Final Report for the Hawai‘i Invasive Species Council FY2023

Part 1. Expansion of management and techniques for species of highly invasive genus *Avrainvillea*

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Problem statement:

As recently as 2011, Hawai‘i ’s marine resources were valued at $33.57 billion dollars (Bishop et al., 2011) recognizing the many important ecosystem services our coastal reefs provide: coastal zone protection via dissipation of storm energies, as well as food/ protein sources via fishing and subsistence collecting, tourist and residents ocean activities, over 100,000 jobs in Hawai‘i (https://coast.noaa.gov/states/fast-facts/tourism-and-recreation.html). Healthy coastal communities also constitute a sacred part of Hawai‘i’s cultural heritage. By 2020, many residents were familiar with all included threats to coral reefs, but were most familiar with pollution and sunscreen (Allen et al. 2022). Blooms of invasive marine algae however were not apparently considered in that report despite being commonplace over the main Hawaiian Islands (https://dlnr.hawaii.gov/ais/invasivealgae/). Conducted in Kihei Maui, economic surveys as early as 2002 revealed that the invasive algae bloom on Maui caused large losses of real estate value and hotel business; mitigation however could result in benefits of $30 million over time (van Beukering and Cesar 2004). With this background of need for a more knowledgeable public and need for breakthroughs in invasive algal research, marine plant researchers in the School of Life Sciences Botany Graduate Program continue to innovate and test new tools to aid management of these reefs. HISC projects are the principal source of funding.

Globally, several of the most notable tropical invasive algae are evolutionarily related and placed in the green algal order, the Bryopsidales (Williams and Smith, 2007; Vroom and Smith, 2001). An important example of invasive biology is the *Caulerpa taxifolia* (M.Vahl) C. Agardh introduction and spread across the Mediterranean sea after its presumed release from the Monaco aquarium (Jousson et al. 1998). This alga is a popular aquarium hobbyist plant and consequently had been Irresponsibly released into new habitats and appeared in a water-feature, a lagoon in a residential coastal region in southern California. Chemical treatments with chlorine bleach were successful in eradicating this population by administering the bleach to a localized area underneath tarps. While successful in removing *C. taxifolia*, the treatments had negative effects on the other organisms under the tarp (Williams and Schroeder 2004; Anderson 2005).

When dealing with chemical treatments, it is very important to consider all of the organisms in that ecosystem and how reactive products may affect them. In Hawai‘i, this consideration of chemical oxidizers led Scott Van De Verg to introduce a novel protocol for the removal of invasive *Avrainvillea* species on O‘ahu using hydrogen peroxide (H_2O_2) as an oxidation agent (Van De Verg and Smith, 2022).

Two of the more broadly spread invasive species in Hawai‘i today are in the green algal genus, *Avrainvillea. Avrainvillea erecta* (Wade et al., 2018) and *Avrainvillea lacerata* (Brostoff, 1983) outcompete native species despite having differing morphologies and species traits. These species can form substantial mounds (*A. lacerata*) or large meadow areas (*A. erecta*) that can persist in certain key reef regions such as in intertidal reef flats over coral colonies (*A. lacerata*) to various depths near the upper mesophotic region, between 75 (*A. erecta;* L. Wheeler per obsv) to 120 ft (*A. lacerata;* Foster et al., 2019). Thus, this continued project aimed to further understand how to best manage these species at depths while increasing collaboration with stakeholders. The use of hydrogen peroxide as a novel control method was applied to both species and was evaluated for efficacy in eliminating the plant *in situ*. Testing this strategy is useful as it may become more important than manual removal that generates viable fragments in the harvesting effort.

Overall goal:

The objective of this research was to apply hydrogen peroxide on both species of *Avrainvillea* at depth and expand sites where treatment was implemented. From previous work conducted under the HISC grant by two of the grant’s researchers, hydrogen peroxide is a new control agent for this genus of algae. This investigation was to continue testing feasibility of a larger-scale management strategy at different depths of the algae’s range. Further this work proposed to begin baseline studies on Maui and Hawai‘i sites where new populations have been established.

