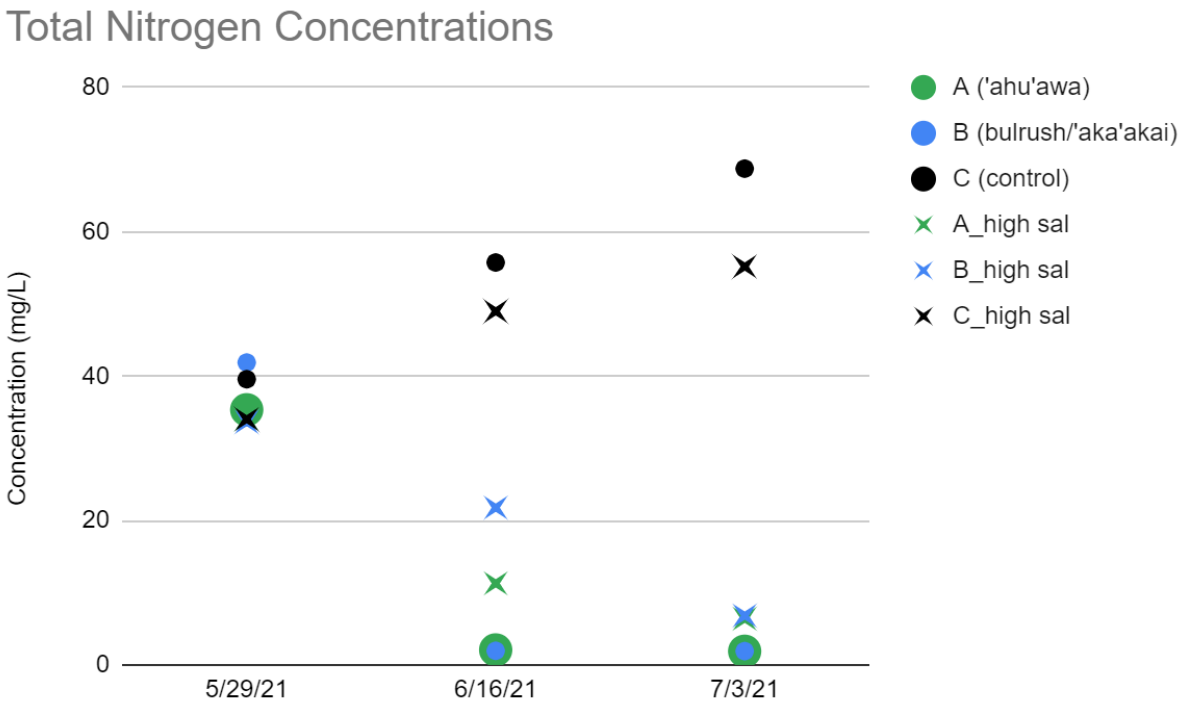


Figure 4: Fertilizer added on May 29th, initial salinities approximately 9 ppt and 13 ppt (high salinity treatment)

