

TESTING NOVEL TECHNIQUES TO MANAGE THE INVASIVE GREEN ALGA *Avrainvillea erecta*
(Berkeley) A.Gepp & E.S.Gepp
ON O'AHU SOUTH SHORES

A THESIS SUBMITTED TO THE GRADUATE DIVISION OF THE UNIVERSITY OF HAWAI'I AT MĀNOA
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE
IN
BOTANY

Liv Wellington Wheeler
May 2025

Committee:
Dr. Celia Smith (Chair)
Dr. Kimberly Peyton
Dr. Alison Sherwood

Acknowledgments

I began working on this degree in 2018. I will do my best to recall the many people who have contributed to this body of research, but inevitably I will be unable to do justice to all that have made this possible. I want to begin with a story. I had been struggling to get my final admission result from the Graduate Division and I was feeling a little despondent. In my ~~full~~ [full-time](#) job as Assistant Diving Safety Officer I had just finished a checkout dive with Dr. Heather Spalding, who got her PhD in the Limu Lab under Dr. Celia Smith back in 2012. I told her of my trouble, and she walked me over to Dr. Smith's office. We knocked on the door, there was no answer. So, she grabbed an old key and opened the door. Dr. Smith did not look happy for the intrusion, but it got me the meeting I needed to find out about my acceptance. I was in!

I want to thank the three women who comprised my committee team: Dr. Alison Sherwood, Dr. Kim Peyton and Dr. Celia Smith. Their support and understanding through all the challenges I have faced made it possible to continue when I truly thought I should quit. I was working full time at the university, pursuing a master's degree and also starting a family. I have had many obstacles; the biggest one being diving and data collection while pregnant. This committee was patient and understanding. They gave me good advice in standardizing the data collection to the divers I had to trust to implement my experimental design. It is through their guidance and patience that I am able to present this body of research, and I am truly thankful for them.

Most of the funding for this research came from the Hawaiian Invasive Species Council (HISC). Special thanks to Chelsea, Layla, Jake, for taking the time to meet with the *Avrainvillea* team to learn about our projects and their importance to Hawai'i near shore environments. Their continued support to fund small research projects on algal invasions has made it possible to investigate the interactions of native psammophytic plants to the newly introduced species of *Avrainvillea erecta*. I also could not have gained access to these grants if it weren't for the help and guidance of my lab mates Scott Van De Verg and Solimar Carrasquillo-Ho.

The first experiment I conducted was the physical removal treatments. It was labor intensive and spanned over 3 years, partially during the pandemic (2019-2022). Each dive site had to be monitored monthly. It was with the partnership of the Aquatic Invasive Species (AIS) team at the Division of Aquatic Resources (DAR) that allowed for monthly monitoring at the Ke'ehi sites. Kim Fuller, who managed the AIS team, was extremely generous with her time. She was very consistent with her data collections and even let us run two more transects to increase replication for the experiment. Without her and her team's countless hours of diving and photo downloading, this experiment would not be a success.

The Limu Lab is an ever-growing tribe of algae lovers. My time with them has introduced me to some wonderful people. Their contribution to my experience as a grad student and a new psychologist is indescribable. The mentorship and comradery that I received from all the Master and Ph.D. candidates before me has filled me with gratitude. Scott Chulakote, Solimar Carrasquillo-Ho, Veronica Gibson, Migiwa Kawachi, Brianna Ornelas, Nicole Yamase, and Dave Spafford, Donna Brown and Angela Richards-Donà all have helped me with my experiments, statistics, understanding of limu and the cultural importance it holds for the Native Hawaiian people. Special thanks to Scott Van De Verg for your mentorship, volunteer hours and friendship you have extended to me.

When I first started to look for this species of algae, I used the sightings reported from other divers. Researchers from UH, PMNM, Bishop Museum, and NOAA sent me GPS points and photos of meadows they had seen. I began systematically looking from Kewalo Basin to Honolulu Harbor and eventually to Ke'ēhi channel. I was pregnant at the time so all I could do was drive the boat and follow dive floats. It was the many volunteer divers, most of whom were previous students of mine that were willing to go searching for the meadows in the vast sandy rubble areas off O'āhu. Thank goodness they love to dive. Thank you, Melissa Butler, Madi Davis, Sam Darin, Ashleigh Epps, Sarah Franklin, Jimmy Fumo, Campbel Gunnell, Matt Holland, Mario Mora, Ethan Nash, Emilio Puga, Jake Reichard, Taylor Williams, and the many others that all volunteered to come out on my research dives. Particular thanks to Denise Oishi, who is my favorite dive buddy. She is an underwater ninja, so reliable and capable of accomplishing the most complicated underwater tasks successfully. Thank you for your tireless efforts and help.

None of the diving could have occurred without the support from my co-workers at the Diving Safety Office. Maria Laamang for navigating me through the many forms required to have a multi-agency project. Mike Pamatat for driving the boat so often and being a rebreather buddy on the deeper site. Dave Pence for volunteering to dive with me on the rebreathers and hunt for sites, set them up, take photos, and all the while being a kind ear to listen to my challenges. Dave was extremely supportive and willing to help me come up with a safe protocol so we could keep the data collection going during COVID.

Once the data collection was over the truly hard part came, the analysis. There was so much data to organize and proof check, and then manipulate, that many times I felt completely buried. I had several friends and colleagues to help teach me beyond my rudimentary understanding of data analysis and manipulation. Jonathan Whitney helped me organize the data so that it could be read by an R script. Wei Shen Lim helped me analyze my Hydrogen Peroxide experiment, and Noam Altman-Kurosaki helped me wade through the 2 years of data from the physical removal experiment. Assaf Azouri help with the graphics that included the south swell data from PacIOOS. Thank you all for your patience and tutelage.

Lastly, I would like to thank my deeper support network, my friends and family. To Jessie and Gina, you are my strength when I am weak, thank you for your support. My brothers for their words of wisdom and encouragement. You always believe in me. My mom, who still proofreads my work, listens to my rehearsal talks and can provide honest feedback. My two wonderful sons who have been a part of this journey from the very beginning and have given me perspective into what is truly important. To my husband, Chris, who was starting his own business when I announced that I was going back to school. He continues to support my endeavors even when his plate is completely overflowing with a growing business and two growing boys.

To my dad, who passed in 2021. He once said, "Just relax so everything can work out the way it should". Field ecology is like that, you must be patient to let nature play out and be an unbiased observer. Here's to you, Dad.

TABLE OF CONTENTS

	Page
ACKNOWLEDGMENTS.....	iii
ii	
LIST OF TABLES.....	iv... vi
LIST OF FIGURES.....	iv... vi
CHAPTER 1. LITERATURE REVIEW.....	7
LITERATURE CITED.....	18
CHAPTER 2. Testing novel techniques to manage <i>Avrainvillea erecta</i> in coastal waters of O’ahu	
ABSTRACT.....	243
INTRODUCTION.....	254
MATERIALS & METHODS.....	3029
RESULTS.....	413
DISCUSSION.....	4
LITERATURE CITED.....	541
CHAPTER 3. SUMMARY AND FUTURE WORK.....	58
APPENDIX.....	601
<i>Appendix 1.</i>	
Graphical representations of the General Linear Mix Model outcomes from the Physical Removal experiment.	

Appendix 2.

Supplementary preliminary biomechanical data collected on *Avrainvillea erecta*

Appendix 3.

Observations of all the different animal families and orders found within the novel meadow community

LIST OF TABLES

Table	Page
1 The dates of the physical removal experiment were conducted..... and when COVID-19 had impacts on the data collection.	34
2 Combined results for the four locations that the physical..... removal experiment occurred General Linear Mixed Model	40

LIST OF FIGURES

Figure	
1 Relative Dominance model.....	25
2 A map of tow research sites Ala Moana and KeKe'ehi that were used.....	30
3 Four treatments of physical removal techniques imposed upon plants..... <i>Avrainvillea erecta</i>	32
4 Facilities at Anuenue Fisheries Research Center (AFRC) open..... flow sea water system	34
5 A map of the chemical removal site outside of Kewalo Basin.....	36
6 Underwater images of hydrogen peroxide injections and the..... equipment used	38
7 Pulse Amplitude Modulated Fluorometer being used by a..... diver, showing the fiber optic clipped onto an <i>Avrainvillea erecta</i> plant	39
8 Surge effects on density of <i>A. erecta</i> from summer south swell.....	41
9 AFRC mesocosm experimental trial 1 measured average ETR_{max} $\mu\text{mols electrons m}^{-2}\text{s}^{-1}$ after H_2O_2 was injected	43
10 Baseline ETR_{max} $\mu\text{mols electrons m}^{-2}\text{s}^{-1}$ for three psammophytic..... plants that co-occur in meadows	44
11 Impacts of the <i>in-situ</i> injections of hydrogen peroxide on densities..... of <i>A. erecta</i> over 10 weeks	45

CHAPTER 1. LITERATURE REVIEW

BACKGROUND

The Hawaiian archipelago is the most isolated landmass on the planet, located in the North Pacific, it is 43,000 km from North America and 64,000 km from Japan (Coles et al. 1999). The species found here today exist from a limited number of colonization events that resulted in reproductive isolation (Price 2004). Over time these species evolved, adapted to Hawaiian habitats and new species emerged. Adaptive radiation is the divergence from an ancestral species to unique descendants that can better exploit different ecological opportunities (Glor 2010). It is this process that explains why the Hawaiian ecosystems have high rates of endemism in terrestrial and aquatic ecosystems with species that occur nowhere else in the world (Eldredge and Smith, 2021, Smith et al. 2004). For a long time, many Hawaiian marine ecosystems were thought to be much less diverse than similar reefs found in the South Pacific (Tsuda, 2014). Recent studies of deeper water algae (Sherwood and Guiry, 2023, Pyle et al. 2016) have flipped that assessment; new genera and species of algae from mesophotic depths have been estimated as high as 40 % of the deeper water flora.

Because native species in Hawaiian marine ecosystems evolved with less pressure of competing against aggressive non-native species, non-natives may have advantages in resource competition, release from predators in addition to the nature of the interspecific competition present with in the ecosystem (Davidson et al. 2018; Davidson and Shoemaker 2023). In addition to the timing or repetitive inoculation of an introduced species, introduced species may have broader habitat tolerances from their home regions as well as higher reproductive output that further their success rate against predation pressures (Carlton 1996, Inglis et al. 2006; Williams and Smith 2007). Further, because our Hawaiian flora lacks large canopy-forming submerged aquatic vascular species (SAV) such as *Thalassia*, competition among the existing functional groups present in Hawaiian marine settings can be seen as fundamentally distinct from successional trajectories in other Pacific Islands. The consequences of our unusual disharmonic distribution of species (Carlquist 1966; Wagner et al. 1990; Midway and Stephan 1996) may have shaped shallow-water coastal communities across the Hawaiian archipelago. Finally, because of this combination of factors, Hawaiian reefs are susceptible to marine invasions.

Marine algal introductions threaten the biodiversity of Hawaiian reefs (Conklin and Smith, 2005; Smith et al., 2004; Wade et al., 2018). With heavy shipping traffic occurring on the south shore of O'ahu, hull foulers, ballast water hitch-hikers and anchor snagged plants are transported to near shore habitats (Carlton, 1985, 1994) because the south shore of O'ahu is also the main metropolis for commerce, tourism, shipping, and urban development (State of Hawai'i 2024; Wade et al. 2018). Coastal development and extreme use has impacted the native

Commented [CS1]: Copy this introduction and insert it in Chap 1. These paragraphs lay out a nice background for the lit rev too,

Commented [CS2]: Added this ref to lit cited

benthic communities by reducing coral cover, removing key fish species, and severely impacting sea grass habitats (Littler et al., 2006, Peyton 2009) as well as increasing nutrient inputs to these regions (e.g. Amato et al., 2016; Gibson et al. 2024). Undisturbed Hawaiian fringing reefs typically have low nutrients and high herbivory intensity – a combination that favors coral species as dominant cover for the 41% of coastal reef that these hard-bottom benthic communities cover in Hawai‘i (Coyne et al., 2003). In fact, this combination is one of four combinations of herbivory intensity and nutrient availability that are found on most reefs. Strikingly, the authors, ~~Diane Mark~~ and ~~Diane Mark~~ Littler, proposed that characteristics incorporated into the Relative Dominance Paradigm (RDP) (Littler and Littler 1984, 1991) predict which component of the benthic cover is likely to be dominant (Littler et al., 2006), based on the number of herbivores that visit an area, coupled with the concentrations of nutrients that are available to primary producers.

INVASIVE SPECIES AND RELATIVE DOMINANCE PARADIGM HYPOTHESIS

The effectiveness of the RDP as a generalizeable model was first tested in experiments at Puakō, Hawai‘i where the newly settled benthic communities were manipulated by exposing them to one of four nutrient/herbivory regimes creating a four-state framework that included ambient controls (Smith et al., 2001). The results from the 12-month study’s community development and outcomes strongly supported Littler and Litter’s RDP in ~~proposing~~ their conceptual model. Both nutrient enrichment and herbivore exclusion had significant effects on the development of different benthic community structures and biomass compared to the control (Smith et al., 2001, 2010). Where herbivores were excluded, filamentous algae dominated on experimental tiles; where nutrients were elevated despite continued intense herbivory, coralline algae dominated on ~~the~~ experimental tiles. When herbivores were excluded and nutrients were elevated, a diverse set of macroalgae dominated experimental tiles. This was the first experimental effort to combine herbivory - a top-down force and nutrient availability as a bottom-up force.

The RDP has become a helpful tool to infer reef health when observing the dominant benthic community and can help further when asking questions about why the reef is structured in a particular way. However, reef communities are best seen as multiple stable states of these four functional groups – macroalgae, turf algae, crustose coralline algae and corals. Species are likely to cycle throughout a year associated with seasonal inputs of nutrients, herbivore recruitment or die off, as well as competition among algae and corals, and life history cycles. The reef itself is influenced by the greater marine system that helps to influence the presence and absence of the top-down and bottom-up forces. An important part of this broader marine system is the sand-dwelling, or psammophytic communities.

Psammophytic sea grass / algal meadows adjacent to coral reefs occupy as much as 50 % of benthos in Hawaiian shallow waters (<30m) (Coyne et al., 2003). These communities provide refuge and food, and absorb organic nutrients that enhance the health of neighboring hard-bottom reefs (Waycott et al., 2009, Bulmer et al., 2018, Pereda-Briones [et al.](#), 2018). The health of the meadow is important to the larger coastal ecosystem because psammophytic communities sequester carbon, recycle organic nutrients and provide habitat for fish and invertebrates (Bulmer et al., 2018, Pereda-Briones et al., 2018).

EXAMPLES OF RDP

The patterns of dominance described by the RDP have been observed on reefs around the world under similar large-scale impacts. One example is the effects of nutrient input on coral reef community structure in Kāneʻohe Bay, Oʻahu in the mid 1970's (Smith et al., 1981). Effluent had continuously been discharged into the bay as the surrounding suburban community was growing. As a result of increased nutrients, the green alga *Dictyosphaeria cavernosa* (Forsskål) Børgesen became prolific and smothered the coral colonies that once dominated patch reefs (Done 1992, Stimson 2015, Stimson, 2018). This community reverted to a mix of algal / coral dominance after the effluent was diverted from the bay.