Approach and methods:

To test hydrogen peroxide on *Avrainvillea erecta,* scientific diving had to be used to locate and apply treatment *in situ* at 19m depth. Scientific Divers laid a 30m transect at 19m within the co-occurring meadow of the two targeted *Avrainvillea spp* (Figure 1). Individual plants of *A. erecta* and *A. lacerata* are marked at every 2 meters along the 30 meter transect. The beginning and end of the transect were marked by a 0.75m aluminum stakes that were hammered into the deep sand.

Figure 1. Aerial map of Ala Wai boat harbor and fringing reef with Ala Moana Beach Park to the rear. Site was established on the Kaka'ako side of the channel.

A Walz Diving Pulse Amplitude Modulated Fluorometer (PAM) was used to measure saturated pulses of photosystem II. The DIVING PAM sends eight saturated light pulses through a fiberoptic clamp that is attached to the targeted plant and measures its maximum electron transport (ETR_{max}) and reports a rapid light curve (RLC) (Beer, 2004). These two measurements can provide information on the maximal rates of photosynthesis and irradiance required to saturate photosynthesis in targeted plants (Dummermuth, 2003). The values of ETR_{max} and E_k were selected as physiological indicators (Smith et al. 2004) and these measurements will serve as parameters to measure photosynthetic success in these communities. Additional measurements used were the photosynthesis maximum (P_{max}), which was calculated through the equation $P_{max} = (\alpha * E_k)$ (Richard Dona et al., 2023; Silsbe and Kromkampp, 2012).

Hydrogen Peroxide Application Protocol: Hydrogen peroxide was diluted from a 30% concentration to a 3% concentration from lab grade H_2O_2 using deionized water (formula: $V_1C_1=V_2C_2$, V = Volume and C = Concentration of Hydrogen Peroxide). 1000mL of the 3% solution was poured into a catheter bladder and attached to a ¼ inch tubing that led to a horse injector gun (QC Supply) available with a 16-gauge needle (via Amazon; Figure 4). This system allowed for direct application of 30mL of 3% H_2O_2 to be injected into each plant. The site of

injection for *A. erecta* was into the stipe, below the photosynthetic part of the blade. The site of injection for *A. lacerata* was into the holdfast, which carries most of the mass of the plant and is connected to other blades of the plant.

Monitoring of injected plants: Plants were marked at each 2 meter mark along a 30 meter transect using two fishing weights and wire wrapped to encircle the base of the targeted plant. Each plant was observed for initial ETR_{max} and E_k values using the DIVING PAM prior to any treatments with a photograph that also measured the height (cm) and number of blades present. *A. erecta* plants also noted the blade width (cm). Plants were injected with 30mL of 3% H_2O_2 and field workers returned for additional PAM measurements 2 days and 7 days post-treatment alongside photographs. These photos were compared against pre-treatment photographs to show any discoloration or necrosis of plant tissue.

Site descriptions: The site chosen for this experiment was at approximately 19 meters depth. This study area is located outside of Magic Island approximately one mile outside the Ala Wai boat harbor (Figure 1). This area has dense populations of *Avrainvillea erecta* and *Halimeda kanaloana* beds with growing populations of *Avrainvillea lacerata. A. erecta* is a psammophytic (sand dwelling) species that can occur at depths as shallow as 15 m (Figure 2, 3). *Avrainvillea lacerata* can be prevalent in sandy bottoms, coral rubble, or within crevices of rock or live coral (SCH per obs.).

Access to this location was via small boat which utilized live boating, which requires the boat captain to maintain position proximate to divers without the use of moorings or anchors. This location is a heavily used corridor for vessel travel between Kewalo Basin and popular Waikiki dive sites.