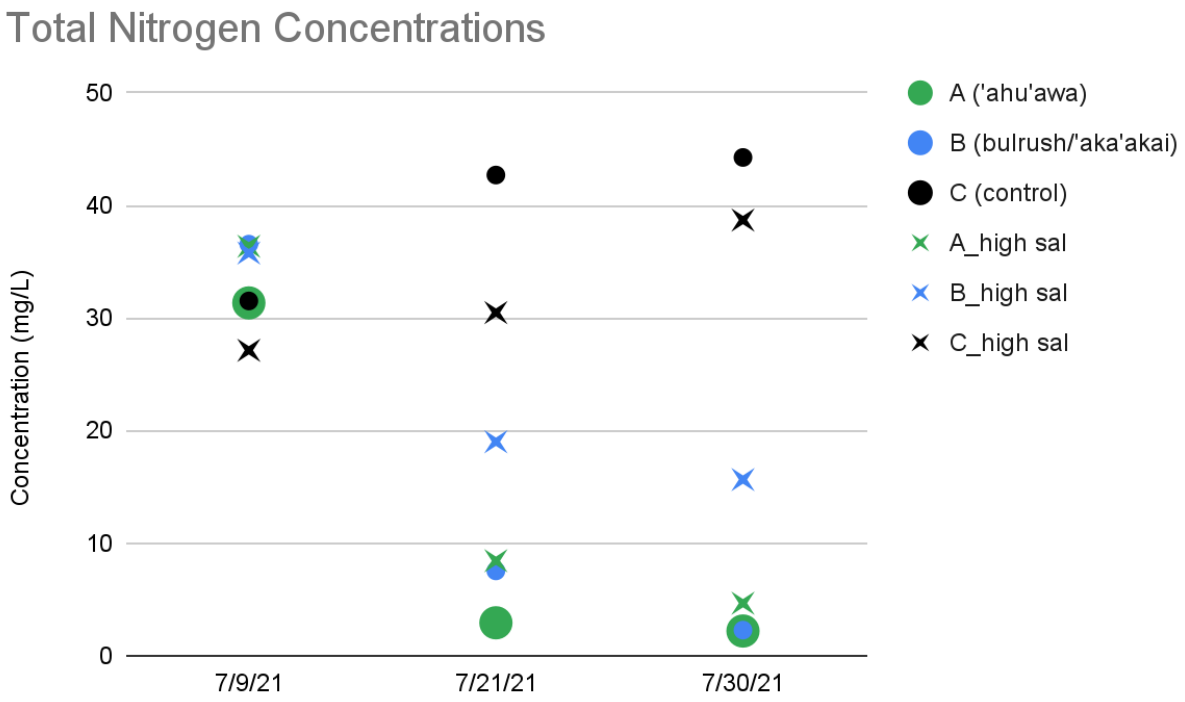


Figure 5: Fertilizer added on July 9th, initial salinities approximately 10 ppt and 14 ppt (high salinity treatment)

| | Round 1 | | | Round 2 | | | | | | Round 3 | | | | | | Round 4 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Low Salinity | | | High Salinity | | | Low Salinity | | | High Salinity | | | Low Salinity | | | High Salinity | | |
| | (A) | (B) | (C) | (A) | (B) | (C) | (A) | (B) | (C) | (A) | (B) | (C) | (A) | (B) | (C) | (A) | (B) | (C) | (A) | (B) | (C) |
| Starting N Concentration (mg/L) | 28.8 | 23.7 | 36.0 | 7.6 | 8.3 | 9.6 | 7.1 | 7.9 | 8.9 | 35.4 | 41.9 | 39.6 | 34.1 | 33.7 | 34.0 | 31.3 | 36.6 | 31.5 | 36.4 | 35.8 | 27.1 |
| Starting Salinity (ppt) | 6.2 | 6.9 | 6.9 | 8.8 | 8.9 | 8.7 | 12.7 | 12.6 | 12.2 | 8.6 | 8.9 | 8.8 | 12.6 | 12.9 | 12.6 | 9.7 | 10.3 | 9.6 | 14.6 | 14.6 | 13.0 |
| Ending N Concentration (mg/L) | 3.3 | 1.6 | 46.3 | 1.0 | 1.4 | 15.3 | 1.0 | 1.3 | 16.6 | 1.9 | 1.9 | 68.8 | 6.4 | 6.8 | 55.2 | 2.2 | 2.3 | 44.3 | 4.7 | 15.7 | 38.7 |
| Ending Salinity (ppt) | 9.4 | 15.2 | 9.1 | 14.2 | 16.4 | 10.4 | 16.2 | 17.9 | 14.9 | 18.1 | 19.8 | 12.1 | 20.0 | 20.6 | 17.3 | 12.8 | 13.5 | 10.5 | 16.6 | 16.3 | 14.0 |
| % Nitrogen Removal | 88% | 93% | -29% | 87% | 83% | -59% | 86% | 83% | -86% | 95% | 95% | -74% | 81% | 80% | -62% | 93% | 94% | -40% | 87% | 56% | -43% |
| Total Nitrogen Removal (mg/L) | 25.4 | 22.0 | -10.3 | 6.6 | 6.8 | -5.7 | 6.1 | 6.6 | -7.65 | 33.4 | 40.0 | -29.2 | 27.7 | 26.8 | -21.2 | 29.1 | 34.3 | -12.7 | 31.7 | 20.1 | -11.6 |
| Sampling Period (days) | 42 | 42 | 42 | 34 | 34 | 34 | 34 | 34 | 34 | 36 | 36 | 36 | 36 | 36 | 36 | 22 | 22 | 22 | 22 | 22 | 22 |
| Average Biomass (g) | | | | 84 | 116 | - | 94 | 104 | - | - | - | - | - | - | - | 648 | 724 | - | 463 | 490 | - |
| Biomass Change (g) (over 134 days) | | | | | | | | | | | | | | | | 564 | 608 | - | 371 | 386 | - |