An example of herbivory exclusion effects on algal overgrowth occurred in Jamaica after the loss of a keystone herbivore, the long spine sea urchin, *Diadema antillarum* (Philippi, 1845) in 1983. The loss of this dominant herbivore along with heavy fishing pressures allowed macroalgae to grow unchecked and began a phase shift, a change of the dominant functional form from a coral reef to algal meadows (Mumby, 2009; Mumby et al., 2007).

To give an example of nutrient enhancement and herbivory suppression, we can turn to the invasion of *Caulerpa taxifolia* Vahl C. Agardh (Order Bryopsidales). *C. taxifolia* was accidentally introduced to the Mediterranean Sea in 1984 (Jaubert et al., 2003; Meinesz et al., 2001). In just under 20 years this alga covered 121 km² of benthos and spread across the coastlines of six countries (Meinesz et al., 2001). This strain was also identified in southern California in 2000 (Williams and Schroeder, 2004) likely as an accidental hobby aquarist release. This species is one of the top 100 invasive algal species (International Union for the Conservation of Nature, [www.issg.org/ booklet.pdf](http://www.issg.org/booklet.pdf)). The success of *C. taxifolia* may have to do with multiple factors represented in RDP. However, the suitable habitat and lack of herbivory in a new environment strongly suggest that this is an aggressive competitor (Thibaut et al., 2004). Other Bryopsidalean algae have successfully invaded cooler waters such as *Codium fragile* (Sur.) Hariot spp. *tomentosoides* (Van Goor) Silva in the Eastern United States and Canada (Chapman, 1998). Additionally, *Avrainvillea lacerata* J. Agardh has established itself within sub-tropical habitats Hawai'i most notably, the shallow reefs of O'ahu replacing seagrass habitat with homogenous meadows of *A. lacerata* (Van De Verg and Smith, 2022). Coastal areas with high

Commented [A3]: species authority needed

Commented [A4]: ? Is this a citation? Need to include year, if so.

Commented [KP5]: If I follow the point of this sentence, this comma doesn't belong here.

development may have increased nutrient input, and once characteristic of invasive species is the lack of predation.

ALGAL INVASIONS IN HAWAII

The main Hawaiian islands have probably received many introductions, 19 of which have been documented ; however, only a subset exhibit invasive traits (Smith et al., 2001; Smith et al., 2004). These weedy species in Hawai'i include the rhodophytes *Acanthophora spicifera* (Vahl) Børgesen, *Gracilaria salicornia* (C. Agardh) E. Y. Dawson, *Hypnea musciformis* (Wulfen) J. V. Lamouroux, *Kappaphycus alvarezii* (Doty) Doty ex P. C. Silva, *K. striatum* (F. Schmitz) Doty ex P. C. Silva, and the chlorophyte *Avrainvillea lacerata* (J. Agardh). Of these introductions, *H. musciformis*, *Kappaphycus* spp. and *G. salicornia* were a result of experimental aquaculture farming for ~~carrageenan agar, or agar or carrageenan~~ production in the mid 1970's (Smith et al., 2004). In addition, the hull fouler *A. spicifera* was brought to Pearl Harbor from Guam in 1950 via a military fuel barge (Doty, 1961) and has spread to other Main Hawaiian islands (Weijerman et al., 2008).

For invasive benthic algae living on rock or coral substrates, the RDP easily explains their dominance under conditions of low herbivory pressure and elevated nutrients. ~~O'ahu~~~~The impacts of these invasives~~~~invasive algae is exacerbated by O'ahu waters being are~~ increasingly susceptible to invasions partly associated with degradation of reef habitat from urbanization and over fishing. ~~Further, -and partly because of Honolulu harbor being is a heavily trafficked an~~ international port ~~- a hub for hub in the Pacific, and there is has a near -~~ continual delivery of ~~new~~ non-native taxa ~~delivered~~ by a variety of vessels arriving from diverse locations (Carlton, 1996).

AVRAINVILLEA

In marked contrast to those multicellular, saxicolous taxa that arrived as part of mariculture or hull fouling, siphonous *Avrainvillea lacerata* was discovered in 1980's by Brostoff, originally reported as *A. amadelpa* (Mont.) Gepp and Gepp (Brostoff 1989) in deeper water locations at two O'ahu locations, Portlock and Ewa Beach. The mode of introduction is still unknown for *A. lacerata* but because of this plant's large size and preference of soft bottom habitats, *A. lacerata* was an unlikely hull fouler or ballast water species. Perhaps this species arrived via solid ballast, on anchor chain or in anchor holds of earlier maritime vessels. Its origin of discovery near the Portlock, near the Hawai'i Kai Harbor ~~-or Ewa, close to Pearl Harbor,~~ where boats landed after traveling from other Pacific Islands might suggests ~~it~~~~A. lacerata~~ did arrive from vessel transport. ~~T; further,~~ this plant dominates large regions in deep water off Ewa Beach, suggesting that the arrival of this alga was not recent (Brostoff 1989). Peyton added important perspectives for *A. lacerata* with her research that documents slow growth of this species at Paikō reef as well as its ability to occupy hard and soft-bottom areas (Peyton 2009). In more recent studies,

this alga was recognized as *Avrainvillea lacerata* J. Agardh (Wade et al., 2018). The siphonous organization of this alga, along with *Caulerpa taxifolia* and *Codium*, introduced earlier seems an unusual coincidence and aligns with the suggestion that siphonous algae in the Order Bryopsidales may be more invasive than expected (Williams and Smith, 2007). This morphology will be discussed in another section below.

MARINE PLANTS IN PSAMMOPHYTIC HABITATS: SIPHONOUS ALGAE AND SEAGRASS

Little is known about how top-down and bottom-up forces affect psammophytic (soft bottom) habitats that are dominated by submerged aquatic vascular plants (SAV) – our seagrasses *Halophila hawaiiiana* and *H. decipiens* (Order Hydrocharitaceae) are two that inhabit the near shore sandy bottom habitats on O’ahu (Abbott and Huisman 2007; Nordlund et al., 2016, Peyton 2009). Their ecosystem services range from contributing to sea turtles’ diet and detritus-based food webs by oxygenating the upper sediment layer and up taking inorganic nutrients. These meadows also provide refugia for juvenile fish and invertebrates and breeding grounds for shellfish (Bulmer et al., 2018, Pereda-Briones et al., 2018), and stabilizing sediment while contributing to carbon and nutrient assimilation and cycling (Waycott et al., 2009, Peyton 2009).

In contrast to the SAV in these communities, a dominant canopy-forming plant in psammophytic habitats is the native siphonous alga, *Halimeda kanaloana* Vroom. This alga is another member of the Order Bryopsidales (Vroom and Smith 2001, Vroom et al., 2003). Plants in this order are single cell macro-organisms that will be discussed later within this first chapter. Species of *Halimeda* generally form large bulbous holdfasts to anchor in sand, as seen with native *H. kanaloana* anchoring and forming large meadows extending from 18 to 90 m (Littler et al., 1985, Vroom et al. 2002, Spalding 2012,). This ~ 30 cm tall species contributes to fish and invertebrate habitat, sand production, sediment stabilization, and carbon sequestering as ecosystem services (Beach et al., 2003; Vroom et al., 2002).

Until recently, *H. kanaloana* was absent from O’ahu’s south shore (Spalding 2012). Since early 2000, University of Hawaii Diving Safety Program has been using the south shore of O’ahu as a training site for open circuit SCUBA and closed-circuit rebreather technologies. The depths that former Diving Safety Officer D. Pence covered range from 10 m to approx. 100 m. His extensive personal observations are not published but are credible as a source of historical information and a starting point from which to measure change. Since 2014, meadows have expanded in the south shore area, providing an increasingly lush “lei” that likely stabilizes sand along the coastline (D. Pence pers. comm.). The psammophytic habitats can help increase fish populations, enhance nutrient absorption and recycling. Though there are not many studies of the interaction between the coral reef and psammophytic habitats within the Hawaiian Islands, there is community interaction and energy exchange between the two habitats that is

important to the health of both ecosystems (Peyton 2009, Langston and Spalding, 2017). The presence of these co-occurring marine plants off the coast of the Honolulu metropolis could bring a positive influence on the adjacent heavily impacted reef, supporting juvenile herbivorous fish and invertebrates. It is important to monitor the health and community structure including seasonal shifts to understand the role this habitat plays in the health of our coastline.

PSAMMOPHYTIC ECOLOGY AND INTRODUCED SPECIES

Besides the factors identified in the RDP, other complexity comes with the balance between disturbance and stress in the environment. Dominant species may be seasonal on a reef depending on the cycles in that area. However, when an aggressive new species arrives, the natural ebb and flow of benthic community structure starts usually with subtle changes that eventually reveal the introduced species as weedy and thus categorized as invasive. Invasive species can have devastating effects on native communities (Bax et al. 2003, Smith et al. 2004, Martinez et al. 2012, Wade et al. 2018). Invasive organisms can alter the community structure, disrupt the food web, and ultimately change the functional role of the ecosystem (Carlton, 1994; Epstein and Smale, 2017; Littler et al., 2006; Van De Verg and Smith, 2022; Wade et al., 2018). Hawai'i has seen ~~several~~ multiple algal invasions in the last 60 years that led to phase shifts ~~-;~~ the change in the stable state of an ecosystem that abruptly shifts to a different stable state, favoring a different set of organisms, such as coral dominated to algal dominated communities (Martinez, 2012, Mumby, 2009; Smith et al., 2002).

INVASIVE ALGAE CONTROL TECHNIQUES

Managing algal blooms is not a new problem. Whether it's weeding your garden or managing a forest, historically pulling out undesirable plants has been a common, inexpensive, and easy to implement method. However, the success of this technique in the marine environment is dependent on a species' ability to regrow from fragments versus success in removing a vast majority of fragments.

Unlike invasive terrestrial plants that can grow back from fragments of roots and rhizomes, algae have the ability to grow back from fragments of thalli that can be transported to new locations through the water column (Smith et al., 2002, 2004). During the Smith ~~et al.~~ et al. study in 2003 to 2004 there were five major clean up events for the invasive *Gracilaria salicornia* resulting in 20,000 kg of wet algal biomass being removed from the reef seaward of the Natatorium War Memorial in Waikiki. There was maximum effort to reduce fragments of biomass in the water column. However since its introduction in the 1970's the alga has spread both up and down to Ala Moana and to Diamond Head (Smith et al., 2004). Because of limited

Commented [KP6]: Do you mean undesirable plants?

workforce to remove algal biomass and the vegetative propagation success of this species, *G. salicornia* has become ubiquitous among O'ahu's south shores.

In the case of *Acanthophora spicifera*, multiple control methods were used in comparison of efficacy within the Kaloko fishpond on Hawai'i Island (Weijerman et al., 2008). Using effectiveness and effort as a measurement of success to inform managers, the researchers were able to quantify techniques of physical removal strategies, shading and increasing herbivory through native fish. The RDP state that best characterized the fishpond was high nutrients and low herbivory, a perfect combination to produce large macroalgal blooms. In this case the absence of herbivory was a key factor in the abundance and growth of the red alga *A. spicifera*, and using a biocontrol technique of native reef fish had the most success in reducing biomass over time (Weijerman et al., 2008). Another study in Puerto Rico looked at *Diadema antillarum* (long spine sea urchin) as a biocontrol. The *D. antillarum* removed 46% of the red encrusting alga *Ramirusta* spp. and 37% of the brown fleshy algae *Dictyota* spp. over the course of two months of observations (Williams, 2022).

When feasible, biocontrol is a natural way to control algal biomass. However, a characteristic of being invasive is being chemically defended, having unpalatable taste, or having no native predation (Carlton, 1996). The marine alga *Caulerpa taxifolia*, one of the top 100 most invasive species, was discovered in Southern California (Jaubert et al., 2003; Meinesz et al. 2002). A quick response led to an experiment for eradication using high concentrations of chlorine. In the lab, it required 125 ppm for a minimum of 30 min to kill the entire plant (Williams and Schroeder, 2004). In the field, a tarp was secured on the benthos to keep the concentration of chlorine solution near the algae for the minimum time. It is unknown if the concentration was sufficient, or the combination of tarping-light reduction and chlorine made the experiment successful *in situ*. However, the casualties to the seagrass and other benthic organisms were great and cost of chlorine was high (Williams and Schroeder, 2004).

A more localized approach with less impact on the established ecosystem is described in Van De Verg and Smith (2022), as they explored hydrogen peroxide effects on intertidal populations of *Avrainvillea lacerata*. This study uses injection methods delivering hydrogen peroxide to the fibrous parts of the plant, to reduce the negative effects of hydrogen peroxide on the surrounding community. The use of hydrogen peroxide as a photosynthetic inhibitor has been tested on *A. lacerata* and produces a byproduct of oxygen and water (Van De Verg and Smith, 2022), while impairing photosynthetic capacity that started at 15 rETR_{max} μmols electrons m⁻²s⁻¹ for the controls to 10 rETR_{max} μmols electrons m⁻²s⁻¹ to treated plants. This type of management technique can address the plant above and below the substrate while physical removal and biocontrol does not necessarily address below the sand or remnant rhizoids.

MARINE MEADOWS and the MEADOW SUPPRESSION HYPOTHESIS

Commented [A7]: is it truly a removal technique?

Commented [LW8R7]: We are calling it that, because it removes the living and competitive nature of the plant. But it does leave the plant to be removed by decomposition.

Meadows in sandy settings can be extensive single or mixed species covering over six km at 10%-40% cover of coastal soft bottom habitat (Lyons et al., 2012). The meadow suppression hypothesis states that in habitats suitable for seagrass, green algae are restricted to understory canopy cover and algal meadows without seagrass occur in habitats unsuitable to support seagrass meadows. This is because to become successful among green algae, seagrass had to evolve mechanisms that could out compete the older macroalgae meadows that were already established (Davis and Fourqurean 2001, Peyton 2009). Green algae, specifically bryopsidalean, siphonous algae, have been established in the marine ecosystem for over 750 million years (Vroom and Smith, 2003). As an angiosperm evolving traits to live on land, seagrass evolved traits to return to the marine environment only 70-90 mya (Les et al., 1997). Deriving originally from freshwater habitats, seagrasses developed competitive traits, such as nutrient acquisition to outcompete the established bryopsidalean algae (Davis and Fourqurean, 2001). The meadow suppression hypothesis supports that over time, seagrass has become a dominant species in the psammophytic near-coastal habitats over macroalgae. (Hillis-Colinvaux, 1988). Previous research on O'ahu, conducted by Dr. Kim Peyton compared the competitive nature of *Halophila* spp. with *Avrainvillea lacerata* and found support for the Meadow Suppression Hypothesis (Peyton 2009, Tussenbroek et al., 2006, Williams, 1990). But with current anthropogenic pressures such as increased sedimentation, pollution, and disturbances from anchoring and bottom fishing it is important to monitor the community composition between native seagrasses, native psammophytic algae, and their resilience to invasive algae.