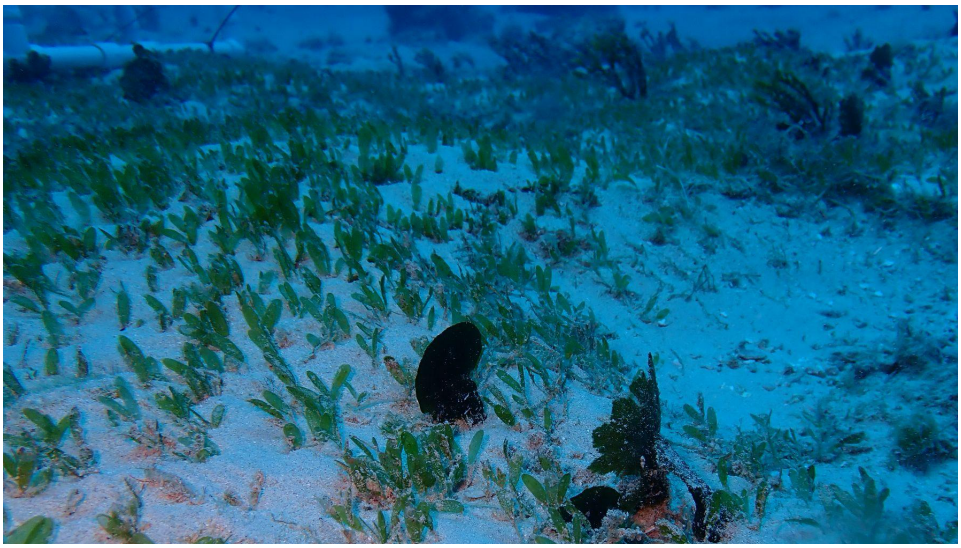

Figure 2. *Avrainvillea erecta* has invaded and now co-occurs in meadows of endemic species of *Halophila hawaiiana* (native seagrass) and *Halimeda kanaloana.*

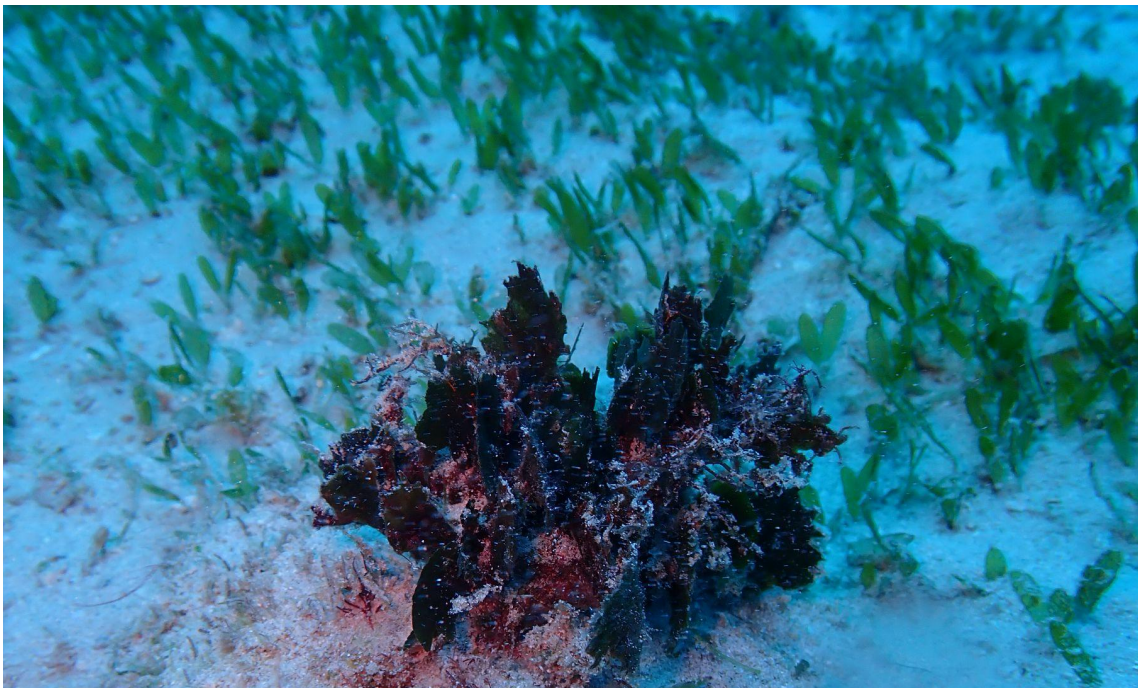

Figure 3. *Avrainvillea erecta* and *A. lacerata* have invaded and co-occurs in meadows of *Halophila hawaiiana* and *Halimeda kanaloana*.