Table 1: Nitrogen removal between selected plants compared to the control and under varying salinities and growth stage

**Effect of Salinity**

It was observed that plants in the lower salinity solution removed nitrogen faster than the higher salinity plants. For example, in Round 3 (June), the high salinity plants did not remove as much of the nitrogen as the lower salinity counterparts (~80% compared to 95% respectively). It appeared that the higher salinity treatments took a little longer to uptake the nutrients than their lower salinity counterparts on average. Towards the end of the experiment, the ʻakaʻakai plants in the higher salinities appeared more stressed than the ʻahuʻawa in higher salinities. Results in Table 1 also reflect that ʻahuʻawa significantly removed more nitrogen under higher salinity concentrations in Round 4.

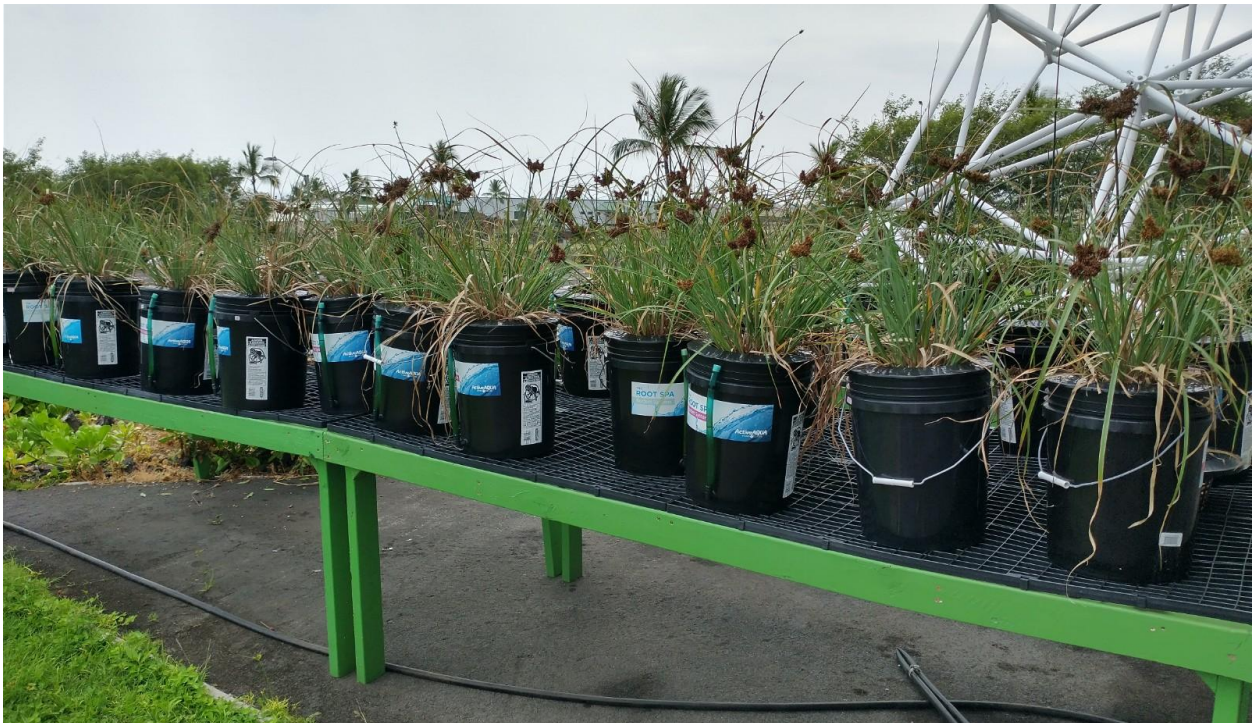

Figure 6: ʻAhuʻawa (A) on the last day of the experiment (July 31, 2021). Higher salinity plants are on the left, lower salinity are the five on the right.

Figure 7: Bulrush/‘aka‘akai (B) on the last day of the experiment (July 31, 2021). Higher salinity plants are in the foreground.

