Final Report for the Hawai‘i Invasive Species Council FY2022

Part 1. Investigating management techniques for two invasive congeneric green algae, *Avrainvillea lacerata* and *A. erecta*

Project Start Date 10/1/2021- Project End Date 5/31/2023.

Prepared by

Celia Smith - celia@hawaii.edu

Solimar Carrasquillo Ho – solimarc@hawaii.edu

Liv Wheeler - liv4@hawaii.edu

Scott Van De Verg – scottvdv@hawaii.edu

University of Hawai‘i at Mānoa

Problem statement:

As recently as 2011, Hawaii'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 a tarp. 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;* LWheeler per obsv) to 120 ft (*A. lacerata;* Foster et al. 2019). Thus, this continued project aimed to understand how to best manage these species at different depths, where they are both found to be abundant. 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. From previous work conducted under the HISC grant by two of the grant's researchers (Scott Van De Verg and Liv Wheeler), hydrogen peroxide is a new control agent for this genus of algae. This investigation was to test feasibility of a larger-scale management strategy at different depths of the algae's range.

Approach and methods:

To test hydrogen peroxide on *Avrainvillea erecta,* scientific diving had to be used to locate and apply treatment *in situ* at 23m depth. Scientific Divers laid a 30m transect at 23m within the co-occurring meadow. Four quadrats were placed randomly along the transect and 0.75m aluminum stakes were hammered into the deep sand to mark each quadrat.

Avrainvillea lacerata plants were identified *in situ* at depths of less than 1m depth, which were accessible from shore. A 30m transect was laid parallel to shore at Kualoa Beach where a 0.5m² quadrat was placed along the 2m mark of each transect to identify plant communities of at least 20% bottom coverage. Each quadrat was separated into four subsections by polyethylene fishing line, and two plants were identified within different subsections for treatments.

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.

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: V1C1=V2C, 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 with a 16 gage needle. This system allowed for direct application of 10mL 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: For *A. erecta* plants: Two sized quadrats to closely monitor each plant's response after injection. The 1m² quadrat was used to define the plot, while a smaller 0.25 m² strung quadrat was used inside the 1m² quadrat to systematically organize the plants into 4 quadrants. The 0.25m² quadrat was strung with evenly spaced strings at 4 cm increments crisscrossing to form 16 squares. The 0.25m² quadrat was placed in the lower left (LL) corner of the 1m² quadrat from the perspective of the transect. Within each 5cm square of the 4 quadrants, the *A. erecta* were counted, measured in height, and injected with 10mL of 3% H_2O_2. The injection targeted the stipe close to where it joined the photosynthetic part of the blade. From there the 0.252 m² quadrat was flipped clockwise to the upper left (UL) of the 1m² quadrat, then to the upper right (UR) and finally to the lower right (LR). Follow up dives occurred weekly to record decline of the plants. A photograph of the main quadrat with the 0.25m² quadrat was taken from above to capture each time the 0.25m² quadrat was flipped. A side view was also taken before each flip of the 0.25m² quadrat to better see individual plants. These photos were repeated on every follow up dive.

This protocol was part of a larger study that included physical removal as a strategy to control *A. erecta* within established meadows and in areas of new invasion. The physical removal mimicked natural events that might remove increasing percentages of the plant until the whole thalli (holdfast, stipe and blade) was pulled out. We used the hydrogen peroxide experiment as a follow up to the physical removal experiment to evaluate efficacy for managers in the field. This study is not complete but we do have some observations that will help managers plan their control efforts.

For *A. lacerata* plants: Two plants within the 0.5m2 quadrat, but found in separate subsections were selected for a treatment and control. These plants were observed for initial ETRm and Ek values using the DIVING PAM prior to any treatments. Field workers returned for additional PAM measurements post-treatment at 1 and 7 alongside photographs. These photos were compared against pre-treatment photographs to show any discoloration or necrosis of plant tissue. Because of the tidal fluctuations and difficulties with marking systems, a majority of the experiments at Kualoa had resulted in lost marks or field workers unable to refind the plants. A total of five marking systems were tested throughout this time to maximize the amount of plants the field team could re-find. With the low re-find rate of marked plants, the field team was unable to monitor plants at Kualoa Beach Park for longer than a two-week period.

Site descriptions: *Avrainvillea erecta* is a psammophytic (sand dwelling) species that occurs at depths as shallow as 15m. The site chosen for this experiment was at 23m. The study area was located off of Kewalo Basin channel, at the end of the pipe that stretches from shore out to sea and ends at 20m (Figure 1). Just beyond this exists an extensive meadow of *A. erecta* co-occurring with native seagrass and *Halimeda kanaloana*. Access to this location was via small boat, utilizing the existing mooring balls that the recreational dive companies tie up to. This site also offers adjacent coral reefs with populations of various reef fish and invertebrates. Observations of fish and invertebrates in the meadow were taken opportunistically to gain insight on the benefits the meadow offers the reef fauna.

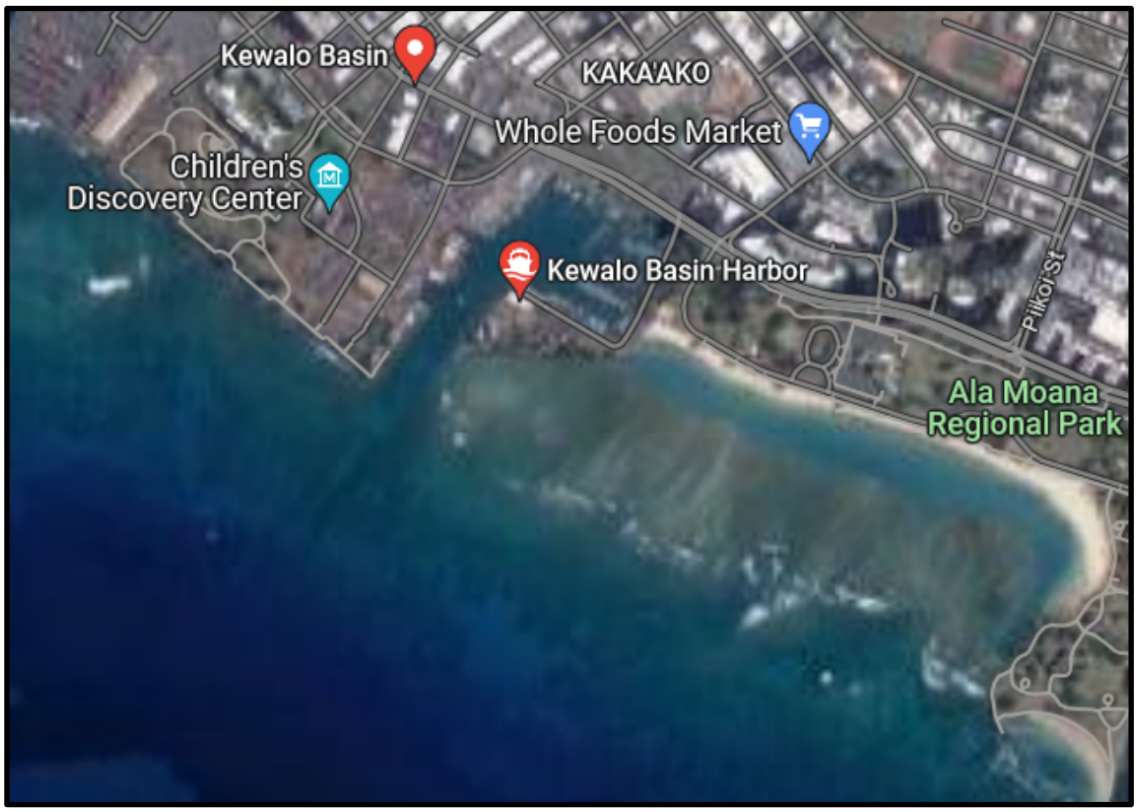

Figure 1. Aerial map of Kewalo basin harbor and fringing reef with Kaka‘ako park to the left and Ala Moana Beach Park to the right. Site was established on the Kaka‘ako side of the channel (site not marked).