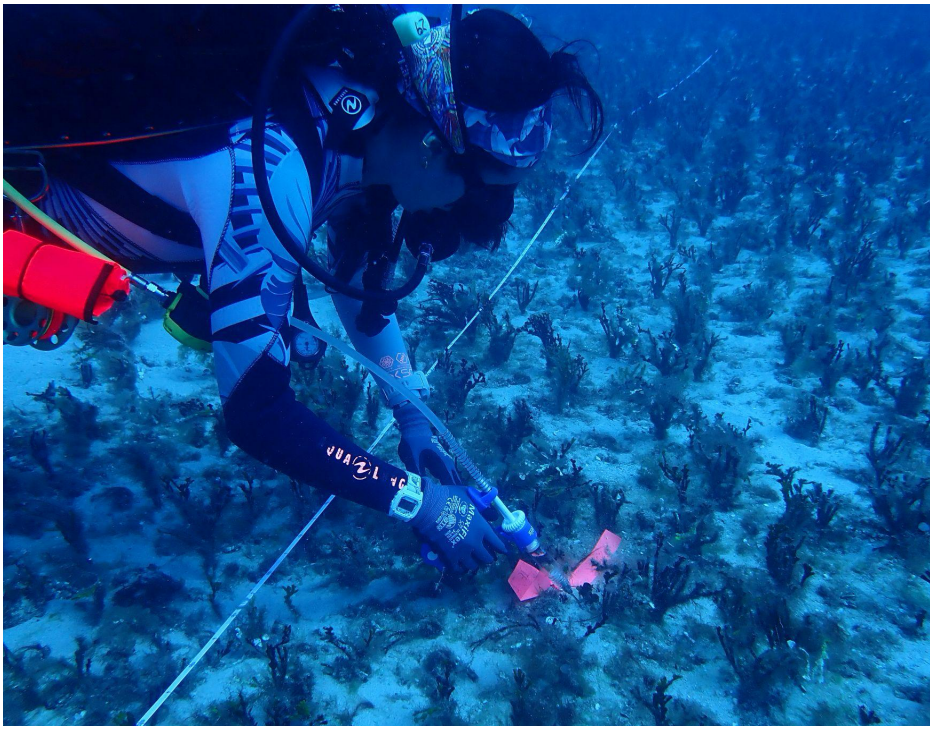

Figure 4. Diver Solimar Carrasquillo Ho injecting *Avrainvillea lacerata* with hydrogen peroxide treatment at 19m depth outside Ala Moana.

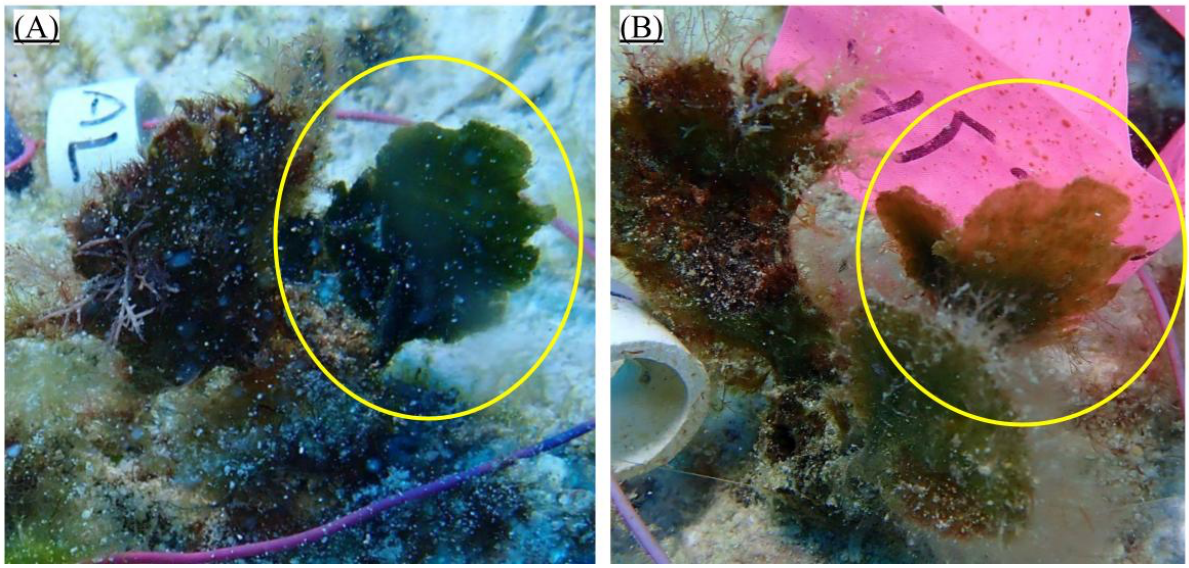

Figure 5. *Avrainvillea lacerata* pre-treatment (A) and one week post-treatment (B).