On the south shore of O'ahu, just past the end of the shallow fringing reef, the psammophytic ecosystem begins. This is a sandy rubble zone that extends from about 18-60m. This area supports plants that grow in the sand and rubble. One species of native limu, *Halimeda kanaloana* has become ubiquitous in this habitat over the last 10 years and can create extensive meadows. It has a wide depth range from 15-60 m (Spalding 2012). Extensively studied on the deep reefs of Maui, *H. kanaloana* was not reported to be on the south shore of O'ahu until recently. In the summer of 2018, it was observed on reconnaissance dives from 15-65m by University of Hawai'i Diving Safety personnel. This expansion of native limu is important to monitor because of the services it provides, forming canopy structure for fauna, stabilizing the sandy substrate, while simultaneously producing sand from its calcareous thallus. Additionally, the native seagrass *Halophila hawaiiiana* also inhabits the upper ranges of the psammophytic area adjacent to the fringing reef. Meadows of *H. hawaiiiana* have been observed to about 32m and co-occur with *H. kanaloana* creating a diverse habitat to help support the complex trophic food web (Hillis-Colinvaux 1974). Concurrently a newly introduced species has also been recorded off the south shore of O'ahu, *Avrainvillea erecta*. Little is known about the ecology of this species and how it will interact with the native psammophytic species. It has been observed in overlapping habitats with *H. kanaloana* and *H. hawaiiiana* from 15-60 m (Wade et al. 2018). Previous work on the invasive *Avrainvillea lacerata* indicated that this

Commented [KP9]: Well said

species could also have weedy qualities and monitoring its range and abundance is important for coastal management and habitat conservation.

UNICELLULAR, MULTINUCLEATE ALGAE AS REMARKABLE COMPETITORS

This thesis research will test novel techniques to remove *Avrainvillea erecta* from mixed meadows of two dominant natives, the psammophytic plants *Halimeda kanaloana* and *Halophila hawaiiiana*. Both algal species, *A. erecta* and *H. kanaloana*, are siphonous algae within the Order Bryopsidales. Siphonous algae are unicellular and multinucleate (Vroom and Smith 2001). Their cell is tube shaped but can be ornately branched to form complex and differentiated bodies. (Littler and Littler 1992, Vroom and Smith 2001). The chloroplast and nuclei are contained within the cytoplasm that surrounds a large central vacuole within the cell, resembling a garden hose (Vroom and Smith 2001). To expand, these plants use sea water's osmotic constituents to create turgor pressure from the central vacuole and expand the cell wall to create more segments or branches. The chloroplast and nuclei can be moved freely to specific areas of the plant to adjust for photosynthesis or to sequester after damage has occurred (Vroom and Smith 2001). If damage occurs to the alga a quick response is triggered, and the wound can be plugged before too much cytoplasm is lost (Barsanti and Gualtieri 2014). Many siphonous species can regrow entire plants from small fragments of tissue (Barsanti and Gualtieri 2014). This can lead to a competitive advantage over other algae and has demonstrated weedy characteristics when introduced to non-native habitats (Smith et al., 2004, Barsanti and Gualtieri 2014).

SPECIES DESCRIPTIONS

Avrainvillea erecta (Berkeley) A. Gepp & E.S. Gepp has a native range of the Philippines, Micronesia, South-east Asia, Australia, New Zealand, South China Sea and Indian Ocean (Guiry and Guiry, 2024). It is an uncalcified siphonous green alga within the order Bryopsidales.

Each plant has one holdfast, a stipe and one to multiple upright blades (Littler and Littler 1992). A holdfast can produce one of two kinds of bodies (1) Blade like with tightly woven siphons to produce a paddle/ fan shaped blade above the substrate (2) Loose aggregates of siphons, unwoven assemblages attached by a single holdfast, which could be a juvenile stage (Wade et al. 2018). Depending on the size of the plant, the holdfast can protrude above ground creating a mound 1-5 cm high. The holdfast can be up to 46% of the total plant height. A single blade can be up to 10 cm tall (Wade et al. 2018). Although reproductive structures in either introduced *Avrainvillea* species are yet to be confirmed in Hawai'i, *Avrainvillea* probably undergoes gametic meiosis with holocarpic reproduction (Guiry & Guiry 2024). Asexual reproduction is a non-sexual reproduction, either through fragmentation where a broken piece from an adult can sprout a new individual or an adult individual can bud a new plant from its thalli (Vroom and Smith 2001).

The preferred habitat for *A. erecta* is psammophytic meadows; this alga inhabits loose sediment substrate and deep sandy bottoms. However, at the base of many observed holdfasts it has a piece of rock or coral attached, suggesting it is saxicolous in its early life stages using rocks to anchor via a holdfast network within the sandy substrate. This alga has been observed from 12 – 60 m on O’ahu (Pyle et al. 2016). The ecology of *A. erecta* suggests it does stabilize substrate by binding sand into its holdfast and provides structure for invertebrates to occupy. It is also likely to be chemically defended by producing a brominated diphenylmethane compound called *avrainvilleol*, that was originally reported by Hao et al. from another species, *A. longicaulis* as a feeding deterrent (Hao et al. 1983, Littler and Littler 1992, Martinez et al. 2012). These species can entrap silt and sediment and create low oxygen environments (Littler et al. 2004). Other species of *Avrainvillea* have been reported to sequester nitrogen from submarine ground water discharge, increasing their competitive advantage among other plants that do not (Albright 2017). The ecology of *Avrainvillea erecta* in [Hawaii-Hawaii](#) is largely unknown as it is newly introduced.

Halimeda kanaloana Vroom is a native species with a range spanning from Japan, Central Polynesia and including the Hawaiian archipelago (Guiry and Guiry, 2024). This calcified green alga is in the order Bryopsidales and forms multiple erect branches of disc-like segments (Vroom and Smith, 2003). The thallus is made of filaments that lack cellular cross walls and are laterally inflated. Between the disc segments are flexible joints called utricles. ~~This alga~~ has a fibrous holdfast that binds the sand and silt to form a solid bulbous clump and can be half the size as the erect photosynthetic thallus (Huisman et al., 2007). Size can reach 15 cm with a maximum of ~ 30 cm (Fukunaga, 2008). When going through sexual reproduction *H. kanaloana* is holocarpic (the entire plant dies in the process of gamete formation) creating compound gametangia resembling broccoli like clusters (Abbott and Huisman 2004). Plants have anisogametes (unequal sized gametes) and follow the gametic meiosis life history. When the plant has released photosynthetic gametes, the calcified remains of the plant axes are bright white and contribute to sand formation (Huisman et al., 2007). Living plants likely undergo fragmentation where a segment will break off, heal wounds, form rhizoids, anchor and regrow (Vroom and Smith 2001). The preferred habitat for *H. kanaloana* is soft sediment with fine sand grains, in shallow and mesophotic (10 – 90m) environments around the Hawaiian Islands (Fukunaga 2008, Spalding 2012).

The ecology and ecosystem benefits of *H. ~~kanaloana~~kanaloana* are that it creates structural habitat for fish (Fukunaga 2008, Spalding 2012) and provides a food source for multiple species such as sea urchins, parrot fish and sea turtles (Spalding 2012), and is substrate for other epiphytic algae (Beach et al. 2003, Vroom et al. 2003, Spalding 2012). Holdfasts help to stabilize the substrate while the thallus above reduces water motion (Fukunaga 2008). The

Commented [KP10]: The original source Hao H. Sun, Valerie J. Paul, William Fenical, Avrainvilleol, a brominated diphenylmethane derivative with feeding deterrent properties from the tropical green alga avrainvillea longicaulis, Phytochemistry, Volume 22, Issue 3, 1983, Pages 743-745, ISSN 0031-9422, [https://doi.org/10.1016/S0031-9422\(00\)86974-5](https://doi.org/10.1016/S0031-9422(00)86974-5).

Commented [CS11]: Not best source for this

calcareous skeleton of *H. kanaloana* is an important producer of sediment for reef lithification and sand production.

Halophila hawaiiiana Doty & B.C. Stone is an endemic species only found within the Hawaiian Islands (Guiry and Guiry, 2024). As with all seagrasses, this marine angiosperm has a complex internal anatomy with xylem, phloem and produces flowers and seeds. Its leaves are ovate, approximately 3-5 cm long with smooth edges (Doty and Stone 1966). This seagrass has true roots and rhizomes that are buried under a muddy or sandy substrate, often leaving only the blades exposed. It forms large ranging meadows connected by many rhizomes. Flowers are produced infrequently, and on separate male and female plants. After pollination, the female will produce a seed that will germinate in the sand and regrow a new individual. Its preferred habitat is sand.

Halophila hawaiiiana has many ecologically important roles. It provides habitat for leaf dwelling organisms as well as organisms found in the sediments. It also provides food for more mobile organisms such as grazing sea turtles, fish, and crustaceans, and it stabilizes substrate (Doty and Stone 1966). In an area where large canopy forming plants do not exist, *H. hawaiiiana* is important for providing structure in an otherwise barren habitat. This seagrass also helps to stabilize substrate that allows organisms to create more permanent residence.

In the next chapter of this thesis, I will address three objectives:

- 1) Monitor *Avrainvillea erecta* density within co-occurring meadows as *Avrainvillea erecta* changes over summer south swell.
- 2) Apply novel physical and chemical removal treatments to *Avrainvillea erecta*.
- 3) Measure recovery and changes in numbers of plants of *Avrainvillea erecta* in experimental quadrats before and after treatment(s).

LITERATURE CITED ~~(Style of Global change Biology, will edit it to Invasion Biology)~~

Abbott, I. A. and J. M. Huisman. (2004). Marine green and brown algae of the ~~Hawaiian islands~~ Hawaiian Islands Bishop Museum Press. ~~(pp. 188 – 190)~~ give total # pages.

Albright, S. (2017). A growing problem: The missing link for ecological success by the invasive ~~Acrainvillea~~ Avrainvillea. A Senior Honors Project. ~~Presented to the Faculty of the Department of Biology, University of Hawai'i, Mānoa.~~ (pp. 1-12) pages.

Bax, N. T., Carlton, A., Mathews-Amos, R. L., Haedrich, F. G., Howarth, J. E., Purcell, A., Rieser, A., Gray ?? (2001). The control of biological invasions in the world's oceans. *Conservation Biology*, 15, 5-1234-1246

Barsanti, I., ~~and~~ and P. Gualtieri, P. (2014). *Algae, anatomy, biochemistry, and biotechnology*. Second edition ~~(p. 13)~~. CRC Press, Taylor and Francis Group, Baton Rouge.

Beach, K., ~~L., Walters, L., P., Vroom, P., S., Smith, C., J., Coyer, J., & C., Hunter, C.~~ (2003). Variability in the ecophysiology of the *Halimeda* spp. (Chlorophyta, Bryopsidales) on Conch Reef, Florida Keys, USA. *Journal of Phycology*, 39, ~~p.~~ 633-643

Bulmer, R. H., Townsend, M., Drylie, T., ~~&~~ & Lohrer, A. M. (2018). Elevated turbidity and the nutrient removal capacity of seagrass. *Frontiers in Marine Science*, 5(462)- <https://doi.org/10.3389/fmars.2018.00462>

Carlton, J. T. (1985). Transoceanic and interoceanic dispersal of coastal marine organisms: The biology of ballast water. *Ocean Mar Biol Ann Rev*, 23, ~~313-374~~ 313-374

Carlton, J. T. (1994). Patterns of transoceanic marine biological invasions in the Pacific Ocean. In E. A. Kay, *A Natural History of the Hawaiian Islands* (pp. 504–518). University of Hawaii Press. <https://doi.org/10.1515/9780824844264-043>

Carlton, J. T. (1996). Pattern, process, and prediction in marine invasion ecology. *Biol Cons*, 78, ~~97-106~~ 97-106 [https://doi.org/10.1016/0006-3207\(96\)00020-1](https://doi.org/10.1016/0006-3207(96)00020-1)

Chapman, A. S. (1998). From introduced species to invader: What determines variation in the success of *Codium fragile* ssp. *tomentosoides* (Chlorophyta) in the North Atlantic Ocean? *Helgoländer Meeresuntersuchungen*, 52(3-4), 277–289 <https://doi.org/10.1007/BF02908902>

Conklin, E. J., ~~and~~ and Smith, J. E. (2005). Abundance and spread of the invasive red alga, *Kappaphycus* spp., in Kane'ohe Bay, Hawai'i and an experimental assessment of

Commented [A12]: check journal name

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Commented [A13]: make sure your reference formatting is consistent

Formatted: Font: Italic

Formatted: Font: Italic

management options. *Biological Invasions*, 7(6), 1029–1039
<https://doi.org/10.1007/s10530-004-3125-x>

Coyne, M. S., ~~T. A.~~ Battista, ~~T. A.~~, M. Anderson, ~~S. M.~~, ~~J.~~ Waddell, ~~J. W.~~ Smith, ~~W. P.~~ Jokiel, ~~P. M. S.~~ Kendall, ~~M. S.~~, ~~and~~ ~~and~~ ~~M. E.~~ Monaco, ~~M. E.~~, (2003). NOAA Technical Memorandum NOS NCCOS CCMA 152 (Online). Benthic habitats of the Main Hawaiian Islands.
<http://biogeo.nos.noaa.gov/projects/mapping/pacific/>

Davis, B. C., ~~and~~ Fourqurean, J. W. (2001). Competition between the tropical alga, *Halimeda incrassata*, and the seagrass, *Thalassia testudinum*. *Aquatic Botany*, 71(3), 217–232.
[https://doi.org/10.1016/S0304-3770\(01\)00179-6](https://doi.org/10.1016/S0304-3770(01)00179-6)

Commented [A14]: italicize through where necessary

Done, T. J., (1992). Phase shifts in coral reef communities and their ecological significance. *Hydrobiologia* 247, 121-132

Doty, M. S., & Stone, B. C. (1966). Two new species of *Halophila* (Hydrocharitaceae). *Brittonia*, 18(4), 303–306. <https://doi.org/10.2307/2805146>

Epstein, G., & Smale, D. A. (2017). *Undaria pinnatifida*: A case study to highlight challenges in marine invasion ecology and management. *Ecology and Evolution*, 7(20), 8624–8642.
<https://doi.org/10.1002/ece3.3430>

Fukunaga, A. (2008). Invertebrate community associated with macroalga *Halimeda*

Gibson, V. L., A. ~~Dedloff~~, ~~L. J.~~ ~~Dedloff~~, ~~L. J.~~ Miller, & C. M. Smith. (2024). Integrated physiological response by four species of Rhodophyta to submarine groundwater discharge reveals complex patterns among closely-related species. *Sci Rept* 14(23547)
<https://doi.org/10.1038/s41598-024-74555-6>

Guiry, M. D. ~~and~~ ~~&~~ G. M. Guiry. (2024). AlgaeBase. World-wide electronic publication, Galway. <https://www.algaebase.org/>; searched on 9/20/2024.