## In-situ Measurements

In-situ measurements were recorded for each plant and averaged for each sample day. Salinity during the experiment increased up to approximately 20 ppt for certain plants. See figures 8 through 11 below to see how parameters fluctuated over the course of the experiment.

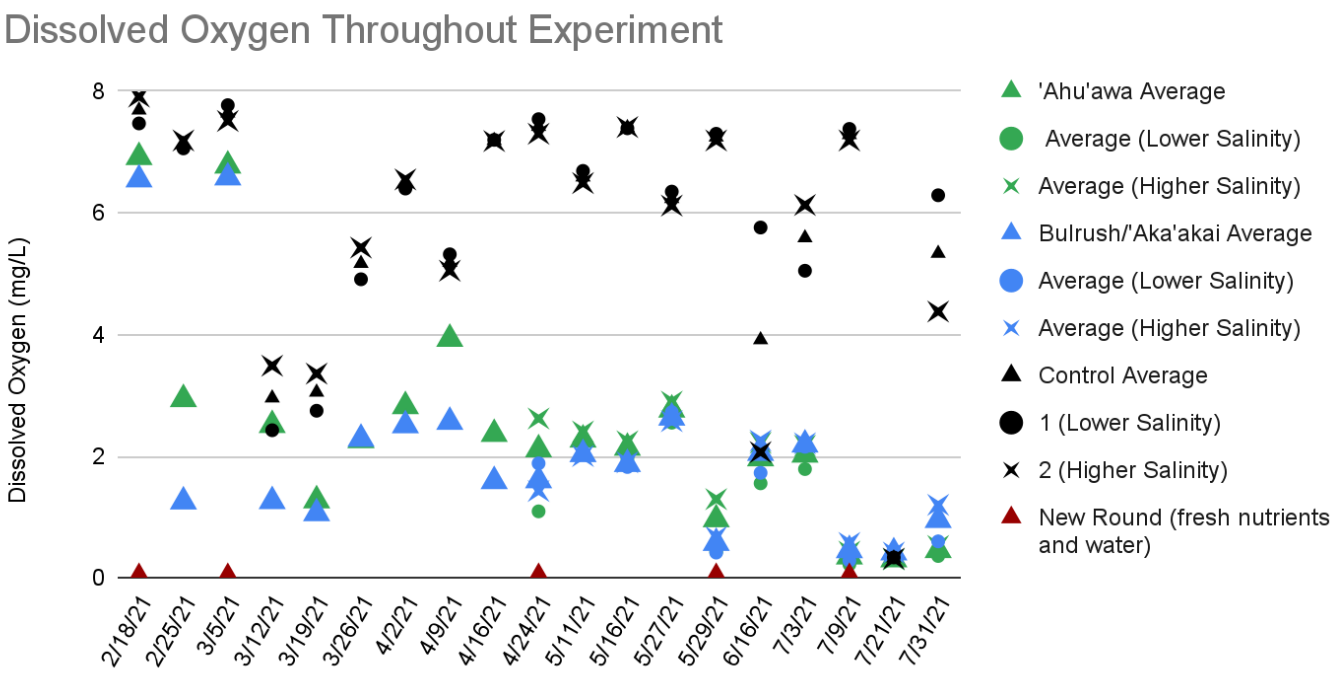


Figure 8: Dissolved oxygen measurements

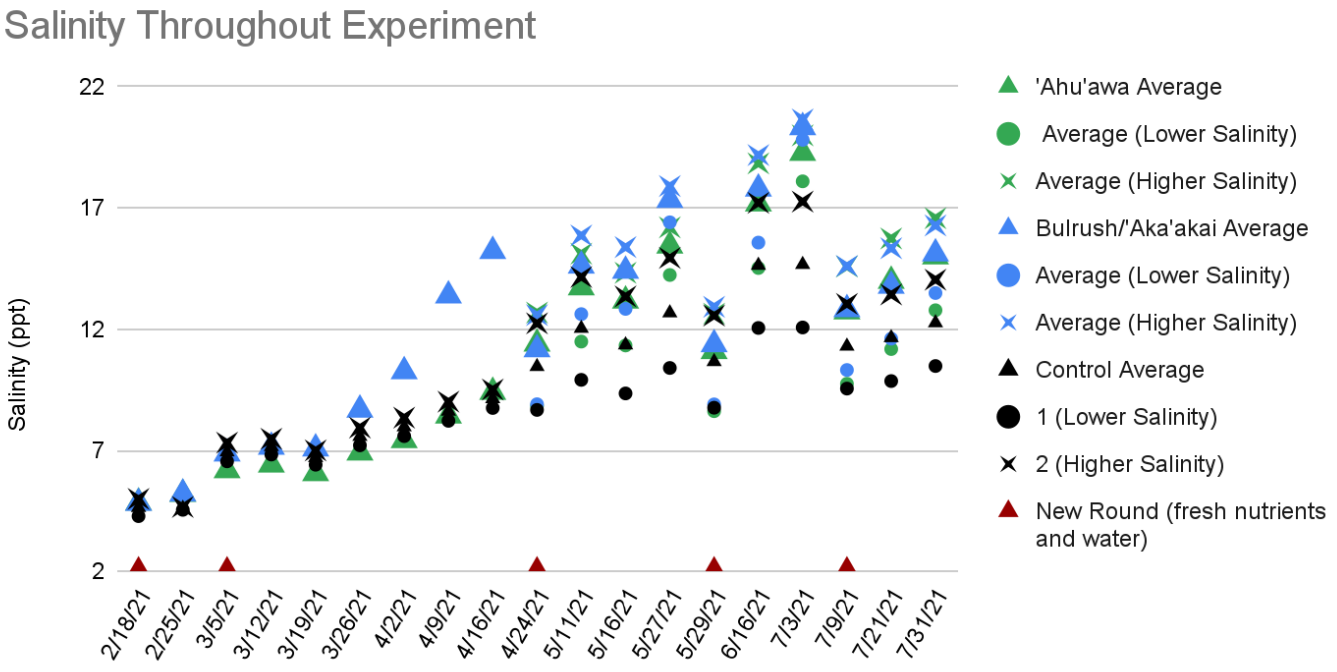


Figure 9: Salinity measurements

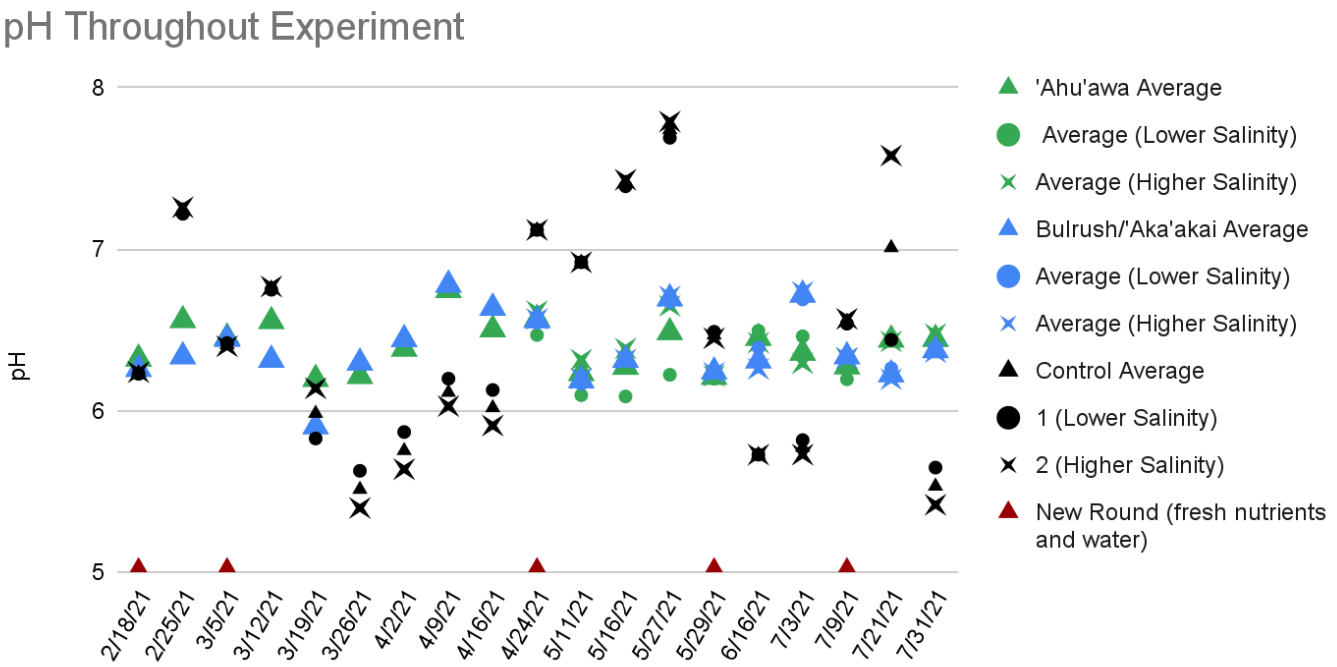


Figure 10: pH measurements

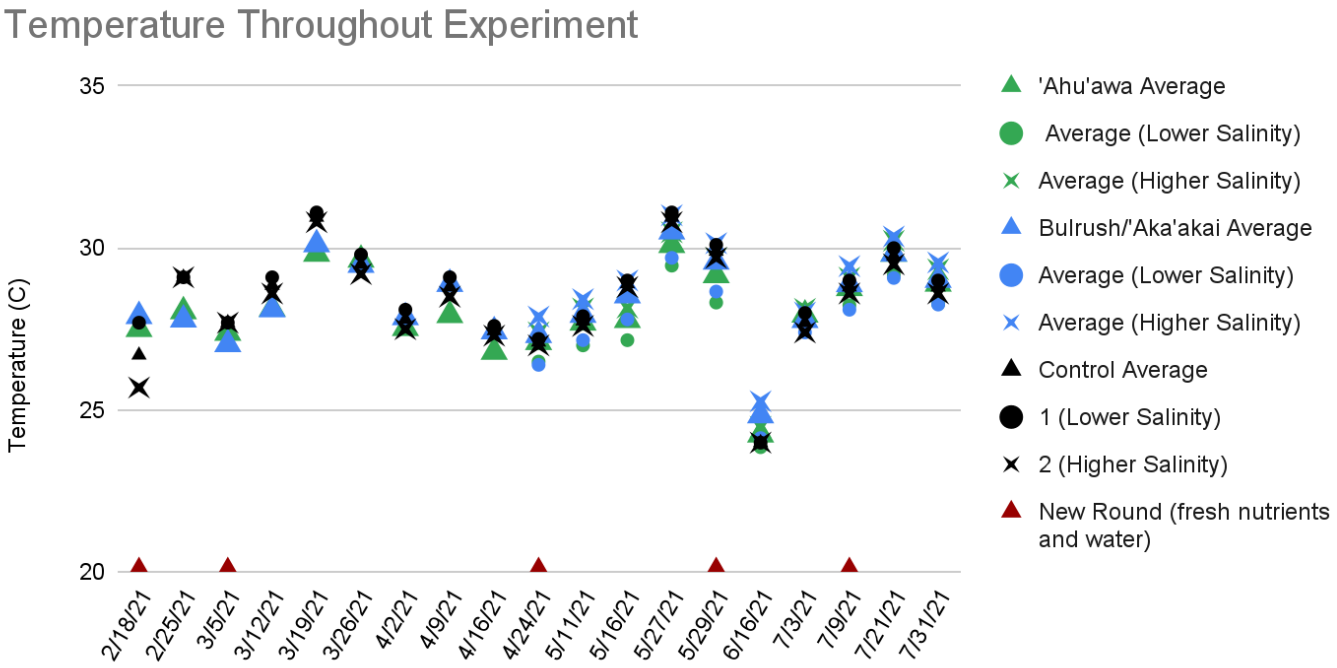


Figure 11: Temperature measurements

## Biomass

Over the period of six months, the plants grew and became more established. Evidence suggests that more plant biomass will remove more nitrogen more effectively. However, to prevent unwanted shock to the plants, the plants were only weighed at the beginning and end of the experiment.

There was a general trend that more nitrogen was removed as the plants grew. This may imply that the greater biomass (roots and above ground tissue) contributed to faster nitrogen uptake. However, this would need to be further studied, because not all of the sampling rounds had a high enough sampling resolution to be able to determine how strong this trend was. Biomass as a function of uptake was observed more in the lower salinity treatments than the higher salinity solutions. No consistent patterns were seen between species. As seen in Table 2, both species grew comparable amounts on average. The bulrush (B) put on more mass on average than ʻahuʻawa (A), however, the ʻahuʻawa growth rate was higher (394% on average) compared to the bulrush (318% on average). On average, ʻakaʻakai grew visibly more roots compared to the ʻahuʻawa. See Table 2 below for the weight results from the beginning of the experiment to the end and Figures 12 though 17 for photos of the plants over the course of the experiment.