Avrainvillea lacerata was studied at various distances from Kualoa Beach Park (Figure 2). Kualoa is a site of considerable cultural significance to Hawaii and is heavily influenced by the presence of the invasive alga. The site is used by fishers and for recreational purposes and is an important landmark for traditional voyaging practices. The reef-flat region extends approximately 1 mile from the beach and is likely habitat for continual growth of this invasive species moving forward, including a potential source for coastal regions northward. Although the reef-flat is considerably shallow, the extent of wave energy and tidal fluctuations make working in this site difficult to mark and re-visit plants for long-term monitoring of treatments. All *A. lacerata* communities were accessible from shore and were located in depths of less than 1m that were completely submerged in water during extreme low-tides (-1.0 feet).

Figure 2. Aerial photograph of Kualoa Beach Park. Yellow lines indicate approximately where 30 meter transects were placed for injections.

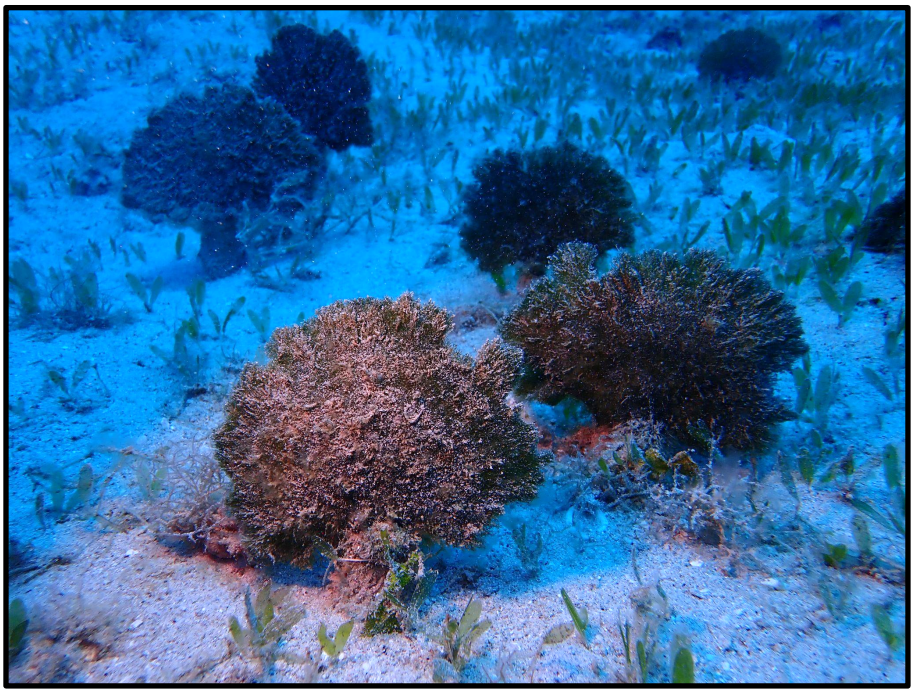

Figure 3. *Avrainvillea erecta* has invaded and now co-occurs in meadows of *Halophila hawaiiana* (native seagrass).

Figure 4. Divers Gavin Lewis and Solimar Carrasquillo Ho extracting *Avrainvillea erecta* samples at 18m depth off Kewalo Pipe.

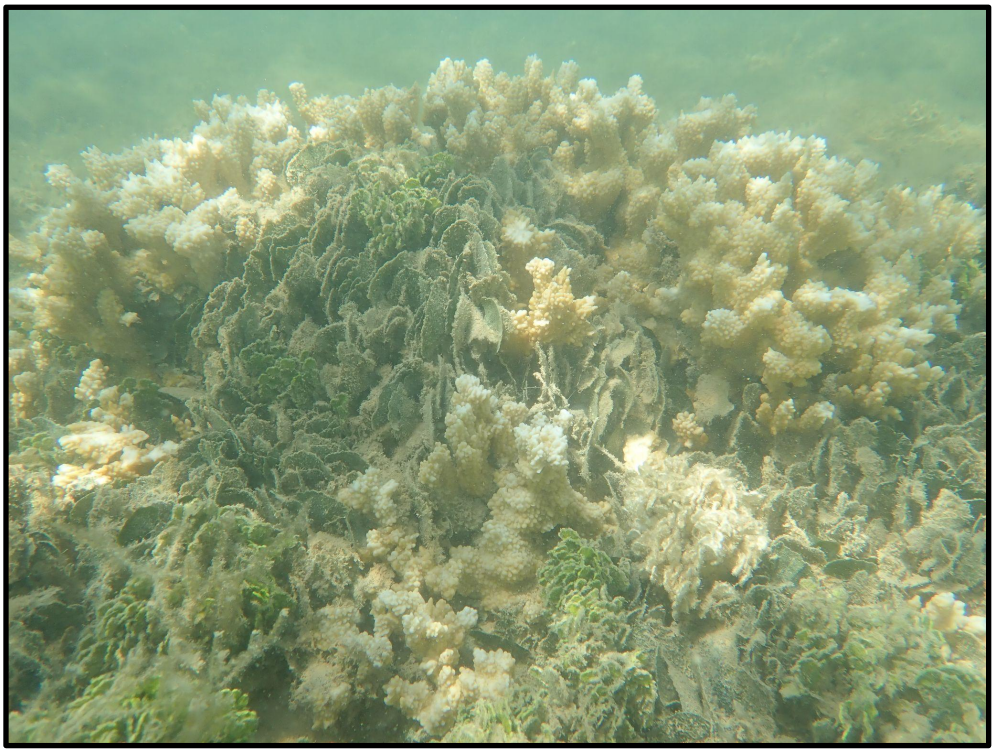

Figure 5. *Avrainvillea lacerata* growing on coral in shallow water..

Collections of *A. erecta:*

10 to 15 individual *Avrainvillea erecta* samples were collected from an algae meadow at ~20 m located near the Ala Wai channel at a marked GPS location (Figure 1). The site was made accessible by a University of Hawai‘i Boston Whaler and a team of scientific divers using nitrox scuba. Photos and maximum electron transport rate (ETRm) measurements of the individual plants were taken at depth using a GoPro Hero3+ (GoPro Inc, San Mateo, California, USA) and a blue actinic irradiance (Figure 3). Diving Pulse Amplitude Modulated Fluorometer (D-PAM; Heinz Walz GmbH, Pfullingen, Germany). After ETRm was measured, the plants were dug out of the sandy substrate using a blue actinic (DPAM #2) Diving Pulse Amplitude Modulated Fluorometer (D-PAM; Heinz Walz GmbH, Pfullingen, Germany). After ETRm was measured, the plants were dug out of the sand with a PVC corer (Figure 4). The photosynthetic blade as well as the holdfast of the plants were placed into plastic bags with some of the attached substrate. The samples were kept in a bucket and were transferred immediately after arriving back into the Ala Wai Harbor to Ānuenue Fisheries Research Center run by Division of Aquatic Resources (DAR). Upon arrival the samples were placed into designated aquaria and allowed to acclimate for five days.

Mesocosm studies at AFRC:

The Ānuenue Fisheries Research Center is run by the Division of Aquatic Resources (DAR) and is located on Sand Island, O‘ahu. A partnership between Dr. Celia Smith, Professor at University of Hawaii, and DAR has allowed students to access and run experiments using mesocosms and smaller aquaria the AFRC open flow seawater system. This system works by extracting water from a depth of 8 m out of the Honolulu Harbor. PVC pipes bring it to the facility where it runs through a sand filter. Further filtration is ensured by a three-stage cartridge filter system. Filtered water then runs through five different headers that lead to 2-inch PVC piping perforated with 1/8 inch black water delivery tubes. The tubes delivered seawater into two mesocosm baths. Five tubes led into each bath and were attached into five individual one gallon aquaria for a total of ten individual aquaria. An individual *Avrainvillea erecta* sample was housed per aquarium.Standpipes were placed over the drains to create a water bath with the overflow from the aquaria.