~~Hao H. Sun, V. J. Paul, W. Fenical. 1983. Avrainvilleol, a brominated diphenylmethane derivative with feeding deterrent properties from the tropical green alga Avrainvillea longicaulis. Phytochemistry 22(3) 743–745~~

Hillis-Colinvaux, L. (1974). Productivity of the coral reef alga *Halimeda* (Order Siphonales). *Proceedings of the Second International Coral Reef Symposium 1. Great Barrier Reef Committee, Brisbane, Australia 1974*, (1) 35–41

Hillis-Colinvaux, L. (1988). Characteristics of *Halimeda* meadows, with emphasis on a meadow near Eniwetok islet, Eniwetok atoll, (Marshall Island). *Proceedings of the 6th International Coral Reef Symposium, Australia, 1988*, (3) 119–123

- Huisman, J. H., ~~L.A. Abbott, I.A., and C.M. Smith, C.M.~~ (2007). *Hawaiian reef plants*. University of Hawai'i Sea Grant Program, pp. ~~74-75,###~~
- Jaubert, J., Chisholm, J., Minghelli-Roman, A., Marchioretti, M., Morrow, J., & Ripley, H. (2003). Re-evaluation of the extent of *Caulerpa taxifolia* development in the northern Mediterranean using airborne spectrographic sensing. *Marine Ecology Progress Series*, 263, 75–82. <https://doi.org/10.3354/meps263075>
- Langston, R. C., & Spalding, H. L. (2017). A survey of fishes associated with Hawaiian deep-water *Halimeda kanaloana* (Bryopsidales: Halimedaceae) and *Avrainvillea* sp. (Bryopsidales: Udoteaceae) meadows. *PeerJ*, 5, e3307. <https://doi.org/10.7717/peerj.3307>
- Les, D. H., Cleland, M. A., & Waycott, M. (1997). Phylogenetic studies in Alismatidae, II: Evolution of marine angiosperms (seagrasses) and hydrophily. *Systematic Botany*, 22(3), 443–463. <https://doi.org/10.2307/2419820>
- Littler, M. M., Littler, D. S., & Brooks, B. L. (2006). Harmful algae on tropical coral reefs: Bottom-up eutrophication and top-down herbivory. *Harmful Algae*, 5(5), 565–585. <https://doi.org/10.1016/j.hal.2005.11.003>
- Littler, M. M., Littler, D. S., & Titlyanov, E. A. (1991). Comparisons of N- and P-limited productivity between high granitic islands versus low carbonate atolls in the Seychelles Archipelago: A test of the relative-dominance paradigm. *Coral Reefs*, 10(4), 199–209. <https://doi.org/10.1007/BF00336775>
- Martinez, J. A., 2012. The physical and physiological impacts of the invasive red macroalga *Gracilaria salicornia* Dawson to coral reef habitat quality and coral health. Ph. D. Dissertation, Botany. University of Hawai'i at Mānoa. Honolulu, Hawai'i, pp 1 – 8, [115](#) ~~pages usually cite # pages for whole doc~~
- Nordlund-L. M., E. W., Koch, E. B., Barbier, J. C., Creed, Koch, ~~E. W., Barbier, E. B., & Creed, J. C.~~ (2017) Correction: Seagrass Ecosystem Services and Their Variability across Genera and Geographical Regions. *PLOS ONE* 12(1): e0169942. <https://doi.org/10.1371/journal.pone.0169942>
- Mumby, P. J. (2009). Phase shifts and the stability of macroalgal communities on Caribbean coral reefs. *Coral Reefs*, 28(3), 761–773. <https://doi.org/10.1007/s00338-009-0506-8>
- Mumby, P. J., Hastings, A., & Edwards, H. J. (2007). Thresholds and the resilience of Caribbean coral reefs. *Nature*, 450(7166), 98–101. <https://doi.org/10.1038/nature06252>
- NOAA Benthic Habitat Mapping of Main Hawaiian Islands. NCCOS Project <https://coastalscience.noaa.gov/project/benthic-habitat-mapping-main-hawaiian-islands>.

- Pereda-Briones, L., Tomas, F., & Terrados, J. (2018). Field transplanted of seagrass (*Posidonia oceanica*) seedlings: Effects of invasive algae and nutrients. *Marine Pollution Bulletin*, 134, 160–165. <https://doi.org/10.1016/j.marpolbul.2017.09.034>
- Peyton, K. 2009. Aquatic invasive species impacts in Hawaiian soft sediment habitats. Ph.
- Smith, J. E., Hunter, C. L., Conklin, E. J., Most, R., Sauvage, T., & Squair, C. & Smith, C. M. (2004). Ecology of the invasive red alga *Gracilaria salicornia* (Rhodophyta) on O'ahu, Hawai'i. *Pacific Science*, 58(2), 325-341
- Smith, J. E., Hunter, C. L., & Smith, C. M. (2010). The effects of top-down versus bottom-up control on benthic coral reef community structure. *Oecologia*, 163(2), 497–507. <https://doi.org/10.1007/s00442-009-1546-z>
- Smith, J., Smith, C., & Hunter, C. (2001). An experimental analysis of the effects of herbivory and nutrient enrichment on benthic community dynamics on a Hawaiian reef. *Coral Reefs*, 19(4), 332–342. <https://doi.org/10.1007/s003380000124>
- Smith, S. V. (1981). Responses of Kaneohe Bay, Hawaii, to relaxation of sewage stress. In B. J. Neilson & L. E. Cronin (Eds.), *Estuaries and Nutrients*, pp. 391-410. Humana Press. https://doi.org/10.1007/978-1-4612-5826-1_18
- Stimson, J. (2015). Long-term record of nutrient concentrations in Kaneohe Bay, O'ahu, Hawai'i, and its relevance to onset and end of a phase shift involving an indigenous alga, *Dictyosphaeria cavernosa*. *Pacific Science*, 69(3), 319–339 <https://doi.org/10.2984/69.3.3>
- Stimson, J. (2018). Recovery of coral cover in records spanning 44 yr. for reefs in Kaneohe Bay, O'ahu, Hawai'i. *Coral Reefs*, 37(1), 55–69. <https://doi.org/10.1007/s00338-017-1633-2>
- Sun, H. H., V. J. Paul, W. Fenical. (1983). Avrainvilleol, a brominated diphenylmethane derivative with feeding deterrent properties from the tropical green alga *Avrainvillea longicaulis*. *Phytochemistry*, 22(3) 743 - 745
- Thibaut, T., A., Meinesz, P., Coquillard (2004). Biomass seasonality of *Caulerpa taxifolia* in Mediterranean Sea. *Aquatic Botany*, 80(4), 291-297
- Tussenbroek, B. I., Van, Vonk, J. A., Stapel, J., Erfteimeijer, P. L. A., Middelburg, J. J., & Zieman, J. C. (2006). The biology of *Thalassia*: Paradigms and recent advances in research. In *Seagrasses: Biology, ecology and conservation*. (pp. 409–439). Springer Netherlands. https://doi.org/10.1007/978-1-4020-2983-7_18

- Van De Verg, S. E., & Smith, C. M. (2022). Protocol to control the invasive alga *Avrainvillea lacerata* in a shallow Hawaiian reef flat. *Applications in Plant Sciences*, 10(4), e11490. <https://doi.org/10.1002/aps3.11490>
- Vroom, P. S., & Smith, C. M. (2003). Life Without Cells. *Coral Reef Ecosystem Investigation* ~~###~~ University of Hawai'i, Honolulu, USA. *Biologists*, 50(5) 222 - 226
- Vroom, P. S., Smith, C. M., Coyer, J. A., Walters, L. J., Hunter, C. L., Beach, K. S., & Smith, J. E. (2002). Field biology of *Halimeda tuna* (Bryopsidales, Chlorophyta) across a depth gradient: Comparative growth, survivorship, recruitment, and reproduction. *Hydrobiologia*, 501, 149 - 166
- Wade, R., Spalding, H., Peyton, K., Foster, K., Sauvage, T., Ross, M., & Sherwood, A. (2018). A new record of *Avrainvillea* cf. *erecta* (Berkeley) A. Gepp & E.S. Gepp (Bryopsidales, Chlorophyta) from urbanized estuaries in the Hawaiian Islands. *Biodiversity Data Journal*, 6, e21617. <https://doi.org/10.3897/BDJ.6.e21617>
- Waycott, M., Duarte, C. M., Carruthers, T. J. B., Orth, R. J., Dennison, W. C., Olyarnik, S., Calladine, A., Fourqurean, J. W., Heck, K. L., Hughes, A. R., Kendrick, G. A., Kenworthy, W. J., Short, F. T., & Williams, S. L. (2009). Accelerating loss of seagrasses across the globe threatens coastal ecosystems. *Proceedings of the National Academy of Sciences*, 106(30), 12377–12381. <https://doi.org/10.1073/pnas.0905620106>
- Weijerman, M., Most, R., Wong, K., & Beavers, S. (2008). Attempt to control the invasive red alga *Acanthophora spicifera* (Rhodophyta: Ceramiales) in a Hawaiian fishpond: An assessment of removal techniques and Management Options. *Pacific Science*, 62(4), 517–532. [https://doi.org/10.2984/1534-6188\(2008\)62\[517:ATCTIR\]2.0.CO;2](https://doi.org/10.2984/1534-6188(2008)62[517:ATCTIR]2.0.CO;2)
- Williams, S. L. (1990). Experimental studies of Caribbean seagrass bed development. *Ecological Monographs*, 60(4), 449–469. <https://doi.org/10.2307/1943015>
- Williams, S. L. (2007). Introduced species in seagrass ecosystems: Status and concerns. *Journal of Experimental Marine Biology and Ecology*, 350(1–2), 89–110. <https://doi.org/10.1016/j.jembe.2007.05.032>
- Williams, S. M. (2022). The reduction of harmful algae on Caribbean coral reefs through the reintroduction of a keystone herbivore, the long-spine sea urchin *Diadema antillarum*. *Restoration Ecology*, 30(1), e13475. <https://doi.org/10.1111/rec.13475>
- Williams, S., & Schroeder, S. (2004). Eradication of the invasive seaweed *Caulerpa taxifolia* by chlorine bleach. *Marine Ecology Progress Series*, 272, 69–76. <https://doi.org/10.3354/meps272069>

CHAPTER 2. TESTING NOVEL TECHNIQUES TO MANAGE *Avrainvillea erecta* IN COASTAL WATERS OF O'AHU

ABSTRACT

Marine algal introductions threaten the biodiversity of Hawaiian reefs. With heavy shipping traffic occurring on the south shore of O'ahu, plants are transported to near shore habitats. Coastal development and extreme use have impacted the native benthic communities. Psammophytic, or soft bottom habitats occur adjacent to Hawai'i fringing reefs, starting at about 15-18 ~~m~~ meters depth. Sea grass / algal meadows adjacent to coral reefs occupy as much as 50 % of benthos in Hawaiian shallow waters (<30 m). This study focuses on the meadows formed within these habitats. A newly introduced species of green alga called *Avrainvillea erecta* has been establishing and spreading in these meadows. It is the goal of this research to monitor the growth and spread of *A. erecta* and investigate management techniques to control its populations. Physical removal treatments were applied in 2020-2022. None of the removal treatments were able to significantly reduce the recovery of the plant. However, initial abundance positively influenced the number of plants that did recover in a 1m² plot. Chemical management, using 10 ml of 3% hydrogen peroxide, were injected into plants at the Ānuehue Fisheries Research Center (AFRC) mesocosms and *in situ* off Kewalo Basin O'ahu. While the mesocosm experiment did not result in a significant outcome, the [*in-situ-in-situ \[does this combine Ala Moana and Ke'ehi sites?\]*](#) experiment did result in a significant negative impact on plant densities. -After analyzing these results and monitoring the spread of this plant across the main Hawaiian Islands, it is advised to categorize this plant as invasive. Areas frequented by boat anchors are at risk of transporting fragments of plants that can still be viable and propagate in new areas. Hawaiian shores that do not receive an annual storm surge should also be monitored. These quiet water locations specifically within Maui Nui complex are at high risk for invasion by *A. erecta*. Early detection via monitoring and rapid response appears to be the most critical action managers should consider.

INTRODUCTION

The Hawaiian archipelago, located in the North Pacific is 43,000 km from North America and 64,000 km from Japan (Coles et al. 1999). The species found here today exist from a limited number of colonization events that resulted in reproductive isolation from other populations (Price 2004). Over time these species evolved, adapted to Hawaiian habitats and new species emerged. Adaptive radiation is the divergence from an ancestral species to unique descendants that can better exploit different ecological opportunities (Glor 2010). Thus, not surprisingly, Hawaiian ecosystems have high rates of endemism in terrestrial and aquatic ecosystems with species that occur nowhere else in the world (Eldredge and Smith, 2021, Smith et al. 2004) For a long time, many Hawaiian marine ecosystems were thought to be much less diverse than similar reefs found in the South Pacific (Tsuda, 2014). Recent studies of deeper water algae (Sherwood and Guiry, 2023, Pyle et al. 2016) have flipped that assessment; new genera and species of algae from these depths have been estimated as high as 40 % of the deeper water flora.

Because native species in Hawaiian marine ecosystems evolved without aggressive non-native species, natives may not be as effective when competing for resources. When released from predators in home ranges, many non-native species benefit from reduced grazing or other biotic interactions; these changes manifest as advantages, allowing non-natives to again outcompete native species even in native ecosystems (Davidson et al. 2018; Davidson and Shoemaker 2023). In addition to the timing or repetitive introduction of an introduced species, introduced species may also have broader habitat tolerances from their home regions as well as higher reproductive output because changes in grazing / predation pressures, that further their success rate in a new environment (Carlton 1996, Inglis et al. 2006; Williams and Smith 2007).

In particular, because the Hawaiian flora lacks large canopy-forming Submerged Aquatic Vascular species (SAV) such as *Thalassia*, competition among the existing functional groups, e.g. macro and turf algae present in Hawaiian marine settings can be seen as fundamentally distinct from successional trajectories in other Pacific Islands. The consequences of this usual disharmonic distribution of species (Carlquist 1966, Midway and Hodge 2012) may have shaped shallow-water coastal communities across the Hawaiian archipelago. Finally, because of this combination of factors, Hawaiian reefs are susceptible to marine invasions.

Human impacts over the last century have likely accelerated invasions in two ways. First, non-native tropical and subtropical plants that are associated with aquaculture are purposefully introduced with intentions to farm them (*Gracilaria tikvahiae*; species of *Eucheuma* and

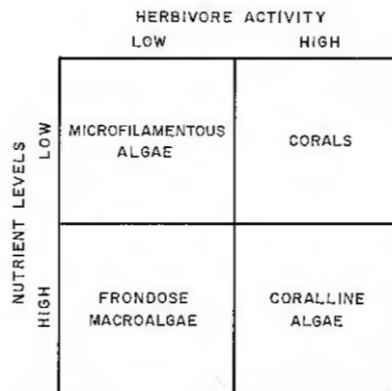
Kappaphycus). Secondly, species that were intentionally introduced to our reefs for fisheries (invasive fish, Peacock Hind or Roi (*Cephalopholis argus*; Giddens et al. 2014) Ta'ape (*Lutjanus kasmira*; Gaither 2012; The Blacktail Snapper, To'au (*Lutjanus fulvus*; Gaither et al. 2012)). These introductions have substantial continued impacts [\(see citations above\)](#).

An underappreciated vector [for the spread of invasive algae](#) is transportation associated with commercial maritime activities including entanglement with fishing or anchoring gear, marine debris, hull fouling and solid ballast (Carlton 1994). The most recent example is *Chondria tumulosa* - a newly described species that may have arrived in Papāhānaumokuākea Marine National Monument, via marine debris, or other flotsam (Sherwood et al., 2020; Fumo et al. 2024).