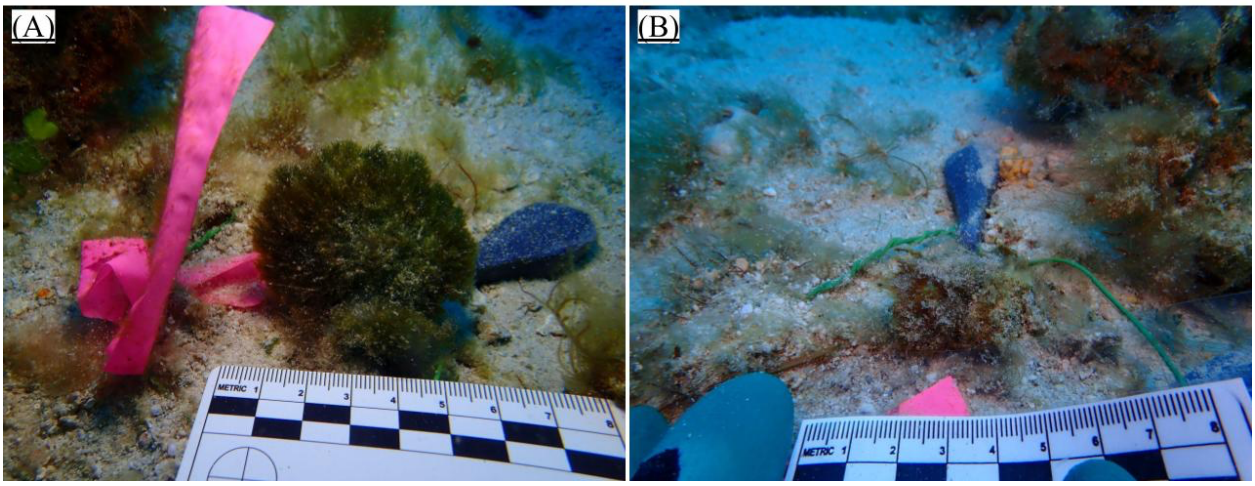

Figure 6. *Avrainvillea erecta* pre-treatment (A) and one week post-treatment (B).