Experimentation with *A. erecta:*

After five days of acclimation, photos were taken using a GoPro Hero3+ and the maximum electron transport rate (ETRm) was measured with a red actinic Diving Pulse Amplitude Modulated Fluorometer (D-PAM). A catheter bladder attached to a ¼ inch tubing that led to a horse injector with a 16 gauge needle was used for treatment injections. The first three treatments were done with seawater. The catheter bladder was filled with filtered seawater and 5 ml, 10 ml, and 20 ml injections were made into three different plants. The injection site was into the thick stipe below the photosynthetic blade. 20 ml treatment consisted of two 10 ml injections on opposing sides of the blade. The next series of injections were carried out with 3% Hydrogen peroxide (H_2O_2) that was prepared using a dilution of lab reagent grade 30% H_2O_2 and deionized water ($V_1P_1=V_2P_2$, V=volume and P=percent of hydrogen peroxide). 500 ml of the H_2O_2 solution was poured into the catheter bladder and injections of 5 ml, 10 ml, 20 ml were made into three individual plants. The remaining plants were used as controls: Plants were either jabbed by the needle but no liquid was injected to account for the effects of puncturing, or were left alone as a negative control to observe potential effects of the mesocosm placement. Photos were taken and ETRm was measured before and ~45 min after treatment for all 10 plants to measure immediate effects of H_2O_2 treatment. To measure long term effects the plants were observed for ten days,

photos and ETRm were taken four times within this period. Plants were discarded at the end of experimentation. This entire process was repeated three times for three different *A. erecta* collections.

Statistical Analysis for mesocosm studies:

Statistical analyses were run on Excel and R version 4.1.1 (R Core Team, 2021). Three one-way ANOVA tests followed by coinciding post-hoc Tukey HSD tests were run. The first measured statistical significance between the rETRm of all treatments 45 minutes post-injection to determine the short term effects of the H_2O_2 treatments. The second was run on the rETRm of all treatments on the final day, day ten, of the experiment to determine the long term effects. The final ANOVA and post-hoc Tukey HSD test was done between the rETRm of all plants between experimental runs on filtered harbor water and runs on well water to account for any possible differences this may have caused. A t-test was conducted for each run to compare the mean rETRm of *A. erecta* specimens at depth (~20 m) to the rETRm of specimens after five days of acclimation at Ānuenue Fisheries Research Center.

Results

For *Avrainvillea lacerata* (Figures 5, 6), the average of ETR_{max} and E_k values pretreatments show an average decrease one day after treatments of the 3% H_2O_2 concentration (Figure 6). There's a 17% reduction in E_k values and 3% reduction in ETR_{max} values after treatment. There is a high variance in the algal communities treatments, which could be expected given the community averages have a high variance in both ETR_{max} and E_k values (Figures 6-8).

Treatments *in situ* for *A. lacerata* involved 10mL which showed a reduction, but our research group questioned the volume of treatments and the impacts on photosynthetic reduction. *A. erecta* plants were collected and transplanted into aquaria at Anuenue Fisheries Research Center to test volumes of 5mL, 10mL, 20mL for 3% H_2O_2 concentration and saltwater. There was nearly a 45% reduction in ETR_{max} values after 5mL of 3% H_2O_2 concentration and a 80-90% reduction in ETR_{max} with 10 to 20mL.

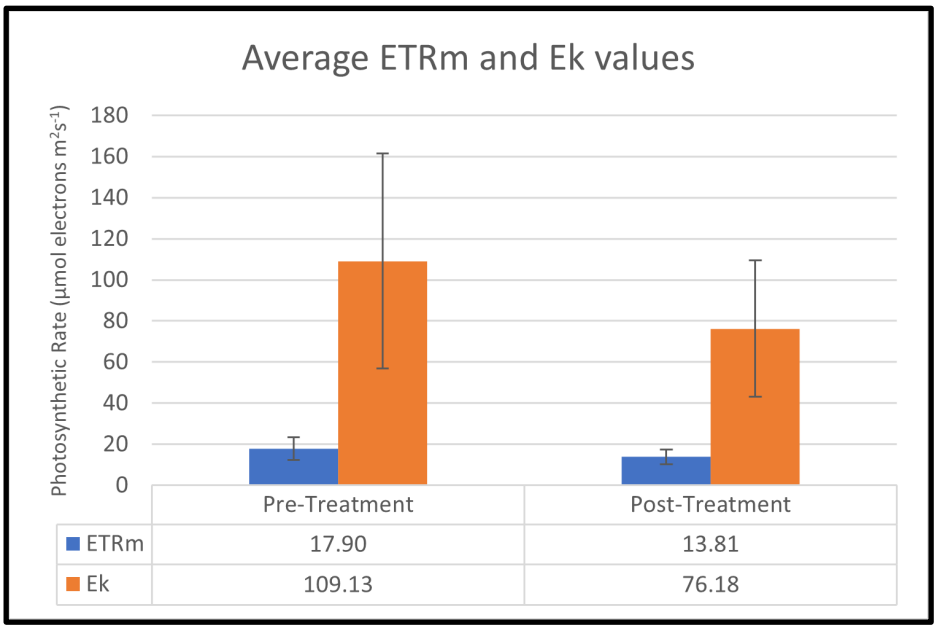

Figure 6. Effects of injecting 10 mL of a 3% H_2O_2 solution on the maximum relative electron transport rate in µmol electrons m^{-2} s^{-1} and Ek values in µmol photons m^{-2} s^{-1} for *Avrainvillea lacerata in situ* at Kualoa Beach Park, O'ahu, Hawai'i (n=19 per treatment). Values represent means and standard errors.

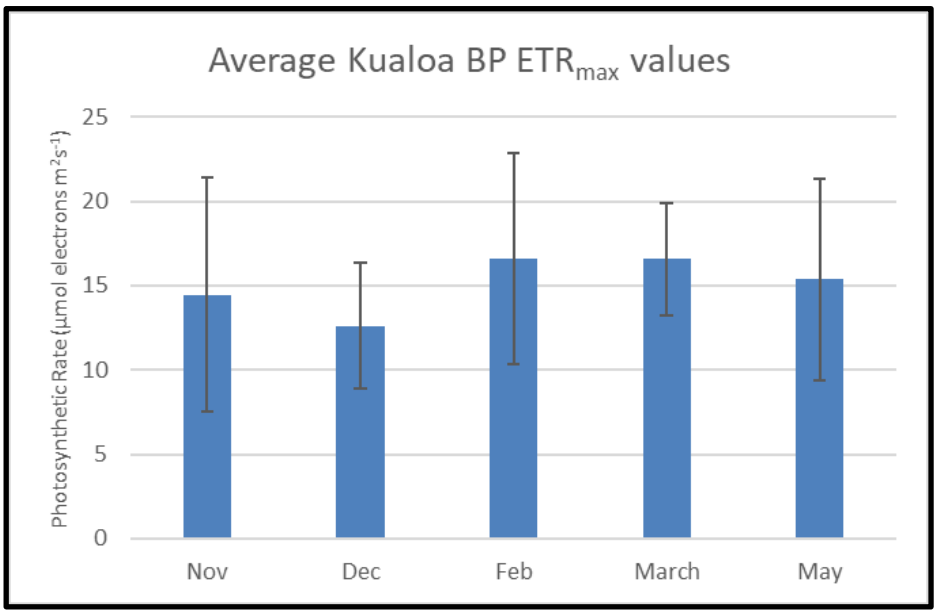

Figure 7. Mean values of ETRmax ± SD, in *Avrainvillea lacerata* communities in situ at Kualoa Beach Park, O'ahu, Hawai'i (n= 90, 22, 20, 18, and 18 respectively).