| MASS | | | | | | |
|---|---|---|---|---|---|---|
| | **ʻAhuʻawa (A)** | | | **Bulrush/ʻAkaʻakai (B)** | | |
| | **Average** | **Lower Salinity*** | **Higher Salinity**** | **Average** | **Lower Salinity*** | **Higher Salinity**** |
| Initial mass (g) (2/12/21) | 90 | 84 | 94 | 113 | 116 | 104 |
| Final mass (g) (8/2/21) | 534 | 648 | 463 | 580 | 724 | 490 |
| % Increase mass | 394% | 573% | 296% | 318% | 425% | 271% |
| Growth (g) (over ~6 months) | 445 | 564 | 371 | 471 | 608 | 386 |
| | **Initial salinities of ~9 ppt* | | | | | |
| | ***Initial salinities of ~14 ppt* | | | | | |

Table 2: The average masses of each group of individuals over the course of the experiment

Figure 12: Photos of ʻahuʻawa plants (left) and bulrush plants (right) in February (start of the experiment).

Figure 13: Photo of ʻahuʻawa plants in July (end of the experiment).

Figure 14: Photo of ʻahuʻawa plants in July (end of the experiment).

On average, ʻakaʻakai grew visibly more roots compared to the ʻahuʻawa

Figure 15: Photos taken in February (4-weeks after plant establishment). On the left are roots from an ʻahuʻawa plant; on the right the roots from an ʻakaʻakai plant


Figure 16: Photos taken at the end of the experiment (7/31/21). On the left are the roots of a low salinity ‘ahu‘awa plant, in the middle and on the right are the roots of high salinity ‘ahu‘awa plants.


Figure 17: Photos taken at the end of the experiment (7/21/21). On the left are the roots of a low salinity ‘aka‘akai plant, in the middle and on the right are the roots of high salinity ‘aka‘akai plants.

## Discussion/Next Steps:

The results of the experiment showed that ‘ahu‘awa and ‘aka‘akai significantly removed nitrogen from the water column compared to the control and that both plants were able to tolerate salinities up to 20 ppt. These results indicate the potential for these plants to be used in phytoremediation efforts where higher nitrogen concentrations may exist. The experiment, however, was limited by budget and timeline constraints. The water quality lab results were only over a period of seven months; the plants had not yet reached full maturity. Extending the experimentation over a period of one to two years would help to determine if and to what

extent the removal of nitrogen continues to increase as the plants continue to grow would be worth exploring. In addition, a longer experiment period would create a higher-resolution data set to better understand the variations in nitrogen uptake that were observed.

Another next potential step in furthering this research of these selected plant species would be to take the information learned from this study and do testing in the field. This would create a richer story of how these plants could support bioremediation in natural conditions. However, in the natural environment, many of the controls that were practiced during this experiment could be highly variable such as nutrient retention, concentrations, salinity and other variables, some of which may be unforeseen. Would these variables change their abilities to uptake nitrogen at similar rates as seen in this study?

Thirdly, the data sets remain very limited in our understanding of native and indigenous wetland plants' ability to remove pollutants. In this study, only nitrogen was assessed. There are numerous other types of pollutants from various point and nonpoint sources that cause damage to waterways and nearshore ecosystems such as heavy metals, phosphorus, petroleum-based, and from pharmaceuticals and personal care products (PPCPs). The further testing of these or other native and indigenous plants for the removal of these types of pollutants would greatly enhance this study given that Hawai'i and many other islands across the Pacific are impacted from sewage-derived pollution.

Lastly, this study has the potential to begin to catalog the ecological services of a variety of native and indigenous plants and the roles they can play in restoration, remediation, erosion control and other areas. Repeating a similar experiment or investigating other potential pollutant removal capacities with other native or indigenous plants could lead to the creation of a field guide for those in the field of conservation, restoration, green infrastructure or other ecological engineering/design fields. By cataloging both historic uses and 21st-century knowledge of native plants, a useful resource could be deployed for land/ocean managers and green infrastructure professionals to select appropriate plants based on the site conditions and pollutants of concern. Having a resource such as this could help save money and time, and importantly help lead to the desired outcome: to repair the water quality in streams, ponds and nearshore coastal environments.