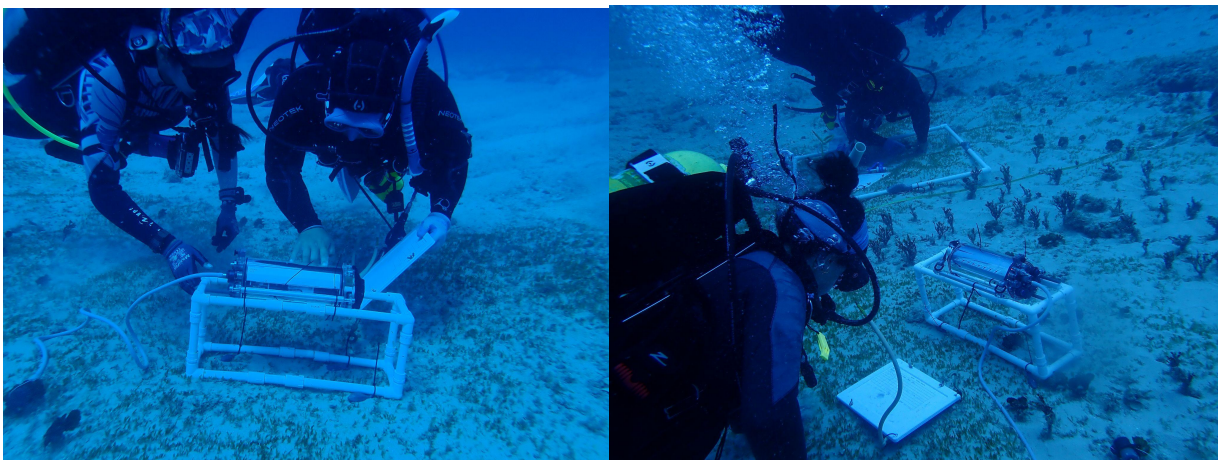

Figure 7. Diver Solimar Carrasquillo Ho guiding Richard Chen (AIS, DAR) while taking PAM measurements on *Avrainvillea erecta* (left). Divers K. Gonzales and R. Chen navigating dive operations on targeted species alongside native species of *Halophila hawaiiana* and *Halimeda kanaloana* (right).

Figure 8. Solimar Carrasquillo demonstrating use of DIVING PAM to the Aquatic Invasive Species team at ʻAnuenue Fisheries Research Center, DAR.

Results

There's less than 1% reduction of photosynthetic parameters (Figure 9, 10) in *Avrainvillea lacerata*. The main impact of hydrogen peroxide treatment was seen through physical discoloration of blades (Figure 5) or through necrosis of blade entirely (Figure 6).

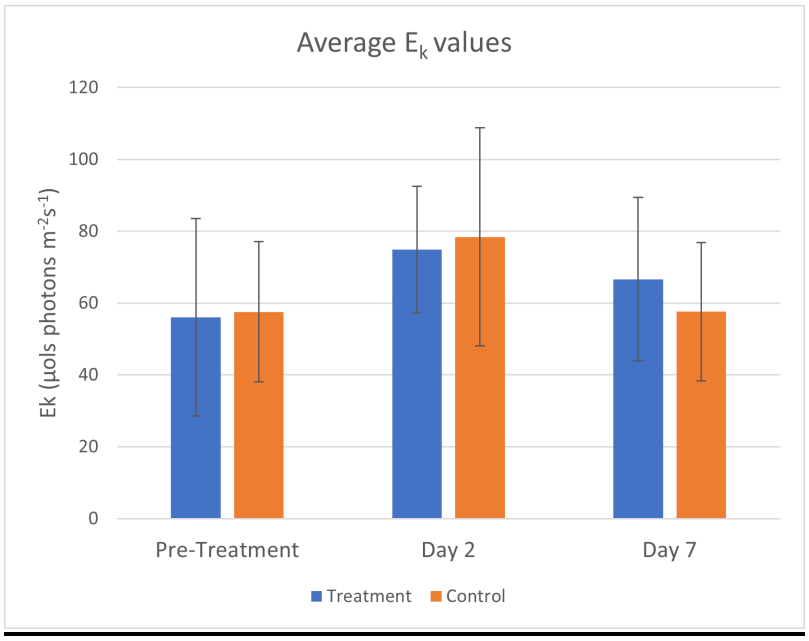

Figure 9. Averaged $E_k \pm$ SD values of *Avrainvillea lacerata* of negative control and treated plants with 30mL of 3% H_2O_2 concentration.