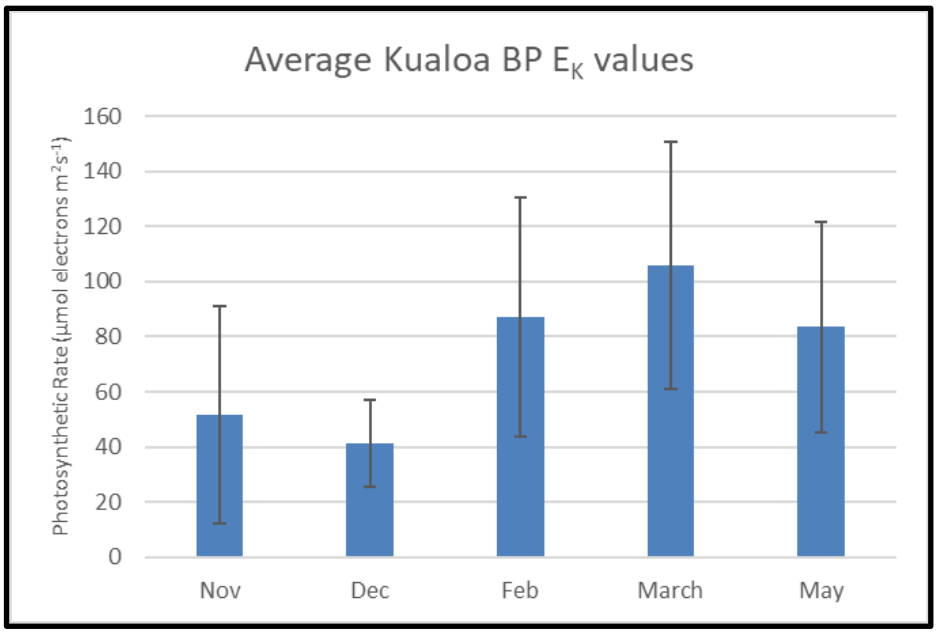

Figure 8. Average values of Ek + SD, for *Avrainvillea lacerata* in situ at Kualoa Beach Park, O‘ahu, Hawai‘i (n= 90, 22, 20, 18, and 18 respectively).

For *Avrainvillea erecta*, ETR_{max} values averaged at 18.5 µmol electrons m^{-2} s^{-1} with an E_k average value of 50.3 µmol photons m^{-2} s^{-1} , n= 65. These measurements were taken *in situ* between 20 and 25 meters over 2022 and 2023 spanning various seasons and weather conditions. This was used as a baseline for the *A. erecta* collected for the AFRC mesocosm experiment (Figure 9).

For *A. erecta,* average ETRm for 5 ml, 10 ml, and 20 ml H_2O_2 injections after 45 minutes were respectively 8.96 +/- 0.999 SE, 1.91 +/- 1.91 SE, 2.44 +/- 2.44 SE, n = 3 (Figure 9). The rETRm for control treatments (5 ml seawater (SW), 10 ml SW, 20 ml SW, puncture wound, negative control) 45 minutes post-injection were 21.4 +/- 0.842 SE, 19.7 +/- 1.82 SE, 18.9 +/- 1.37 SE, 18.3 +/- 3.36 SE, 20.02 +/- 2.57 SE, n = 3 (Figure 9).

A one-way ANOVA found statistically significant differences in rETRm between at least two treatments ($F(7, 16) = [15.6]$, $p = 4.56 \times 10^{-6}$). A Tukey HSD test found no statistical significance between the rETRm between all three H_2O_2 treatments (p = 0.304, 0.392, 0.899). The same Tukey HSD test found no significant differences between the mean ETRm of all controls (p = 0.899, 0.899, 0.832, 0.899, 0.899, 0.899, 0.899, 0.899, 0.899, 0.899). Statistical significance is reported between 10 ml and 20 ml treatments when compared to all of the controls (p = 0.001, 0.001, 0.001, 0.001, 0.001, 0.001, 0.001, 0.001, 0.001, 0.001). The mean ETRm of the 5 ml treatment was statistically significant from every control except the puncture wound treatment (p = 0.007, 0.013, 0.004, 0.116, 0.02).

Initial analysis suggests that densities of *A. erecta* decline with length of time since treatment with hydrogen peroxide, *in situ* (Figure 10). Densities of plants following hydrogen peroxide treatments in situ trended downward with time (Figure 10), in field settings on O‘ahu south shore. Data analysis continues and more results with statistical analyses will be forthcoming.

From these mesocosm studies, field observations and the previous work done on physically removing parts of *A. erecta*, hydrogen peroxide treatment at 10 and 20 ml are an effective method for control treatment.While data analysis is still underway, *A. erecta* is able to regrow from most disturbances, showing remarkable resilience. Small fragments appear to be able to regrow after holdfast removal.

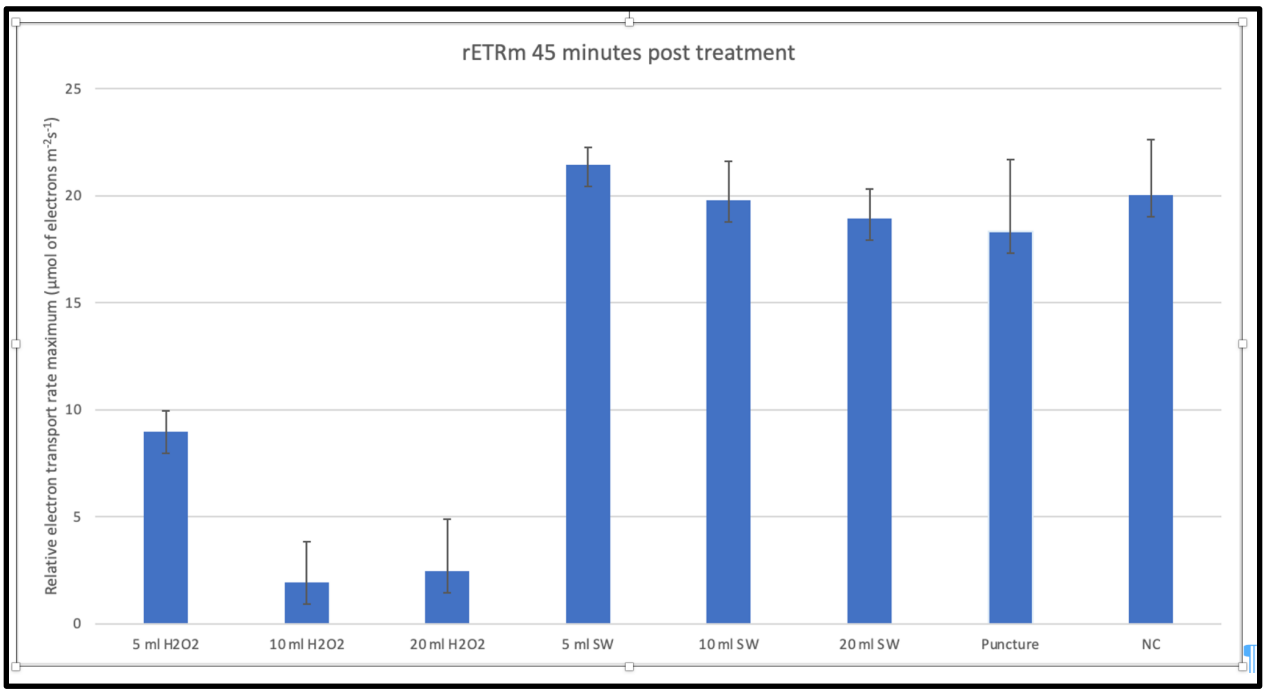

Figure 9. The short term effects of H_2O_2 treatments on the relative electron transport rate maximum in *Avrainvillea erecta* held ex situ at Ānuenue Fisheries Research Center. Treatments listed from left to right: 5 ml H_2O_2, 10 ml H_2O_2, 20 ml H_2O_2, 5 ml seawater (SW), 10 ml SW, 20 ml SW, puncture, negative control (NC), n = 3. Values represent means and standard errors.