RELATIVE DOMINANCE PARADIGM

The photosynthetic benthic communities of tropical reefs compete for abiotic factors; the type of substrate for settlement, sunlight for photosynthesis, availability to nutrients for growth. Disturbances such as intensity of herbivory or wave action as well as nutrient availability combine to create a four-state matrix first proposed by Mark and Diane Littler (Littler and Littler 1984), now known as the Relative Dominance Paradigm (RDP). Complex benthic communities found on shallow reefs were predicted to fall into one of four states based on two levels of these two factors (Figure 1; from Littler and Littler 1984).

Figure 1. The Relative Dominance Model – Four potentially predominant space-occupying groups of sessile reef organisms are hypothesized to interact as a function of (1) long-term nutrient levels, referred to as bottom-up factors and (2) disturbances such as herbivore activity, referred to as top-down factors. The latter is considered the more important direct controller of standing stocks on undisturbed reefs (Littler and Littler 1984)



A later version of the RDP included intense physical disturbances with the removal of biomass via herbivory (Littler and Littler 1985). While physical disturbances such as wave force can remove plants from a habitat, this force is neutral in the sense that the energy that disrupts a plant is not selective based on palatability or other physiological traits of the plant. Palatability, however, is a consideration with herbivory (Weijerman et al. 2008, Van De Verg and Smith 2022). Both removal types, neutral and biological are complex interactions that could

provide strong selective pressures in longer term evolution of psammophytic (or sand dwelling) species.

Considering the RDP for reef taxa, we can expect that selective responses to tolerate high wave energy combined with elevated nutrients could favor the dominance of heavily calcified algae such as crustose coralline algae as well as articulated coralline algae. Selective responses to withstand intense herbivory of reef plants (but not wave energy) and ambient (low) nutrient inputs could favor branching corals. Selective responses to intense herbivory (but not wave energy) and ambient nutrients could favor green, brown and red algal turfs. Finally, selective responses to low herbivory or wave energy and elevated nutrients could favor green, brown and red macroalgae (Littler and Littler 1984, 1985). While Smith et al. (2001, 2010) support the general application of RDP, there has been little crossover of this paradigm to the psammophytic community. Are there other factors that we can identify that favor functional forms that dominate sandy communities?

MEADOW SUPPRESSION HYPOTHESIS

Meadow Suppression Hypothesis was first stated by Fourqurean (Davis and Fourqurean, 2001) and proposed that the evolutionary success of SAVs was in part the evolution of traits that enabled SAVs to outcompete the diverse and well-established 650-million-year-old bryopsidalean species (Peyton 2009, Tussenbroek et al. 2006, Williams, 1990).

Previous work on O'ahu comparing the competitive nature of *Halophila* with *Avrainvillea* supported the Meadow Suppression Hypothesis (Peyton 2009). Some of these traits could be broader and more efficient photosynthetic ability, seed storage and nutrient uptake from roots (Les et al. 1997). However, the local *Halophila* species are small in stature and have been in decline around O'ahu's nearshore reefs (Peyton 2009, [WacottWaycott et al. 2009](#)). It is not clear if species in this genus are able to out compete plants in the genus *Avrainvillea* in the long term. Additionally, the increased range of large stature *Halimeda kanaloana* now present on O'ahu's south shore increases the competition; *H. hawaiiiana* is now competing with two much taller, canopy-forming algae. The seagrass reaches no more than 5cm tall while both algal species are upwards of 30 cm tall (Wheeler, pers. obs.). In Hawai'i, these seagrasses are understory to algal canopies (Davis and Fourqurean, 2001), reversing the traditional roles formed in the Meadow Suppression Hypothesis. However, the large holdfast of these algae and their overstory could also provide sediment stabilization and reduce wave energy to benefit the seagrass. It could also provide physical structure to enhance root development and anchoring (Pereda-Briones [et al. 2018](#)). Monitoring the change in plant density over time will help us understand how these three species interact in this novel community and if one becomes dominant over the others.

PLANT BIOLOGY

Individual plant biology within the ecosystem plays a role in the success of the competitive dominance between species. *Avrainvillea erecta* has a very different blade structure than *Halimeda kanaloana*. *A. erecta* is phenotypically plastic and can present as a blade like paddle that is leathery and spongy, or as an assemblage of diffuse siphons that is more like *Bryopsis* (Wade et al 2018, Peyton and Wheeler field observations). When observed under a dissecting microscope, blades provide habitat for annelids and mollusks, yet do not seem to collect epiphytic plants. A study in Belize on different *Avrainvillea* species showed that the plants readily abandon and re-grow their blades to rid them of epiphytes through cytoplasmic streaming and translocation of chlorophyll through their siphons (Littler and Littler, 2004). This contributes to the idea that these plants readily undergo asexual propagation and are clonal in Hawai'i. One stipe may appear to have lost its blade while a stipe with a new blade will have emerged next to it. Upon digging up the two plants it is common to find their underground holdfasts attached. This would be a form of asexual propagation, where adult individuals can bud a new plant from their thalli. This is supported by the Perennation Hypothesis from Littler and Littler, adding to a robust and bulbous holdfast that acts as a storage organ that has subterranean rhizoids that spread out laterally connecting many plants together (Littler and Littler 2004). Similar observations were made by Wheeler, when first examining *A. erecta* near Ke'ehi channel. Up to nine individual stipes were connected below the sand by rhizoids. The viability of the below ground rhizoidal connections to bud new plants after large disturbances is part of the current goals of this study to understand.

This blade abandonment trait is not shared by the *H. kanaloana* which co-occurs with *A. erecta*. The native *H. kanaloana* attracts many epiphytes and becomes laden with numerous species. However, the reproductive strategy of *H. kanaloana* is holocarpic, which means the entire plant dies after the gametangia are released. (Verbruggen et al 2006) *H. kanaloana* plants can also reproduce via asexual propagation and fragmentation. It appears that they are multiyear plants until they become fertile (Drew and Able 1988, Verbruggen et al 2006). After the photosynthetic gametes are released all that is left is the white calcium carbonate blades that then contribute to sand production (Drew and Able 1988). *H. kanaloana* meadows spread quickly and use both asexual and sexual reproduction strategies. The cues for sexual reproduction of *H. kanaloana* are not part of this study however understanding the mechanisms that initiate this behavior would be important to the whole patch dynamics of this psammophytic ecosystem.

PHOTOSYNTHESIS AND THE LIGHT REACTIONS

Light penetration and harvesting are one factor that marine plants must compete for. Photosynthetic Active Radiation (PAR) is the irradiance that a plant can absorb and use for photosynthesis (Davis and Fourqurean 2001). It is also referred to as actinic light – light that can

drive photochemistry. Plants are able to absorb within the visual spectrum between 400 and 700 nm. The light seen at 400 nm appears blue to violet in color to the human eye. These are shorter wavelengths that penetrate more deeply into coastal waters; from 600 to 700 nm are orange, yellow and red wavelengths. These wavelengths are longer, have less energy and are absorbed by water as light penetrates.

When light hits the water, attenuation occurs exponentially with depth, according to the Lambert-Beer Law:

$$I_d = I_0 e^{-kd}$$

Where d is depth and I_0 is the incident radiation upon the surface of the water, I_d is the photon flux density at depth and k_d is an extinction coefficient. As light waves pass through the water the lower energy light, red, orange, yellow (650-700nm) are lost at about 10 m (Krause and Weis 1991). The plants in the psammophytic habitat thrive at 20m and below. The spectrum of light they receive is between 400-and 500nm. This type of light limitation affects plants photosynthetic output. When plants photosynthesize, the chlorophyll or other pigments can absorb only a portion of the light spectrum which dictate if it thrives in “sun” high light, or “shade”, low light (Hamel and Smith 2020). These pigments are located within the chloroplast. Photons of light energy absorbed by pigments such as chlorophyll require the cooperation of two complexes, known as the antenna complex, before they can be converted and stored as chemical energy. The antenna complex is where the pigments absorb and transfer the light energy to the reaction center where chemical oxidation and reduction reactions take place to form the energy building blocks NADPH and ATP (Richardson et al. 2021). Within the reaction center there are two photosystems, Photosystem II (PSII) and Photosystem I (PSI). PSII is important because when the photons are absorbed within the antennae complex this system produces a strong oxidant that strips an electron from the water molecule, this releases 4 electrons that are carried from PSII to PSI through the Electron Transport Reaction (ETR) where they are eventually converted to NADPH and ATP (Krause and Weis 1991). This process allows plants to use the sun’s energy with the available water to create and store carbon in the form of sugars and starch. The higher the rate of maximal electron transport via photosynthesis, the more energy it can produce to grow and reproduce. As marine plants compete in low light areas the Electron Transport Rate is important to maximize energy production. In this study we will use maximum capacity of the Electron Transport Rate in a calculation called ETR_{max} using Pulse Amplitude Modulated chlorophyll fluorometry (PAM). This device allows us to estimate the plants’ photosynthetic ability after different treatments in the lab and *in situ*.

Hydrogen peroxide is a source of Reactive Oxygen Species (ROS) that can damage the membranes within the photosynthetic organelle of plants. Hydrogen peroxide also passes quickly through membranes by diffusion, inhibits many of the enzymes within the electron

transport system and damages the cellular structures of lipids, proteins, and nucleic acids leading to cell death (Dummermuth et al. 2003). Hydrogen peroxide was tested on the species *Avrainvillea lacerata* J. Agardh, another invasive green alga to Hawai'i. In a study by Scott Van De Verg, both 10% and 3% H₂O₂ had a negative effect on the output of rETR_{max}. Both 10% and 3% concentrations were able to impair the plant to the point that the plants lost their blades (Van De Verg and Smith 2022). This study builds upon the results that 3% H₂O₂ at 10 ml volumes can damage *Avrainvillea erecta* and be used to control population densities.

RESEARCH FOCUS

Guided by the possibility that *A. erecta* has invasive qualities similar to other weedy species such as *A. lacerata*, I anticipate that *A. erecta* will increase in per-cent cover and eventually dominate available niche space regardless of the occurrence of *Halophila spp.* and *Halimeda kanaloana*. In this thesis I propose to test two emerging approaches to examine vulnerabilities of *A. erecta* through manipulations that could aid management. In this way, this thesis provides context for the possibility that *A. erecta* poses a threat to the existing meadows of native *H. kanaloana* and *H. hawaiiiana*.

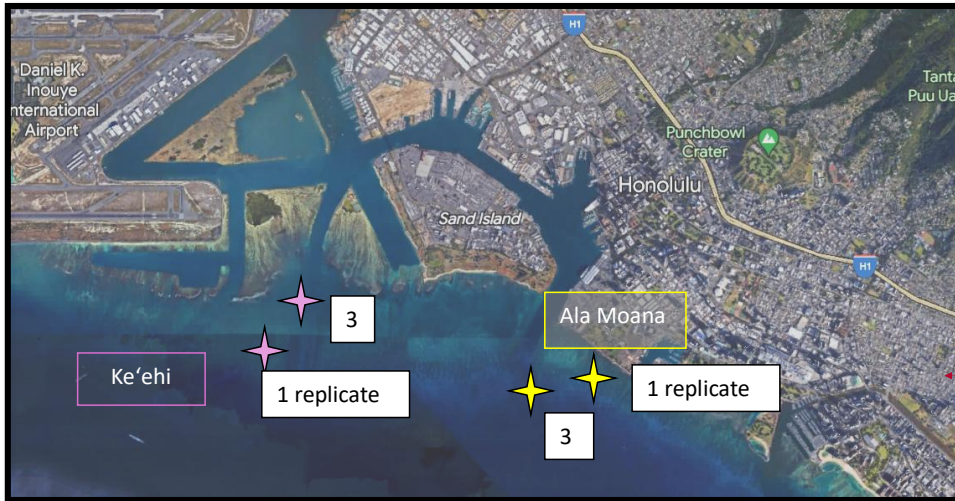
This work will be guided by three research hypotheses:

- 1) *A. erecta* will not grow back after its blade or stipe have been removed.
- 2) If the plants' holdfast is removed, *A. erecta* will not grow back from remnant rhizoids.
- 3) If hydrogen peroxide is injected into *A. erecta*, *A. erecta* will sustain long-term (> 1 wk) reductions in photosynthesis.

MATERIALS AND METHODS

STUDY AREA

In the fall of 2018, we began our reconnaissance of the south shore of O'ahu for *Avrainvillea erecta* based on sightings that had been reported earlier that year from colleagues in the field. We systematically searched from about 13 to 25 m depths between Ala Wai channel and Ke'ehi channel (approx. 10 km). From this extensive survey we chose our locations to implement the physical removal experiment in the Fall of 2019. Two depths, 20 and 25 m, were chosen based on the plant's abundance (Figure 2). These were where we observed the plants to be well established and were within reasonable diving accessibility. The two depths also accounted for potential differences in light attenuation and wave energy. At each depth four transects were established spanning from Ala Wai to Ke'ehi channel. We chose to span across that area of the south shore to capture different influences of wave events. The sites also are on either side of the original sighting of *A. erecta* which was Honolulu Harbor.



Formatted: Centered

Figure 2. Locations for two research sites on the south shore of O‘ahu. Ala Moana reef has two locations marked in yellow: **20 m**: N 21° 16.6323', W 157° 51.071'; at **25 m**: N21° 16.179, W 157° 51.302'). Ke‘ehi reef has two locations marked in purple: **20 m**: N 21° 17.236' W 157° 53.904'; at **25 m**: N 21° 17.173' W 157° 53.941. Each star represents a meadow that received treatments. Stars that are near shore are 20m depth and the offshore stars are 25m depth respectively.

Commented [KP15]: You named each meadow or site. Consider adding labels to designate Ala Moana from Keehi on the image. Consider using a different color for the meadow discovered later in the research.

PHYSICAL REMOVAL EXPERIMENT

Four replicate transects were established at 20 and at 25m along O‘ahu’s south shore. The number of replicates of treatments were unevenly distributed due to meadow density, size of a meadow and dive team accessibility. Because of this, three replicate transects were placed at Ke‘ehi 20 m, and Ala Moana 25 m, and only one replicate transect was established at Ke‘ehi, 25 m and Ala Moana 20 m. The sites located in front of Ke‘ehi Harbor were monitored by Division of Aquatic Resources (DAR), Department of Land and Natural Resources (DLNR), State of Hawai‘i. The 20 and 25 m depths in front of Ala Moana Beach Park were monitored by University of Hawai‘i in compliance with the University of Hawaii Diving Safety Program. The transect locations were marked with GPS (Figure 2). At each transect, four different treatments of physical removal were tested as possible management tools for use with the invasive green alga *A. erecta*. Each treatment was imposed on all *A. erecta* plants above 1 cm in stature within a 1 m² quadrat. Each treatment physically removed a portion of the plant and measured effectiveness and possible rate of regrowth after treatment.

Treatments for Physical Removal Experiment (Figure 3.)

Treatment 1: Cut algae blade off above the top of the stipe shown in Figure 3, includes removal of photosynthetic portion of above ground biomass, leaving stipe and below ground biomass in place.

Treatment 2: Removal of the stipe and blade Shown in Figure 3, includes removal of all above ground biomass, leaving below ground biomass intact.

Treatment 3: Removal of the entire plant shown in Figure 3, includes removal of above ground and below ground biomass, but not all the rhizoids.

Treatment 4: Procedural Control Shown in Figure 3, includes physically touching the blades between thumb and pointer finger and gently rubbing them. Mimicking other disturbances created by divers deploying the experiment such as sediment movement, kneeling, laying out transect, and handling of thalli.