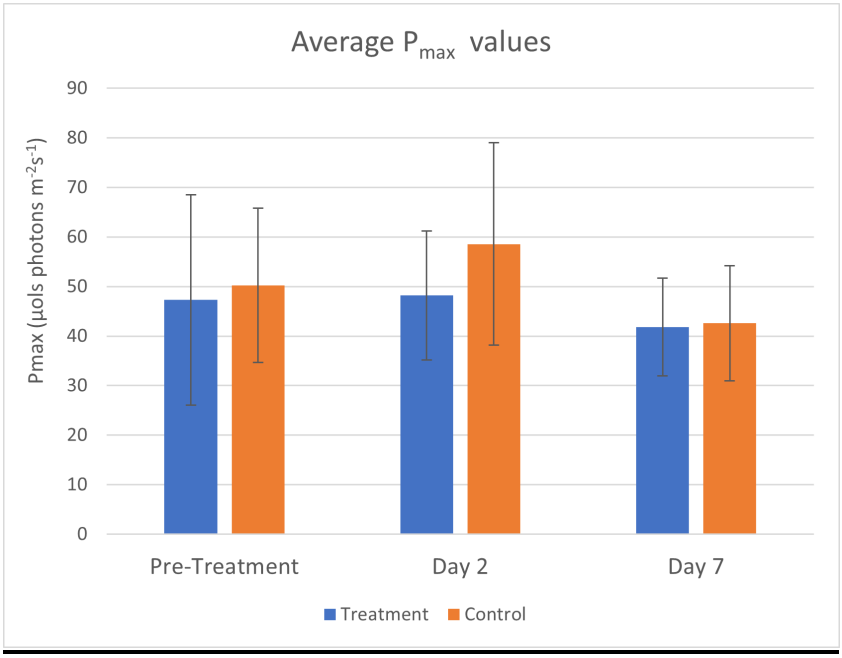

Figure 10. Average $P_{max} \pm$ SD values of *Avrainvillea lacerata* of negative control and treated plants with 30mL of 3% H_2O_2 concentration.

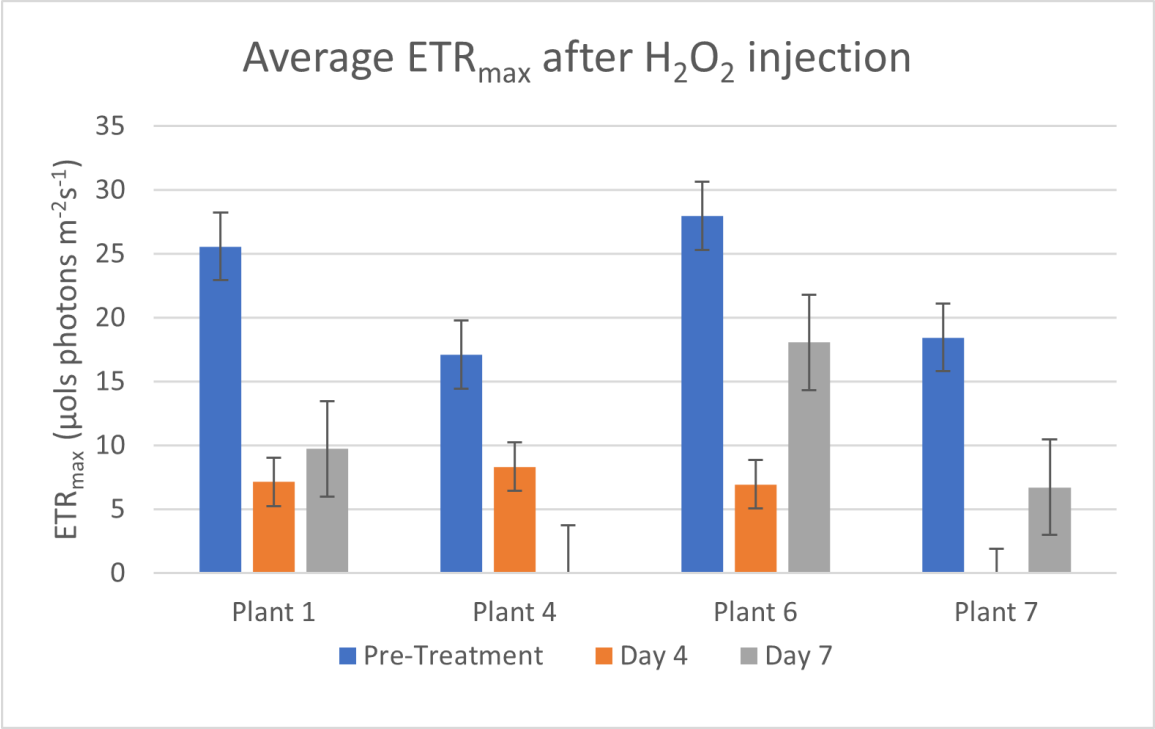

Figure 11. Historical data for average ETR_{max} (µmol photons $m^{-2}s^{-1}$) in *Avrainvillea erecta* post-treatment of 10 mL at 3% H_2O_2 across 7 days.

Discussion

Diving operations for this research project began in October 2023 as a result of distribution of funding from administration. This left approximately 6 months to carry out a field-extensive research project heavily reliant on scuba diving. Any research dives from this project was approved under the auspices of the University of Hawai‘i Dive and Safety Program (UHDSP). The fall/winter diving season on the south side of O‘ahu can come with high winds (>30 kts) and strong currents that make diving dangerous. Prior to dive operations of the day the conditions were assessed and postponed for one week until deemed safe for operations. This reduced the amount of diving days available within the short window of time we had to accomplish our research goals.