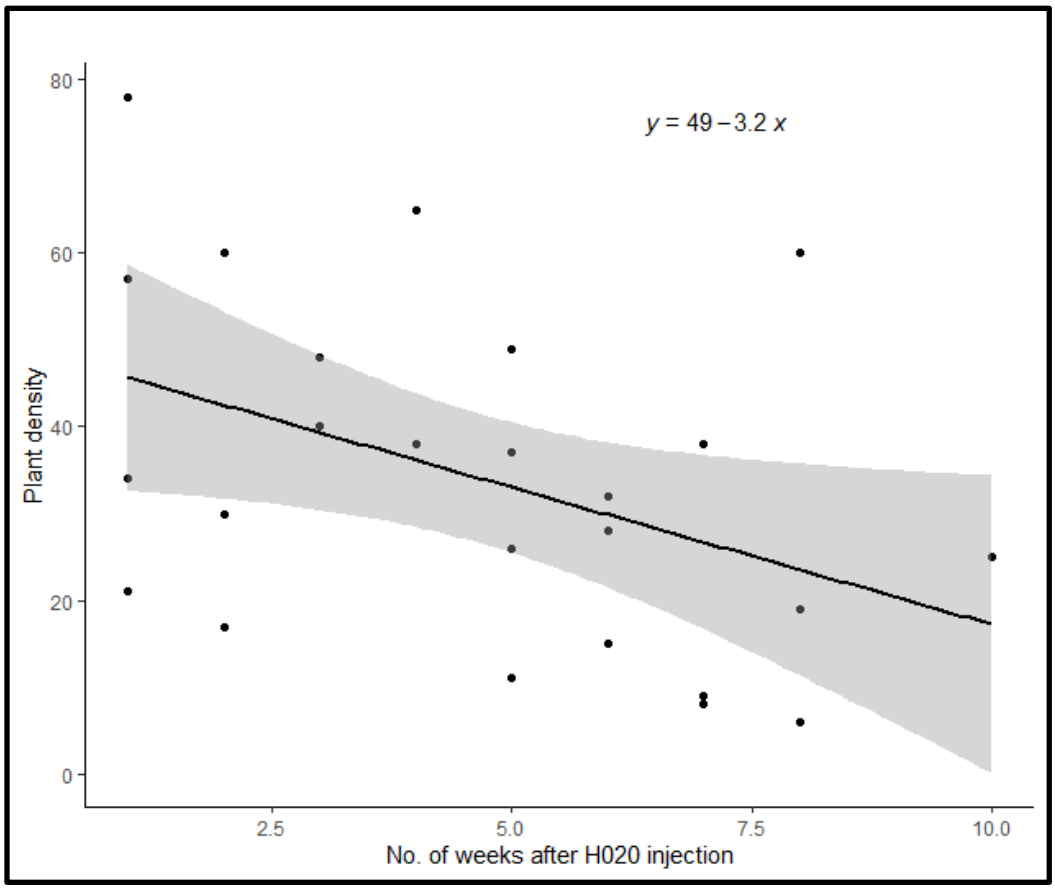

Figure 10. Changes in densities of *Avrainvillea erecta* plants, in response to H_2O_2 injection at 23 meters over 10 weeks of observation. The treatment injected 10 ml of 3% H_2O_2.

Future Work

We will continue to research the impacts of hydrogen peroxide treatments using higher volumes as shown in the AFRC experiments as this technique can serve managers to control populations that reach higher densities, are invading new areas and encroach on critical habitat for *Halophila hawaiiana*. Treatments will continue at Kualoa Beach Park and on the south shores of Oahu to observe impacts of treatments and across further seasons. Additionally, we will expand study sites for these treatments to locations on Maui and Hawaii islands where *A. erecta* populations are present and describe those habitats using current HISC funding awarded from 2023 cycle.

Deliverable 1: 90 (45) mature *Avrainvillea lacerata*

>130 *Avrainvillea erecta* individuals treated with hydrogen peroxide

- Approximately 30 individuals removed for *ex situ* treatment at AFRC
- 45 *Avrainvillea lacerata* plants treated with hydrogen peroxide

Deliverable 2: 0.02 acres treated

- *Avrainvillea erecta* transect = 30m
    - 4 1x1 m^2 quadrats established
    - total area covered by *A. erecta* 0.0034
- *Avrainvillea lacerata* transects = 30m
    - 60 0.25m^2 quadrats established
    - total area covered by *A. lacerata* 0.0037
- Total acres treated: **0.0071**

Deliverable 3: 2 education events

- For the Love of Limu
- Earth Day Cleanup 4/21/2022 at Makai Pier

Deliverable 4: 2 outreach materials produced

- Diving PAM field instructions
- Wanted poster (post full image into document)

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

- Diving PAM workshop w/ Division of Aquatic Resources
- *Avrainvillea* cleanup w/ Trees to Seas and Restore with Resilience

Part II. Building a risk assessment framework for invasive algae

Project Start Date 10/1/2021- Project End Date 5/31/2023,

Prepared by

Celia Smith - celia@hawaii.edu

Angela Richards Dona - angelard@hawaii.edu

University of Hawai‘i at Mānoa

with collaborators: Leah Bremer, Jade Delevaux, Brytne Okuhata

Problem statement:

As recently as 2011, Hawaii'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 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.

Formally, "Risk Assessments" include both the risk of introduction, risk of establishment, risk of uncontrolled spread, and then the ecological and/or economic impact it would have once established in an area. Earlier species distribution models did not take into account a number of fundamental parameters including underlying growth responses.

Given that more than five invasive species are readily collected via asexual fragments, we can ask - under what conditions are these invasive species most likely to thrive and therefore impact/outcompete local species? We proposed to measure growth and competitive dynamics,

using the marine greenhouse at St John Plant Science Building, UHMānoa to gather data towards a robust assessment of competition dynamics under at least one climate change scenarios.

Our choice of invasive / native species pair was initially focused on two species of *Gracilaria*, because of the availablity of co-occurring native *limu manauea* (*G. coronopifolia)*, and two invasive congeners, *G. salicornia* and *G. tikvahiae*. Initial field work however showed that the availability of wild collected limu manauea limits that pairwise comparison. We proposed an alternate species pair at that time and settled on the invasive species of the red alga *Hypnea musciformis* and a native green alga *Ulva lactuca* might be needed, if we are unable to collect sufficient biomass of *Gracilaria* native species. This was the case.

The comparison between *Hypnea* and *Ulva* pose interesting similarities and contrasts. These two species are both bloom species in coastal regions on Kihei and Lahaina Maui (Dailer et al 2010) despite their distinct evolutionary (Rhodophyta vs Chlorophyta) and morphological (cylindrical form vs blade form) differences. On Maui, the bloom sites have a mix of *Hypnea* and *Ulva*, two species that co-occur, in these nutrient rich regions. Yet *Ulva lactuca* is also a well known and highly prized native species, limu palahalaha, in groundwater dependent regions of the Kona coast, where *Hypnea musciformis* does not apparently occur.

This complex of similarities and complexities suggested strong physiological basis for competition mediated by growth and photosynthesis as initially characterized by Dailer et al (2012).

Overall goal:

The objective of this research was to gather physiological and growth data for contrasting native and non-native algae, to better understand how climate change might impact distributions of these two species. From previous work conducted under the other funding, we had initial physiological characterization of these two taxa (Dailer et al 2012).

Approach and methods:

Kona SGD Coastline Habitat

Ulva commonly co-occurs in persistent coastal blooms with the highly branched, cylindrical rhodophyte *Hypnea,* which is now found at numerous sites around the Main Hawaiian Islands, especially in Maui coastal waters (Dailer et al 2010; 2012). In Hawaiian ecosystems, both species are readily grazed—exhibiting similar top-down vulnerabilities (Conklin pers comm)—and both have similar uptake rates of limiting macronutrients (Dailer et al 2010). Strikingly, *Hypnea* is not found in the intertidal region of the Kona coast (Smith et al 2002), despite numerous vectors for delivery. We calibrated our experimental treatment parameters to the intertidal SGD conditions at Kona, Hawai‘i to test the photophysiological responses of these two species to a range of salinity/nutrient combinations following a gradient from full oceanic to groundwater seep locations.