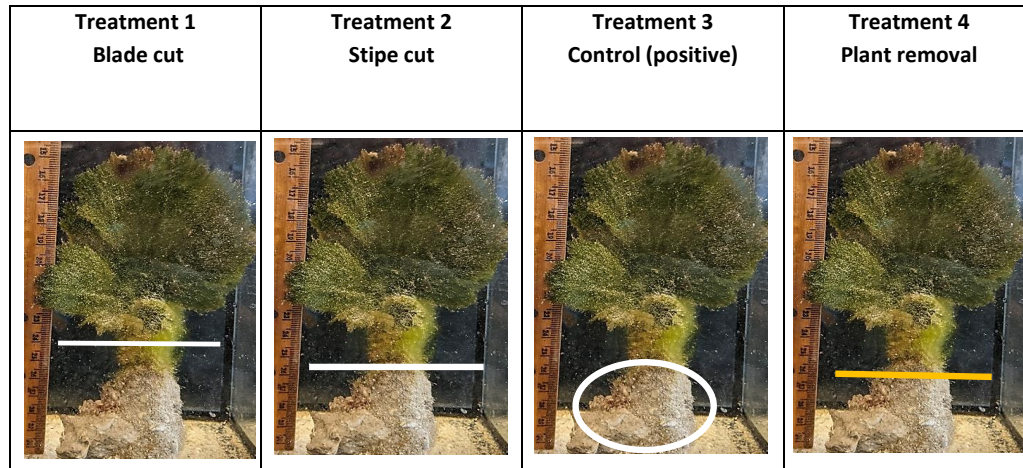


Figure 3. Four treatments were imposed on plants to test the effectiveness of physical removal as management tool to control *Avrainvillea erecta*. Treatment #1 Cut blade above the stipe, Treatment #2 Cut stipe just above the sand, Treatment #3 Pull the entire holdfast out of the sand removing the whole plant, and Treatment #4 Rub blade between fingers: Control.

Site Set up and follow up for physical removal experiment

A full-size concrete block was placed at depth, for 25 months. Each concrete block had laminated tags for identification and marked the start of each transect/s. From the concrete block the 30m transect was deployed on a bearing that traversed through the meadow (bearing changed with each site). At the end of the 30m transect, a corkscrew dog stake was inserted into the sand with a floating buoy attached. The bearing for that specific transect was recorded to reestablish it at each follow up dive. Keehi 20 and Ala Moana 25 both had two additional transects running perpendicular to the primary transect. These were placed at 15m and 25m marks along the transect to avoid proximity to any quadrats.

Once the transect was established, a 1m² quadrat was placed every 10 meters, in total each transect received four quadrats. One of the four treatments were applied within each quadrat. Custom made 0.75m long aluminum with ¼ inch diameter were hammered into the deep sand to mark the corners of each of the quadrats. Two photos were taken before the treatment was applied. One photo was taken from approximately 1 m away from the top view and one photo from the side view at the level of the sandy substrate. The same photos were repeated on every follow-up dive to visually capture the change in the benthic composition. Plants of *A. erecta* were counted and the average height was measured by using a ruler to measure at least 1/3rd of the plants present and then averaged the height during data entry. Within the quadrat the percent cover of *Halophila hawaiiiana* and the number of co-occurring *Halimeda kanaloana* were also enumerated. Follow up dives planned to occur monthly and spanned across 25 months.

The COVID pandemic did present some challenges with consistency of sampling, which left some gaps in the first year of data collection when all diving activity was halted until safe procedures were established. Because we were interested in seasonal effects, we extended our data collection out an extra year (Table 2.). Our original goal was 11 months of consistent sampling however, we ended up with 25 months. By extending data collections, objectives were met by capturing seasonal changes, periods of growth and senescence among the three co-occurring dominant species of this benthic community.

Commented [KP16]: For the duration of the experiment?

Commented [KP17]: For how long?

Commented [KP18]: Diameter?

Commented [KP19]: Consider providing more details of how this was done, unless you have it explained elsewhere.

Commented [KP20]: Consider including dates of the start of the experiments, could be a table. Ignore if you have this information elsewhere.

Table 1. The dates that the physical removal experiment was conducted and when the COVID-19 shutdown overlapped and when dive restrictions were implemented and then removed.

						Key for colors:						
						Start/End	Normal Dive	No Diving	Restricted			
Year	Jan	Feb	March	April	May	June	July	Aug	Sep	Oct	Nov	Dec
2020	Start data 1/5	Normal diving	Covid no diving	Covid no diving	Covid no diving	Covid diving	Covid diving	Covid diving	Covid diving	Covid diving	Covid diving	Covid diving
2021	Covid diving	Covid diving	Covid diving	Covid diving	Covid diving	Covid diving	Covid diving	Normal diving	Normal diving	Normal diving	Normal diving	Normal diving
2022	Normal diving	Normal diving	Normal diving	End data 4/22								

METHODS FOR HYDROGEN PEROXIDE APPLICATION IN MESOCOSM

The Ānuenuue Fisheries Research Center (AFRC) run by Department of Aquatic Resources (DAR) is located on Sand Island, Oʻahu. Partnership between invasive seaweed research in the Limu Lab, University of Hawaiʻi and DAR has allowed student access to the facilities to run experiments using an Open Flow Sea Water System designed after another flow through mesocosm built for algal studies (e.g. Larned 1990). The AFRC system pulls water from 8 m below the surface of Honolulu Harbor. Water is pumped through a sand filter before it is filtered again through a three-stage cartridge filter system of 5, 20 and 20 μm filters. After filtration, water runs through five different headers that lead to 2-inch PVC piping that is perforated with 1/8 in flexible black tubes that deliver the water into desired aquaria.



Figure 4. Facilities at AFRC Mesocosm use an Open Flow Sea Water System. From left to right; Pump house that takes in sea water from an 8 m depth. Mesocosm with PVC piping to distribute filtered sea water. Aquaria within mesocosm that housed plants during experiment.

One mesocosm was filled with sea water. Within that mesocosm, eight 1 gal aquaria were placed inside and also filled (Figure 4). Aquaria were constantly filled and spilled into the mesocosm which drained back into the harbor. Each aquarium was used to house one *A. erecta* plant. This isolated the plants from each other but kept them all at a constant temperature. Two layers of 1 mm black mesh net were placed over the bath to mimic the same Photosynthetic Active Radiance (PAR) the plants received at depth (see Li Cor measurements [section for procedures](#)).

Li Cor measurements

The LiCor 1500 data logger with a [LiCor](#) 4 pi sensor Logger (SPQA 6035) and a 30 m cable were used to measure PAR on Mar 5, 2021. The LiCor 1500 sensor was attached to a metal rigging that allowed the sensor to sit upright as it was lowered overboard the 19ft UH Boston Whaler at the Ala Moana 20 m site. The conditions for the day were fair, light winds, calm seas, mostly clear with occasional clouds. At 13:52 we deployed the sensor. At 10 m we obtained an average reading of 538.6 PAR. The same sensor and logger were used at AFRC to quantify the

PAR in each aquaria filled with seawater within the mesocosm under the 1mm shade tarp that we used to cover the plants. This allowed us to approximate the PAR the plants received at depth.

Plant collection/ injection procedures

During field observation days, plants that were not in the experimental quadrats were collected at a minimum of 5m away from the transect line and placed into a zip lock bag and then placed into a black plastic bag to avoid sun overexposure. Upon surfacing the plants were put into a cooler filled with sea water and then taken to AFRC. Plants were collected around experimental sites for Ke'ehi 20, Ala Moana 20 and Ala Moana 25 meters. In total 24 plants were collected 8 at each site. Each set of 8 plants were monitored for three weeks. After the plants were collected, they were given three days to acclimate in the mesocosm. On the fourth day their photosynthetic output was measured using the Jr. Walz Pulse Amplitude Fluorometer (Jr. PAM). The Jr. PAM measures the Electron Transport Rate (ETR_{max}) among many other parameters. This rate helps to understand the plant's capacity to photosynthesize (See section *Photosynthesis and Light reactions*). After this initial measurement four of eight plants had 10 ml of sea water taken directly from the filtered mesocosm and injected into their thalli just below the blade of the plant. This acted as a control for effects of the puncture on plant health and osmoregulation. The other four plants were injected with 10ml of 3% hydrogen peroxide (See *Dilution of H_2O_2 and applicator, below*). Jr. PAM was used to measure ETR_{max} , four days after both injections. Photographs and ETR_{max} measurements were taken twice a week for three weeks, ~ approx. four days apart. A total of 12 plants were injected with 3% H_2O_2 and a total of 12 plants were injected with filtered Sea Water.

METHODS FOR HYDROGEN PEROXIDE APPLICATION *IN SITU*

Site Set up

In the summer of 2021, a new meadow was discovered at 23 m off the Kewalo channel (Fig 5.). This site was thick with all three species of plants (*Avrainvillea erecta*, *Halimeda kanaloana*, *Halophila hawaiiiana*). It was also an in between depth from the other two sites at 23 meters. This gave an opportunity to observe the plants at a new depth.

Commented [KP21]: Is this meadow on Fig 2?

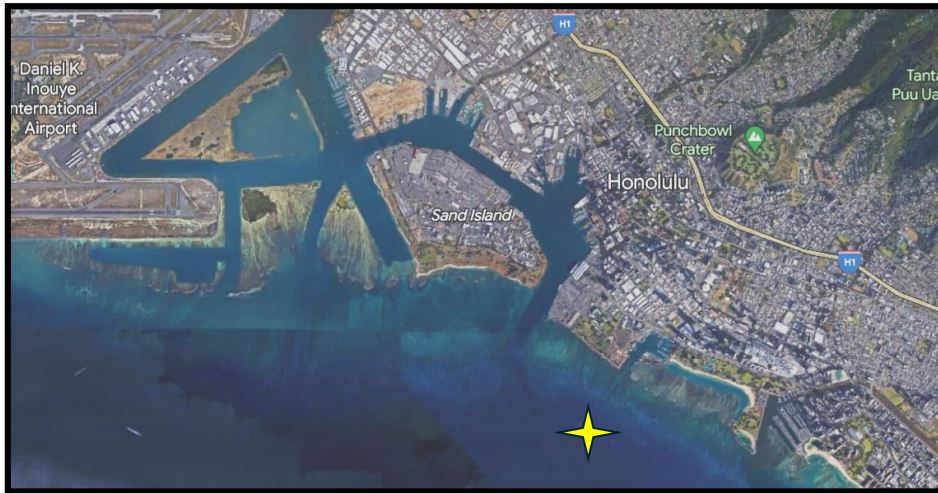


Figure 5. Location at 23 m, outside of Kewalo Basin Harbor channel. This site was used to observe wave influence on meadow composition and conduct chemical removal experiment.

Scientific Divers laid a 30 m transect at 23 m depth within the co-occurring meadow. Four quadrats were placed randomly to capture the relative plant composition along the transect. 0.75m aluminum stakes were hammered into the deep sand to mark each quadrat. Once the site was established the south shore swells started to arrive on O‘ahu. During the months of May - August 2022 there were four big swell events with wave height >3 meters (<https://www.pacioos.hawaii.edu/waves/buoy-pearl/>).

This four-month period was used to monitor the change in plant density without administering any removal treatment. In between each swell event we counted the number of *A. erecta*, *H. kanaloana* and *H. hawaiiiana*. This provided data to compare with the physical removal experiment and the hydrogen peroxide experiment. This unique time of year can bring upwards of 7m waves. This has profound effects on the species in the psammophytic habitat as the sand shifts many centimeters to bury or uncover entire plants. Once the south swells had subsided, we began our hydrogen peroxide experiment on both adult plants and juveniles.

Dilution of Hydrogen Peroxide and application

Hydrogen peroxide was diluted from a 30% concentration to a 3% concentration from Reagent grade H_2O_2 using deionized water (formula: $V_1C_1=V_2C_2$, V=volume and C=concentration Hydrogen Peroxide). One liter of the 3% solution was poured into a camelback bladder and attached to a ¼ inch tubing that led to a horse injector with a 16-gauge needle. This system

allowed for direct application of 10 ml of 3% H₂O₂ to be injected into each plant. The site of injection was into the stipe below the photosynthetic part of the blade.

Constructing an injector included camelback bladders available via Amazon: [CKE-Hydration-70-Ounce to 100-Ounce Backpack](#); Rofferflex Crystal tubing ½ inner diameter to 5/8th inch outer diameter (available via Amazon: [Rollerflex-Crystal-Tubing-2-Inch-8-Inch/dp/B07D9BZHKG?th=1](#)); a Dectomaz Injector (available online via: <https://www.qcsupply.com/540165-dectomaz-injector.html>); with stainless 16 gauge needles (available via Amazon: <https://www.amazon.com/12-Pack-Dispensing-Needle-Stainless/dp/B01LM8LTTO>).

Monitoring of injected plants

In September of 2022, after the south swells had subsided we began our hydrogen peroxide experiment at the Kewalo site. We used two different sized quadrats to closely monitor each plant's response after injection. The 1 m² quadrat was used to define the plot, while a smaller 0.25 m² strung quadrat was used inside the 1 m² quadrat to systematically organize the plants into four quadrants. The 0.25 m² quadrat was strung with evenly spaced strings at 4 cm increments crisscrossing to form 16 squares. The 0.25 m² quadrat was placed in the lower left (LL) corner of the 1 m² quadrat from the perspective of the transect. Within each 5 cm square of the 4 quadrants, the *A. erecta* were counted, measured in height, and injected with 10ml of 3% H₂O₂ (Figure 5). The injection targeted the stipe close to where it joined the photosynthetic part of the blade. From there the 0.25 m² quadrat was flipped clockwise to the upper left (UL) of the 1 m² quadrat, then to the upper right (UR) and finally to the lower right (LR). Follow up dives occurred weekly to record the state of the plants. A photograph of the main quadrat with the 0.25 m² quadrat was taken from above to capture each time the 0.25 m² quadrat was flipped. A side view was also taken before each flip of the 0.25 m² quadrat to better see individual plants (Figure 5). These photos were repeated on every follow up dive. Each quadrat was monitored for six weeks, a total of 10 weeks for the experiment.



Figure 6. Injection of hydrogen peroxide into an *A. erecta* plant (left) within the dual quadrat system (right).

USING PULSE AMPLITUDE MODULATED FLOUROMETER (PAM) TO MEASURE PHOTOSYNTHESIS IN TREATED AND CONTROL PLANTS

Scientific Divers placed a 1 m² quadrat randomly within a meadow of *A. erecta*. 0.75 m aluminum stakes were driven into the deep sand to mark the quadrat. Pre-labeled plant tags 1- five were placed around five individual *A. erecta* plants. The plant tags were made with two 3 gm fishing weights using two zip ties to connect them. Before injection, photosynthetic rates were measured using a Diving Pulse Amplitude modulated Fluorometer (Walz Co. with blue actinic light, DPAM) to determine relative Maximum Electron Transport Rate (rETR_{max}). Each plant received an injection of 10ml of 3% H₂O₂ into the upper stipe close to the photosynthetic part of the plant. Measurements of DPAM and photographs of the plant's physical decline were taken on weekly increments for three weeks (Figure 6). Three replicate quadrats with five plants injected occurred resulting in 15 plants having DPAM data pre and post Injection.