One of the major changes of objectives of this project was the outer island efforts. Traveling to west Maui was overshadowed by the Lahaina tragedy. This tragedy had lingering effects as the pollution to the water became a primary concern for divers because of runoff from chemicals into the coastal water from impacted areas. Our research group shifted its priorities to focus on fostering the relationships with state personnel (Division of Aquatic Resources), while coordinating training activities that would provide professional development for their teams. Further expansion of experimental sites to other islands will be addressed internally with DAR representatives.

The training activities involved presentations regarding on-going research within the research group, open discussion regarding algal related issues in marine ecosystems in Hawai‘i , and training on the use of DIVING PAM for the Aquatic Invasive Species (AIS) team at DAR. A field day was coordinated in collaboration with AIS to take two team members diving to practice the use of proposed methods of hydrogen peroxide treatment and DIVING PAM measurements (Figure 7). Funds that were allocated towards travel were diverted to more extensive diving on O‘ahu.

Photosynthetic measurements using the DIVING PAM on *Avrainvillea erecta* were not achievable as the blades would completely disintegrate and were unable to have accurate measurements (Figure 6). The holdfast was still present slightly exposed above the substrate, but it was not observed to regrow over a one month period (SCH per obs.). The growth rates of *A. erecta* is not well studied, but further work would be important to understand how this treatment would impact regrowth of the plant. Previous work with *A. erecta* shows a reduction in photosynthetic parameters 7 days post-treatment of 10mL of 3% H_2O_2 concentrations. The increase of volumes from 10mL to 30mL caused blades to disintegrate at a faster rate, which could indicate a more effective treatment method for this species.

Treatments of hydrogen peroxide in *Avrainvillea lacerata* caused significant discoloration and necrosis in some blades (Figure 7). Photosynthetic measurements showed less than 1% reduction 7 days post-treatment (Figure 9, 10). The low reduction in photosynthetic parameters indicate alternative factors are impacting the efficiency of this treatment and the need for further testing. The anatomy of the holdfast which allows it to trap sediment beneath the substrate could have made it difficult to target injections and rather place the treatment in sediment. Alternatively, diffusion of the treatment with ocean water upon injection could have occurred and diluted treatment and reduced impacts targeted plants. The issue of diffusion was observed in mesocosms in previous work, but the combination of higher volume and concentration of treatment proved to cause a significant reduction in photosynthetic parameters (Van De Verg and Smith, 2022).

The data presented in this report represent the data for two Master of Science graduate students in the Botany Graduate Program. These data are undergoing further detailed analysis to understand how this method impacts organisms. Future work should consider impacts on sediment and microbial communities along with further studies on reproductive life strategies. Continuation of these studies will help understand the ecology of this plant and its encroaching impacts on native species *Halophila hawaiian* and *Halimeda kanaloana*. Treatments will continue outside of Ala Moana and on the south shores of O‘ahu .

Deliverable 1: (120) mature *Avrainvillea lacerata* and (120) *Avrainvillea erecta* individuals removed,

-*A. lacerata* removal completed w/ Earth Day Beach Clean Up (approximately 30 lbs)

Deliverable 2: 0. 03 acres treated,

- 0.038 acres treated

Deliverable 3: 4 education events

- American Association of University Women: STEM Day with limu ID (Nov 2023)

- UHawai‘i at Mānoa Day, at the Legislature, Limu and Invasive Seaweed ID (Jan 2024)

- *Avrainvillea* cleanup w/ Trees to Seas and Restore with Resilience (March 2024)

- Ho‘ōla Hani‘o Community Day, Limu and Invasive seaweed ID (May 2024)

Deliverable 4: 2 of outreach materials produced

-*Avrainvillea* WANTED poster

-DIVING PAM information packet for DAR

Deliverable 5: 2 of training sessions (student helpers / community)

-Training: Diving PAM workshop w/ Division of Aquatic Resources at AFRC

-Training: Application of H_2O_2 injections in field w/ Division of Aquatic Resources