Algal Manipulative Experiments

Eight identical experimental runs were conducted from in the Fall of 2021 to arrive at 16 total replicates per treatment and temperature of two co-occurring algal species. Each run began with

the collection of six individual plants of *Hypnea* and *Ulva* in the intertidal zone during a negative low tide at Wāwāmalu Beach and Ka‘ala Wai, O‘ahu. Plants were transported in seawater to the Marine Macrophyte Lab greenhouse at UH Mānoa to acclimate and consume tissue nutrients for eight days before beginning the experiment. Acclimating plants were kept in approximately 3 L of unfiltered seawater in covered and aerated 10 L aquaria in a partially shaded area of the lab outdoor terrace. Tanks were divided in half with white plastic egg crate to keep plants—one of each species— separate, and seawater was not changed during the drawdown phase. Plant placement within the tanks was rotated every two days to encourage homogeneous light acclimation by both species.

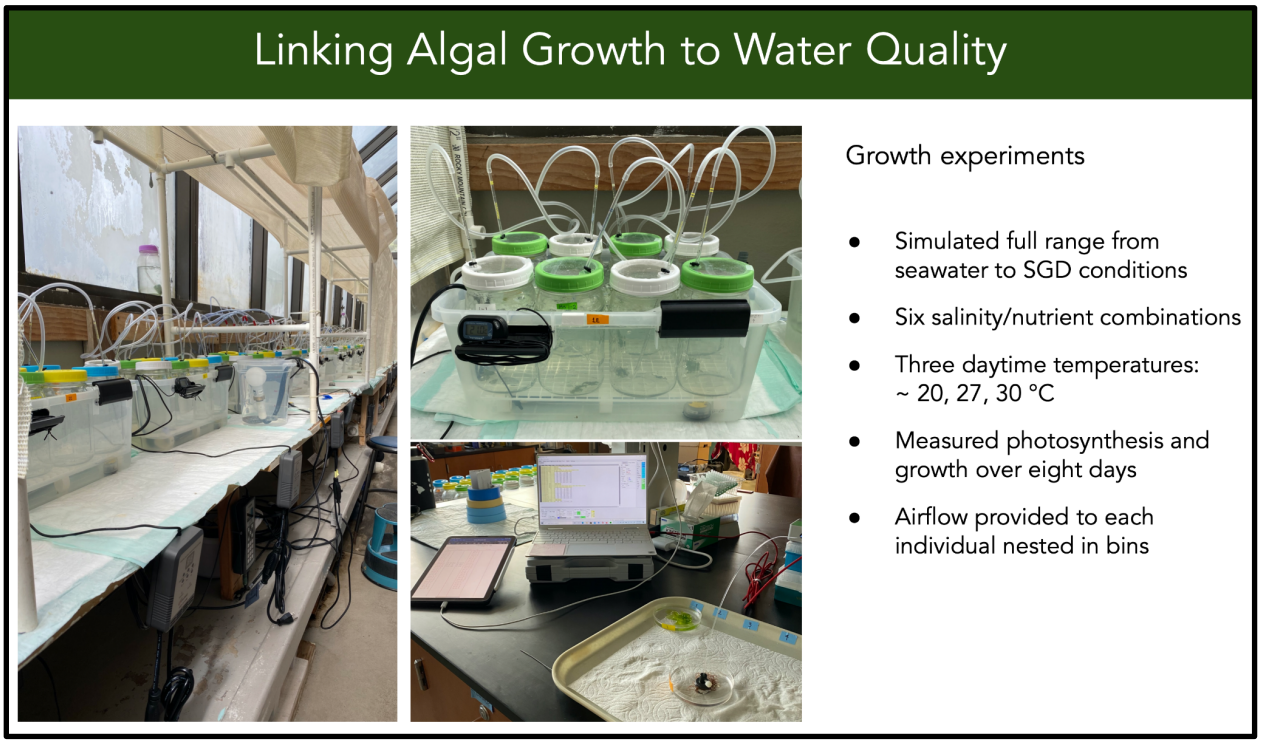

Figure 11. The set up on our marine lanai for growth and photosynthesis experiments with *Ulva lactuca* and *Hypnea musciformis.* Photosynthesis measurements are made in the adjacent laboratory in the St John Plant Science Building, UHM.

Plants were sectioned into four replicate pieces - each 0.28–0.3 g in wet weight—and returned to the tanks with the extra plant biomass removed. The experimental run commenced the following morning at 08:00 with photographs, rapid light curves (RLCs), and wet weighing for each individual (24 per species). RLCs were run on randomly chosen replicates with a Junior PAM-III using WinControl version 3.29 software (Walz, GmbH, Germany). Optimal settings were pre-determined for concurrent use with both species: Actinic light length was 0:30, intensity values (E_{par}) were set at 0, 65, 90, 125, 190, 285, 420, 625, and 820, and the measuring light intensity was set to 8. F_t was offset to fluctuate around zero in seawater before beginning RLCs and probe location was chosen when the F_t value was in the normal range for each species and/or was in a consistent range at a minimum of three locations on the replicate.

Immediately after each RLC, replicates were carefully dried with paper towel three times and weighed with a Sartorius TE214S digital analytical balance (precision to 0.0001g; Sartorius AG, Göttingen, Germany) then placed in 700 mL of treatment seawater in an individual 0.946 L (1 qt) glass jar per plant after Dailer et al. (2012). Seawater was pre-filtered with a 0.22 µm Millepore Stericup® filtration system (Millepore Sigma, Darmstadt, Germany), diluted with deionized water to reach the treatment salinity dilutions, and stored in darkened 20 L carboys. After all replicates were placed in clear-lidded jars, each received the appropriate addition of nitrate and phosphate and were fitted with air tubes that slightly agitated and aerated the water. Eight jars were nested in clear, plastic, water-filled bins, and were raised on white plastic egg crate above a 500-watt titanium aquarium heater (Finnex TH-05005; Illinois, USA). Three daytime temperatures (~20, 27, and 30 °C) were maintained within the bins from 06:00 to 18:30 every day during the experiment. Evening and nighttime temperatures dropped to ambient (~17–20 °C) in the air-conditioned greenhouse. Bins were aligned under a PVC pipe structure covered in shade cloth that reduced ambient light by ~50% with minimal obstruction.

Data Management and Statistical Analysis

Photosynthesis – P_{max} and E_k

Photosynthesis data from WinControl was first cleaned of extraneous information and formatted in Python 3 script using PyCharm (v 2021.2.4 Community Edition). The output was imported into R Statistical Software (v2021.09.0 R Core Team, 2012) and used the fitWebb model (Webb et al. 1974[49]) in the Phytotools package (Silsbe and Kromkamp 2012[50]) to calculate alpha (α) and E_k. This irradiance-normalized PE model was used to avoid the inherent irradiance dependency of the quantum yield measurements (Silsbe and Kromkamp 2012[50]), which are commonly used to calculate ETR. We calculated photosynthesis max (P_{max}) in the simple equation $P_{max} = \alpha * E_k$ from the model output (Silsbe and Kromkamp 2012[50]). We also calculated relative ETR_{max} (absorption factors for each species were not determined), choosing the highest value during the RLC that satisfied the condition $\Phi_{PSII}>0.1$ (PSII = photosystem II), following Beer & Axelsson (2004) protocol for reliable evolved O^2 to ETR ratios. We compared statistical model fit between P_{max} and $rETR_{max}$ and found P_{max} coefficients of determination were higher for *Hypnea* whereas they were the same for *Ulva*. Thus, we used P_{max} for analysis because it does not violate statistical assumptions of independence and it was a better fit for our statistical models. Day 9 values for both P_{max} and E_k were used for analyses (Figures 12,13).