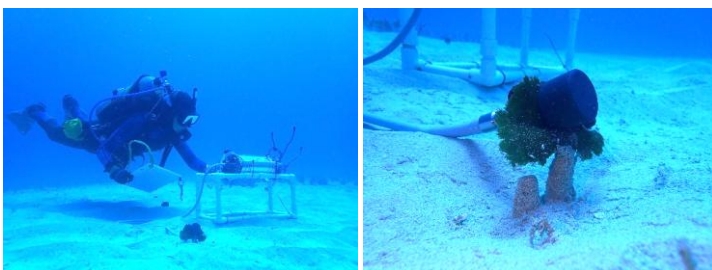


Figure 7. Diving PAM being used by a diver in the field (left) with a close up of the fiber optic cable clipped on a blade of *A. erecta* (right).

TOOLS FOR ANALYSIS

R with RStudio Markdown was used to analyze the data that came from the physical removal experiment. The package `ggmmTMB` was used for the models and Akaike's Information Criterion (AIC_c) for model selection based on the distribution for the data. Model selection was between Poisson, Quasi_poisson, and Negative Binomial as the data were count-based but frequently over dispersed (quasi-poisson mixed effects model, $X^2 = \chi^2_{pqr}$, $P = p$ -value). Model assumptions were checked using DHARMA. In cases where *Halimeda kanaloana* or *Halophila hawaiiiana* were absent or scarce, that variable was dropped from the model,

otherwise *Avrainvillea* abundance and treatment effect were also correlated to abundance of the other native plants.

The number of *A. erecta*, *H. kanaloana* individuals as well as the percentage of *Halophila* cover were all treated as factors within the statistical analysis. Percent cover of *Halophila* was converted from 0-100% to 0-1 for modeling purposes. To check for sampling evenness a table was created for all sites and number of observations of each treatment for each time point (See Appendix 1. in Supplemental Materials.)

RESULTS

Physical removal experiment

There was a significant effect of initial abundance of *A. erecta* before treatment on the outcome of *A. erecta* abundance after recovery over 25 months post-treatment (Poisson General Linear Model, $X^2 = 15.7485$, $Df = 1$, $P \text{ value} = 7.235e-05$). This had a negative impact on plant numbers, decreasing the density per $1m^2$. In marked contrast, no treatment had a significant impact on *A. erecta* abundance, regardless of the location on the south shore or the depth (Table 1).

Commented [NAK22]: Specify kind/family of GLM here

Commented [NAK23]: Repeat for remainder of stats

Commented [NAK24]:

Table 2. Statistical analysis used General Linear Mixed Model. AIC guided which type of Poisson family to use within the model. For Ala Moana 20 meters there were no significant findings from the ‘Treatment effect on *A. erecta*’ numbers. At Ala Moana 25 meters the ‘Initial numbers of *A. erecta*’ had a significant effect on the density of *A. erecta* post treatment. However, ‘Treatment effects on *A. erecta*’ were not significant nor were ‘Treatment effects on numbers of *Halimeda*’. Ke’ehi 20 meters also had a significant effect from the ‘Initial numbers of *A. erecta*’, and no significant effects from the treatments. Ke’ehi 25 meters had no significant effects from the initial *A. erecta* nor the treatments.

Site Location	Ala Moana, 20 m			Ala Moana, 25 m			Ke’ehi, 20 m			Ke’ehi, 25 m		
Type of test used	QuasiPoisson	Df	p value	QuasiPoisson	Df	p value	Negative Binomial	Df	p value	QuasiPoisson	Df	p value
Initial numbers of <i>A. erecta</i>	0.67	1	0.4	9.45	1	0.002	5.9	1	0.01	0.005	1	0.94
Treatment effect on <i>A. erecta</i>	3.16	2	0.2	1.81	3	0.61	5.75	3	0.12	1.69	2	0.42
Initial numbers of <i>Halimeda</i>	=	=		0.07		0.78	=	=	=	=	=	=
Treatment effects on numbers of <i>Halimeda</i>	=	=		4.78		0.18	=	=	=	=	=	=

Formatted: Indent: First line: 0"

Formatted: Centered, Indent: First line: 0.05"

Formatted: Centered, Indent: First line: 0.05"

Monitoring the impact of the south swell on plant densities.

Four quadrats were established along a transect to monitor the effects of large wave energy on the meadow composition (Figure 7). Densities of *A. erecta* decreased after significant (>3 m) swell events during the months of May- Aug 2022 [Poisson GLMR#X² = 4.9, P = 0.02]. In contrast, initial assessment densities of *Halimeda kanaloana* and *Halophila hawaiiiana* densities did not change after the same swell events (*Halimeda* # X² = 0.06, P = 0.8; *Halophila* 2X = 0.9, p = 0.3).

Avrainvillea Abundance vs. Wave Conditions at Pearl Harbor

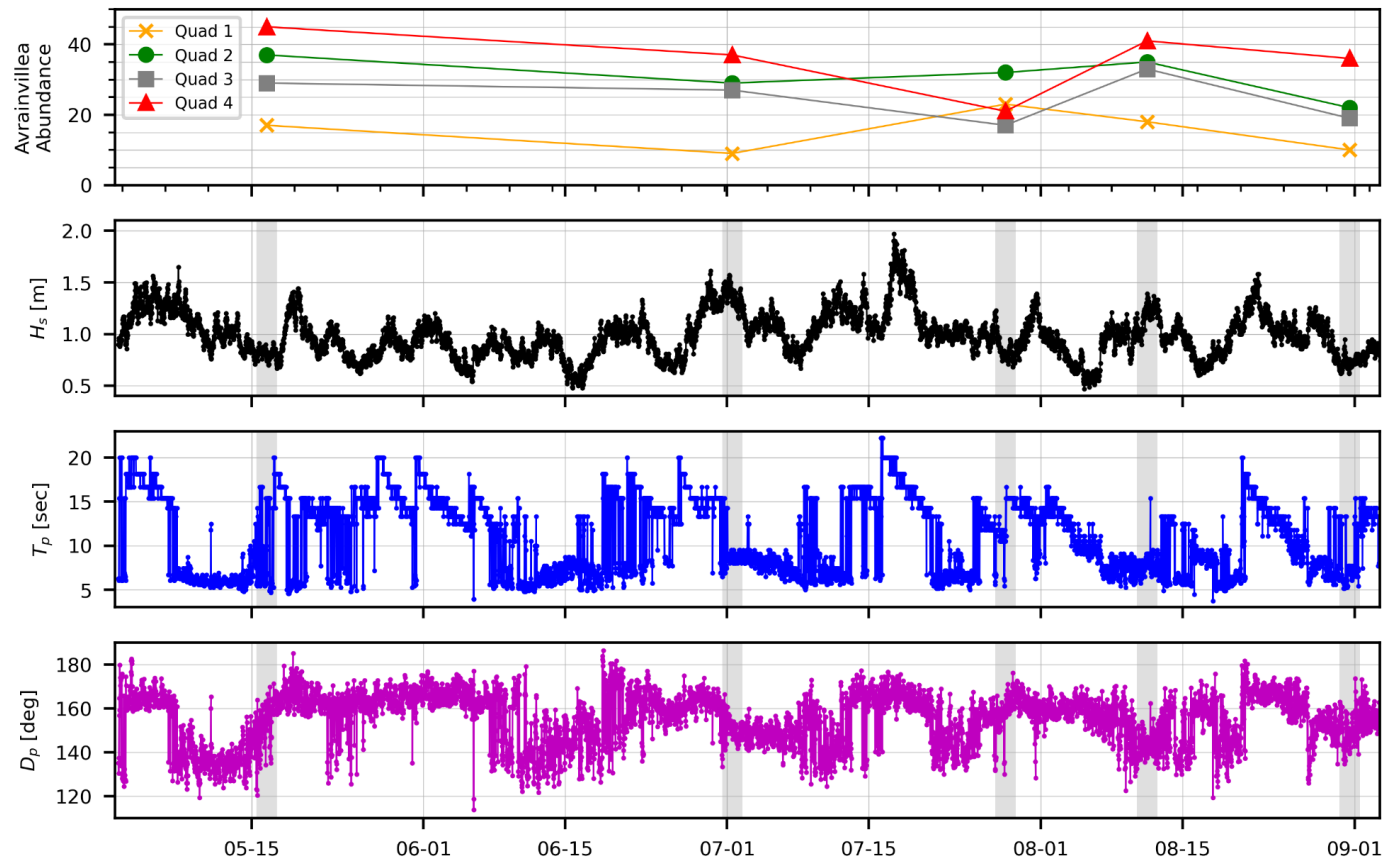


Figure 8. Surge effects on density of *A. erecta*. Average density of *A. erecta* significantly changed from before the swell on May 15th to after each swell. The first panel of the figure is the change in *A. erecta* abundance over the four-month period of observation. The second panel is the height of the waves in meters from the Pearl Harbor buoy on the south shore. The third panel is the period of waves in seconds. The last panel is the directionality of the waves coming from the south and running into the shores of O'ahu

Impacts from application of hydrogen peroxide.

Hydrogen peroxide injections in mesocosm: We compared treatments and controls using a paired T test. There was no significant effect of H₂O₂ injection (Response variable: change ETR_{max}. P = 0.166). However, while treated plants did decrease in ETR_{max} within the first four days of treatment, some experienced a rebound despite their appearance of degrading blade tissue (Figure 9). When taking baseline samples of *A. erecta* photosynthetic parameters, we also sampled the two native plants when dive time permitted. There was a significant difference between the mean calculated ETR_{max} (Figure 10). When H₂O₂ was applied in the field and number of *A. erecta* m⁻² were counted there was a significant decrease in numbers of *A. erecta* over 10 weeks (Figure 11). Using a linear regression model, we detected a significant decrease in *A. erecta* over 10 weeks (p = 0.003, n = 4, df = 1).

2

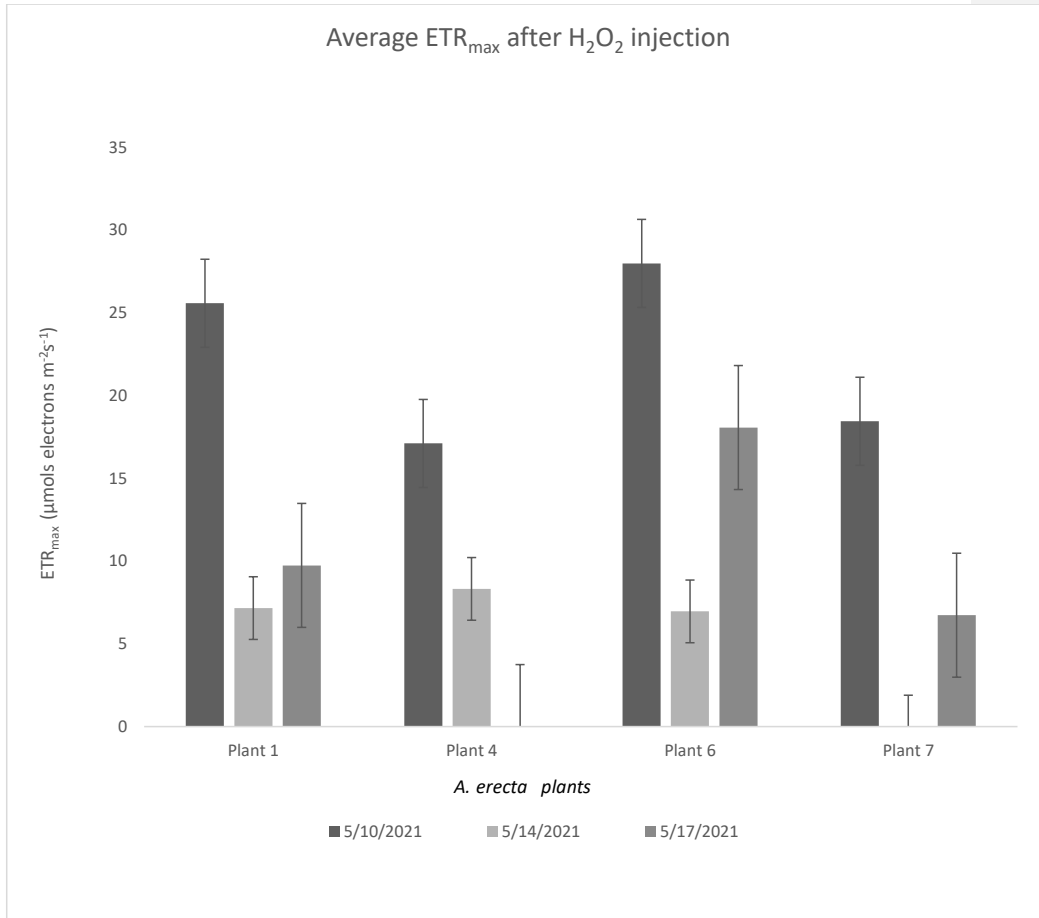


Figure 9. AFRC mesocosm experiment. Average ETR_{max} μmols electrons m⁻²s⁻¹ measurements for trial 1 with plants collected at the 20-meter Ke'ehi site. Data from four individual plants injected with H₂O₂. This figure shows a phenomenon of ETR_{max} bouncing back after an initial marked decline four days after injection and then in some cases increasing ETR_{max} μmols electrons m⁻²s⁻¹ after 7 days.