Relationships between treatment and temperature (fixed effects) and photosynthesis parameters (P_{max}, E_k) and growth were analyzed using linear mixed-effects model (LMM) packages lme4 and lmerTest in R (v1.1-31, Bates et al. 2015). Fixed effects included treatment and temperature for all analyses and for both species. Random effects included: (a) plant ID (to account for natural plant variability), (b) run (potential irradiance changes over time), (c) RLC order (replicate measurement order), and (d) lunar phase that can affect reproductive output. All or a subset of these random effects were used for each analysis to achieve the best model fit and avoid issues of singularity. Plant ID was used in all analyses. Residual plots were visually examined to ensure no deviations from normality or homoscedasticity occurred. The Performance package in R (v 0.10.2, Lüdecke et al. 2021) was used to visualize important model assumptions, i.e., collinearity, influential observations, linearity of residuals, etc. Marginal (fixed effects only; R^2m) and conditional (fixed + random effects; R^2m) coefficients of determination were used to determine goodness of model fit. P-values were obtained via likelihood ratio tests

that compared the full model with a null model that lacked the effect being tested. Lastly, we ran basic linear regressions with growth as the dependent variable and P_{max}, E_k, as predictors to gauge importance as predictors.

Algal Growth

Wet weight was recorded in Google Sheets and saved directly to Google Drive. Growth was calculated using the equation

$$\left(\left(w_f - w_i\right)/w_i\right) \times 100$$

where f = final and i = initial wet weight (w). This equation was used to eliminate the need for assumptions of steady or exponential growth because such assumptions have not been well tested or documented in *Ulva* or *Hypnea*. The parasite *Hypneocolax stellaris* was occasionally found on *Hypnea* and was removed and quantified. See Okuhata et al. (2023) for additional information.

Results

This investigation generated data via replicated assessments of photosynthesis and growth of two contrasting species over nine day runs, for 16 replicates each across three water temperatures and six combinations of salinity and nutrients. We further documented daily irradiances during each run across several seasons.

The variance explained by the linear mixed-effect models (LMM) for all dependent variables analyzed, increased with the addition of the chosen random effects, emphasizing the importance of inherent plant variability (plant ID), irradiance changes over time, and other unmeasured variables. Overall, the LMM was a better fit for *Ulva* than for *Hypnea* for fixed effects (treatment and temperature), explaining up to 62% of the variance in *Ulva* and 38% in *Hypnea*. Both of these highest values were from the E_k model fit. With the addition of random effects, the explained variance increased to a maximum 86% in *Ulva* and 87% in *Hypnea*—both of these from the DSPI model fit. In *Ulva*, treatment had a significant effect on all dependent variables tested, whereas temperature only affected the outcome in one analysis (E_k). The significance results were more complex in *Hypnea* as treatment was an important factor affecting the outcomes in four of the six dependent variables but temperature affected outcomes in all but one (growth).

P_{max} and E_k

P_{max} is a value that describes the maximum light-dependent photosynthetic rate. In *Ulva*, P_{max} linearly increased and more than doubled from T0 to 4 (Figure 12A). The treatment with the highest nutrient input and lowest salinity (4) also had the highest measured P_{max} at 74.3 µmols electrons m^{-2} s^{-1}. Temperature did not have a significant effect on P_{max} (χ^2 (2) = 3.78, p = 0.15).

Treatment affected P_{max} in *Hypnea* (χ^2 (5) = 15.8, p = 0.008) increasing it by a maximum of 14.7 (± 11.5 std. err) in T2.5. The effect of temperature was also significant (χ^2 (2) = 40.1, p <0.001) decreasing P_{max} by 17.3 (± 3.12 std. err) at 27 °C and by 19.4 µmols electrons m^{-2} s^{-1} (± 2.73 std. err) at 30 °C (Fig. 12B). This is notable because 27 °C is closest to ambient seawater temperature in Hawai‘i. The higher P_{max} at 20 °C demonstrates *Hypnea*’s preference for its native temperature range.

Changes in the photosynthetic parameter, E_k, in the amount of irradiance needed to saturate photosynthesis. *Ulva* E_k continued to increase with increasing SGD while *Hypnea* Ek values did not improve (Figures 12C and D).

Growth

Figure 13 reports growth responses by these two species in response to 9 days under the treatment conditions.

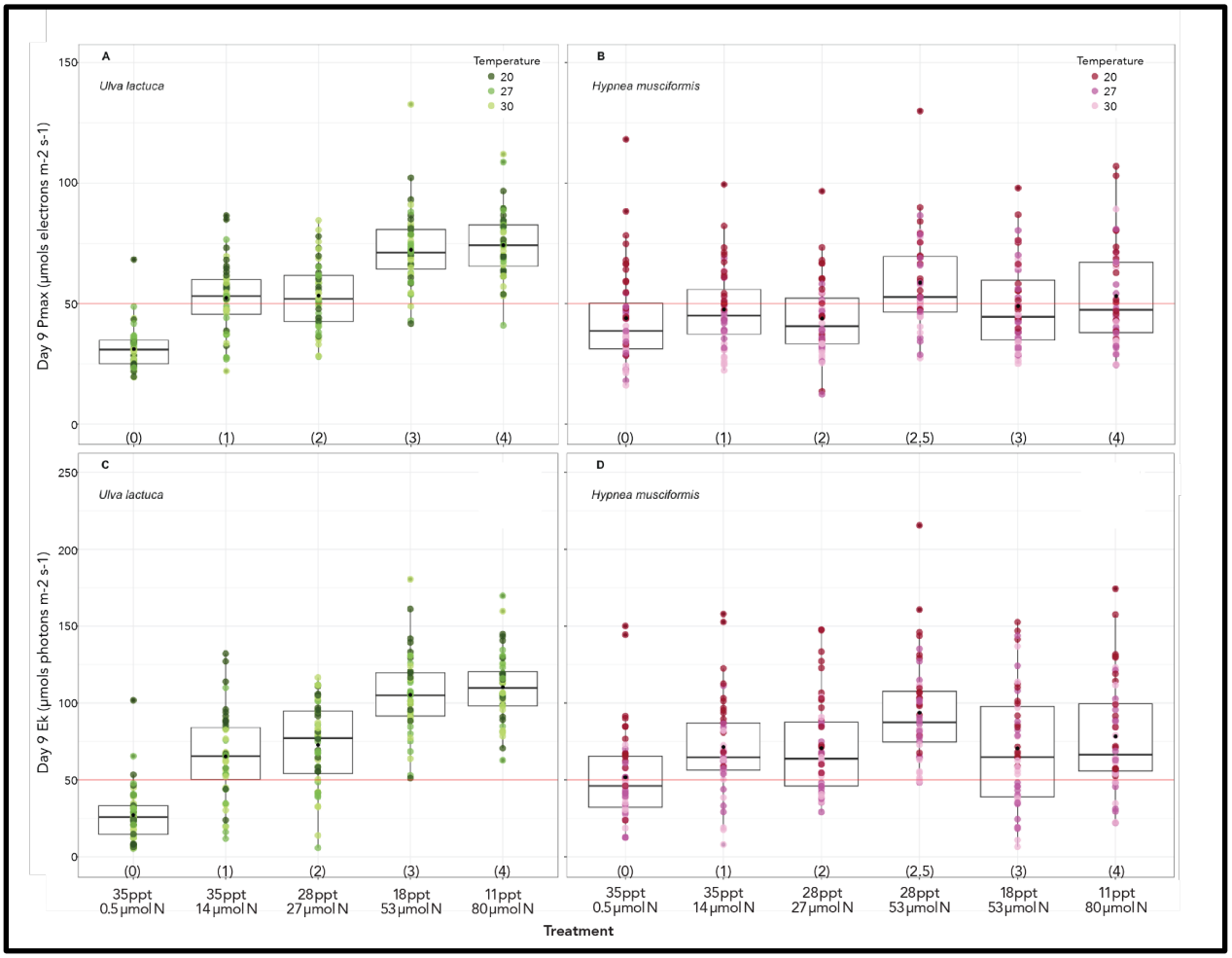

Figure 12. Day 9 P_{max} (A, B) and E_k (C, D) by treatment for *Ulva* and *Hypnea*. Black dots denote mean for each treatment. Treatment description and number (in parentheses) on x-axis. Colored dots represent temperature. Red lines at zero.

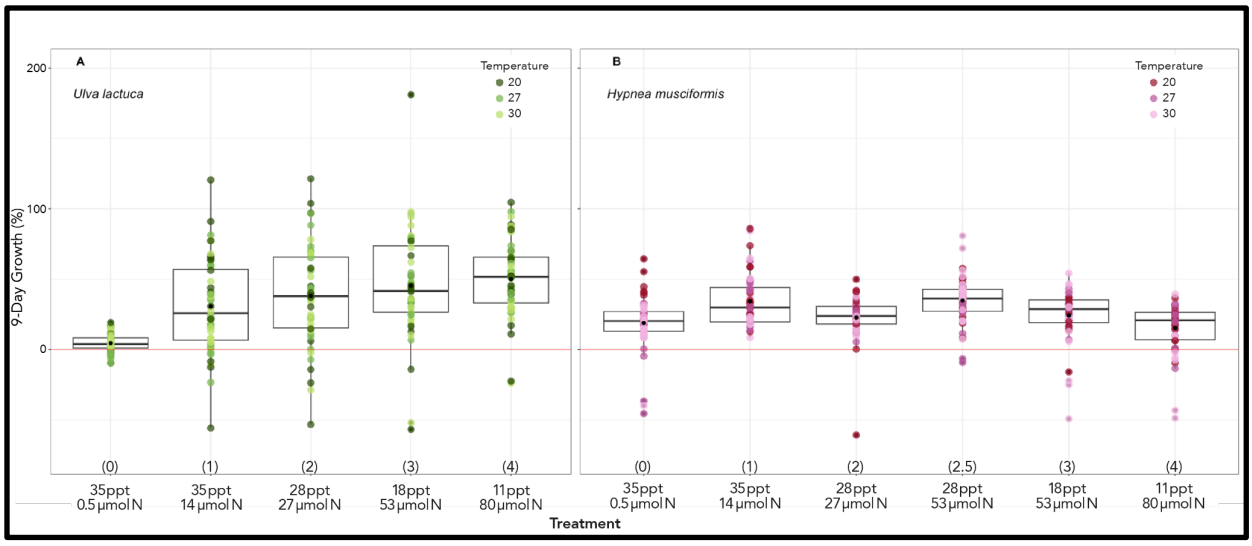

Figure 13. 9-day growth (%) for *Ulva* (A) and *Hypnea* (B). Black dots denote mean for each treatment. Treatment description and number (in parentheses) on x-axis. Colored dots represent temperature.

Collaboration with Dr. Jade Delevaux and others associated with this Kona-based project (Wada, et al, 2022; Okuhata et al 2023) lead to a boosted trees regression analysis based on Dr. Brytne Okuhata's hydrological model for this region (Okuhata et al 2023), the field abundances determined in Kona experimental sites and the physiological growth responses to nutrient temperature and salinity under baseline conditions (Figure 14. left coastline) and with climate change impacts (Figure 14. right coastline).

This powerful approach allows us to calculate the potential change in habitat suitability from *Ulva* to possible *Hypnea* invasion of this coastal region. Decreasing rainfall of climate change is expected to increase prevailing salinities of coastal waters, thereby favoring growth of *Hypnea musciformis* in these coasts that are otherwise populated by native algae such as limu palahalaha.

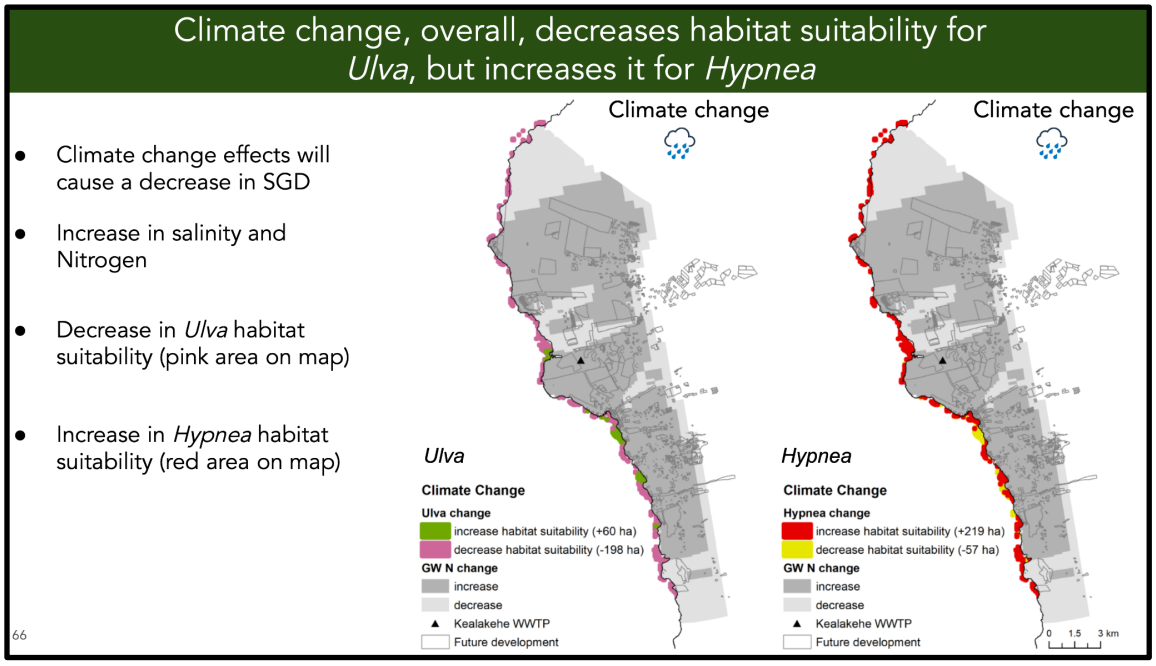

Figure 14. Calculated changes in occurrence of *Ulva lactuca* in the Kona coastline versus the changes

Future work.

Having good success with these new tools has allowed us to gain insight into the vulnerability of an invasive species such as *Hypnea musciformis* being intolerant of the widest possible range of habitat salinities. We now look forward to testing other taxa, native as well as invasive species for similar vulnerabilities that can further guide management of our native habitats. Our hope is that new data from other taxa will highlight additional new management insights for the preservation and conservation of our native marine flora.

Deliverables:

1. Three replicates of minicosms runs in the Marine Greenhouse, UHM for two species of ecologically related taxa that will allow us to compare an invasive and a native species.

Replicated studies have generated large sample sizes, n=16 per treatment, modern code in R for data analysis and statistical tools, to which we applied to data in Okuhata et al as well as Richards Dona et al.

2. Begin the effort to develop process-based model based on growth and competition for a preliminary invasive algae risk assessment framework.

With this effort we have been able to assemble a new set of tools that will help understand the competitive complexities between native limu and invasive algae.

3. Examine risk assessment framework using new data from invasive species.

Okuhata et al 2023 has used boosted trees analysis to model risk assessment from changes in these two algae, associated with our future climate.

4. Results and initial recommendations for next steps.

Water use policies can now be evaluated to insure that sufficient low-salinity groundwater can continue to flow, and maintain these groundwater dependent communities.

5. One peer-reviewed publication:

1. Okuhata, B., J.M.S. Delevaux, A. Richards Donà, C.M. Smith, V.L. Gibson, H. Dulai, A.I. El-Kadi, K. Stamoulis, K.M. Burnett, C.A. Wada, L.L. Bremer. 2023. Effects of multiple drivers of environmental change on native and invasive macroalgae in nearshore groundwater dependent ecosystems. Water Resource Research. Manuscript # 2023WR034593R

2. Richards Dona, A., CM Smith, LL Bremer. in review after revisions. Divergent responses of native and invasive macroalgae to submarine groundwater discharge. Submitted to Scientific Reports.

Spatial Data:

- eight sites in Kona coastline; 10 point intercept transects / site
- 0.625m² quadrat used for 10 quadrats / transect
- 960 data points per site
- Estimated 500 m^2 area assessed across sites, replicates and size of quadrat, **0.12 acre.**

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