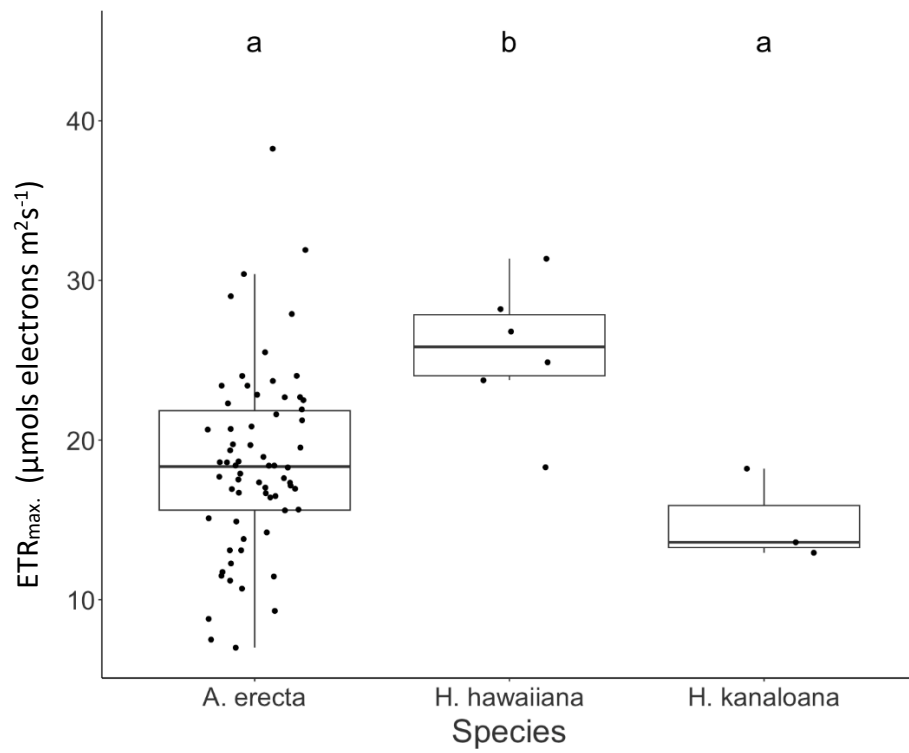
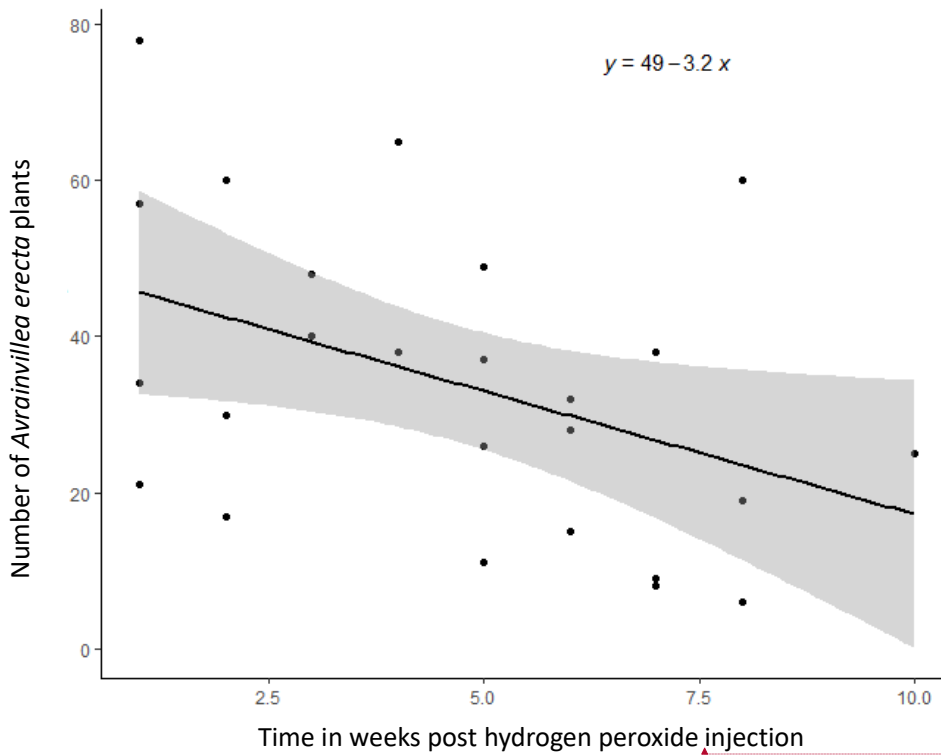


Figure 10. Differences in ETR_{max} among co-occurring species in plots are significant (df = 1, F value = 5.05, P value = 0.008). Baseline ETR_{max} values for *Avrainvillea erecta*, *Halophila hawaiiiana* and *Halimeda hawaiiiana* using Anova Type II test.



Formatted: Font: 14 pt, Not Superscript/ Subscript

Figure 11. Impacts of *in situ* injections of hydrogen peroxide on densities of *A. erecta* over ten weeks. Trendline represents a model fit (\pm 95% Confidence Interval) using a linear regression. Four replicate quadrats were sampled for *A. erecta* densities across a 30m transect, (n=4).

Commented [NAK25]: I'm guessing? Correct if wrong.

DISCUSSION

Overview of recent history

Since the extreme ~~d~~development of south shore of O'ahu starting in 1920, coastal reefs have been increasingly impacted by urban pollution growth and increased sedimentation. Native reef communities suffered reduced coral cover, loss of key fish species and sea grass habitat (Littler et al., 2006, Peyton 2009). Interestingly in 2012, meadows of *Halimeda kanaloana* on the south shore of O'ahu were barely detectable (Pence pers. comm; Spalding 2012). The only meadows that were established at that time consisted of *Halophila* spp. By 2014, there were some small stands of *H. kanaloana* starting (D. Pence pers. comm) which ironically is the same time when the first *A. erecta* plants were noticed in the Honolulu Harbor channel by Dr. Kim Peyton. Over the next four years this novel psammophytic meadow community began to mature, and meadows began to spread along O'ahu's south shore as single and mixed species assemblages.

Commented [KP26]: Intense? Find a better descriptor.

A 2018 dive conducted to the 60m isobath during which video footage was taken, captured both *H. kanaloana* and *A. erecta* co-occurring in a meadow (Wheeler pers. obs.). From this observation, the call went out to researchers from other organizations to report their sightings of *A. erecta*. Photographs with GPS points were reported from the south Shore up to Kahe point, West O'ahu. Within a span of six years this area had been colonized by these canopy-forming algae and are now co-occurring within the seagrass meadows and establishing new meadows.

This ~~psammophytic community~~ is ~~a~~ novel ~~community-benthic community in the~~ ~~Hawai'i's~~~~Hawai'i's~~ marine systems. However, the psammophytic environments in Hawai'i are an understudied part of our coastal ecosystems; these soft sediment habitats provide many services to the adjacent coral reef habitats. The two bryopsidalean algae and the marine angiosperm all have benefits they offer the community, such as primary productivity, supporting detritus-based food webs (Nordland and Nordlund et al. 2018), sediment stabilization; sand production, nutrient cycling and carbon sequestering (Beach et al., 2003; Vroom et al. 2003). They also provide food and habitat for many fish and invertebrates (Bulmer et al., 2018, Pereda-Briones et al., 2018, Waycott et al., 2009, Peyton 2009).

Our native plant species within this habitat are now competing for energy in the form of light, nutrient uptake and space with the newly introduced *Avrainvillea erecta*. Historical data from ~~Hawai'i's~~~~Hawai'i's~~ previous introduction of the species *Avrainvillea lacerata* classifies it as an ecosystem engineer, modifying native habitats which can have significant impacts on the health and stability of ~~psammophytic the~~ environments (Van De Verg and Smith, 2022). As *A. erecta* becomes more established there is more potential for it to move into different habitats.

For now, at least 10 years after its introduction, we only observe it in soft bottom habitats. However, when removing the holdfast of an entire plant very often there is a rock or coral rubble nested within the holdfast (Per Wheeler comms Wheeler pers. obs.). It is possible that the young plant uses the rubble as an initial anchor to then build a siphonous holdfast to anchor itself to the benthos. If this is true, then it could be feasible for these plants to move into coral reef areas where sediment has gathered in depressions of live coral colonies. *Avrainvillea lacerata* is has been observed for this type of intrusion specifically at Makai pier, O'ahu, and it damages the living tissues of the coral colony (Wheeler pers. obs.). The plasticity of the *Avrainvillea* genus should not be underestimated. Because the full ecology of *A. erecta* is unknown, it is prudent to monitor even our coral habitats for invasion. The next 5 years will be critical for managers to begin monitoring and prevent further spread across critical habitat.

Commented [KP27]: In its native range is it known to grow on hardbottom?

Here we sought to investigate the weedy qualities of *Avrainvillea erecta*. Our expectation was: If *A. erecta* has similar traits to *Avrainvillea lacerata*, then *A. erecta* will have greater ability to occupy and dominate available niche space among co-occurring natives *Halophila hawaiiiana* and *Halimeda kanaloana*.

PHYSICAL REMOVAL

The original density of *A. erecta* prior to treatment was the strongest determinant on the number of plants that would regrow after treatment; if the meadow had high density of *A. erecta*, then the plants would regrow to a similar density, and if the density was low, it remained low regardless of treatment. This indicates that Early Detection and Rapid Response (EDRR) is one potential strategy in reducing spread (Fumo et al. 2024).

Commented [KP28]: Another thought is that dense meadows have more rhizomes meaning belowground - that which we cannot see - is critical to factor into management. Feeds into your idea of early detection.

None of the physical removal treatments significantly impacted the density of *A. erecta*, *H. kanaloana* or *H. hawaiiiana* regardless of treatment. Plants were able to regrow from each of the three types of treatments. Because the treatments were intended to mimic natural disturbances that might occur at different frequencies, it would seem that *A. erecta* has adapted great resilience to such disturbances. Natural blade abandonment would account for why it could regrow after treatment 1 (cut blade) (Littler and Littler 2004). This seems to allow it to survive large swell events as well. The ability for it to regrow from fragments of siphons within the substrate also explains why it can regrow after treatment number 2 and 3. Because of cytoplasmic streaming, the chloroplast and nuclei can be stored in any part of the plant, including rhizoids underground, until the plant can regrow new siphons (Smith and Walters 1999, Vroom and Smith 2001).

The Meadow Suppression Hypothesis recognizes the competitive traits needed by seagrasses to out compete green algal species specifically Bryopsidalean algae (Davis and Fourqurean, 2001, Peyton 2009). This hypothesis suggests that *H. hawaiiiana* could have competitive advantage(s) over other Bryopsidalean groups and is supported by *H. hawaiiiana*

having higher average ETR_{max} than both algal species. Higher rates of photosynthesis could link to higher growth rates – a competitive advantage for an understory species as long as incidental irradiances are above E_k . The outcome of no significant effect of treatments to *A. erecta* on the densities of *H. hawaiiiana* suggest either there was not enough suppression of *A. erecta* growth to have indirect positive growth of the seagrass, or that *A. erecta* is not suppressing the seagrass growth. As proposed earlier by Davidson and Shoemaker 2023, the presence of other algae could provide more anchor points and structure for the roots of the seagrass to keep it from being torn up by wave energy. During the swell observations, the native, *H. hawaiiiana*, had no significant changes in plant density following wave energy; *A. erecta* was dramatically impacted. This would take more investigation to answer why, but another indication that *H. hawaiiiana* may be more adapted to disturbances including the arrival and spread of a novel species.

Commented [KP29]: And adapted to disturbances, including the arrival and spread of novel species.

A. erecta and *H. kanaloana* densities showed somewhat cyclical growth patterns, possibly indicating seasonality. *H. kanaloana* is holocarpic and regularly goes through periods of reproductive die-off and regrowth (Drew and Abel 1988). We also frequently observed *A. erecta* regrowth from both treatment and large swell events, often growing a new blade onto the side of an older holdfast where the old blade was either shed, or clearly discolored and being shed (LW pers [eob](#), Littler and Littler, 1999). Blade abandonment was the theory behind *Avrainvillea* plant's ability to shed epiphytic growth and regrow a clean new blade. Both plants undergoing periodic growth and senescence would need more observations to discern if they had any synchrony or pattern to them. There was a non significant trend that correlated treatment #1 with increasing *H. kanaloana* numbers. This could mean that while *A. erecta* regrows its blade, *H. kanaloana* may have an advantage to increase in density. Regardless, the mechanisms governing competitive outcomes and coexistence between the invasive and native species pairing warrants further investigation and could provide insight for managers to assist in promoting more *H. kanaloana* in areas where *H. kanaloana* is already established with *A. erecta*.

On O'ahu, we have observed that high swell events will decrease the *A. erecta* densities. However, in locations such as west Maui, there are no seasonal swell events. This is a protected area with quiet waters. Since the sighting of *A. erecta* in 2019 off Olowalu our source ([Botany Graduate](#), Donna Brown [pers. comm](#), M.S.) has reported a drastic shift in the depth and composition of the native *H. kanaloana* meadow. She observed in this location the depth of the meadow which previously started at 12 m now starts at 20 m. The composition that was once thick *H. kanaloana* is now much sparser [meadow of H. kanaloana](#) and denser *A. erecta* (D. Brown pers. comms). It is possible that without the seasonal swell energy to reduce *A. erecta* densities, *A. erecta* can outcompete native meadows in quiet water locations. This increases the risk of spread to other protected quiet water areas within the Maui Nui complex. EDRR would

Commented [KP30]: This is interesting because of the canopy height difference between these two species. I may ask you about this

be the best solution for prevention. Cooperation across agencies and islands should be discussed to unify the effort.

Injecting 10 ml of 3% hydrogen peroxide into the thalli of *A. erecta* did decrease the density in the 1 m² quadrat over six weeks. This technique is localized in its effects and there were never any observed negative effects on the benthic community beyond the treated thalli. It might therefore be an effective method for invasive bryopsidalean algal management. Compared with other strategies that target the entire benthic community, such as the chlorine tarping that was used to remove invasive algae *Caulerpa taxifolia* in California (Williams & Schroeder 2004), the injection technique does not impact the entire benthic community. There are no other mortalities besides the target species that we observed. Initial DPAM readings of *A. erecta* and adjacent algae were taken before and after injections. This protocol was terminated after it became evident that the D-PAM would not be used for detecting mortality in *A. erecta*. However, those photosynthetic calculations revealed that adjacent plants did not suffer effects from the injection to *A. erecta*. When using this technique, it is best to time it with a strong swell event preferably an injection four to seven days before the swell arrives and one to two weeks after. Using the storm surges to rip out already injected plants, and then inject whatever grows back could take full advantage of the natural perturbations in combination with this novel technique. Additionally, there was a pilot study that was informed by this experiment that increased the volume of H₂O₂ and found that 20ml of 3% H₂O₂ had a greater negative effect on *A. erecta* (G. Lewis unpublished undergraduate research, 2022). This injection method is volumetric, as volume increases, a higher concentration of H₂O₂ perfuse through the plant. A consideration for managers to take when developing the management strategy. This will be further discussed in Chapter 3.

The injection method does require extra equipment. It requires supplies of concentrated 30% hydrogen peroxide to be diluted to 3% and that comes with its own restrictions. It also requires some extra training in the field to prepare the injection bladder for underwater use. The application of hydrogen peroxide into the plant can sometimes be difficult due to the tight tangle of siphons in the stipe. In cases where the treatment was ineffective, it may have been due to diver error. Sometimes the plant was small, or the stipe was narrow and the injection pushed out the other side of the thalli. This can be accounted for and additional injections can be applied.

MECHANISM OF INTRODUCTION OF *A. ERECTA* TO HAWAI'I

The role that *Avrainvillea erecta* will play within our Hawaiian near shore ecosystems still needs investigation. It seems to have weedy characteristics through its ability to spread and colonize new areas, it doesn't seem to have any native herbivore that will eat the entire blade of the plant, and being physically resilient to disturbances in the environment, it is yet to be classified as invasive. It is possible in some locations such as south shore O'ahu, that *A. erecta* may not out-compete the native meadow community because of periodic large swell events reducing *A. erecta* densities. But in areas such as west Maui and other quiet water locations around Maui Nui such as Kāho'olawe, Molokai'i and Lanai'i, the lack of periodic disturbance could give it a competitive advantage; *A. erecta* could quickly out-compete native meadow communities. This is why EDRR in sensitive areas is important. From the time this study began the population density in West Maui went from sparse in 2019 to dense in 2024 (D. Brown pers. comm) in just five years. This type of establishment and spread leans toward the outcome that this plant is highly weedy and given time will be classified as invasive. From 2014-2018 *A. erecta* was not monitored and had only been sighted in Honolulu Harbor. Once this study began to track where it was in 2019, four years after its initial discovery, it had spread along O'ahu's south and west shores (Wheeler pers. obs. 2018). In just five years (2019-2024) it is now on two outer islands and creeping around to east O'ahu. The mechanism for spread is part of the puzzle that needs further investigation to prevent future invasions into our quiet water locations.

To begin to understand how *A. erecta* is able to spread so quickly and across deep channels further investigation on the ecology of *A. erecta* needs to be understood. The native range of *A. erecta* is quite broad throughout the Philippine Islands, Micronesia, Southeast Asia, Australia, New Zealand, and China Sea (Guiry and Guiry, 2024). Because of this broad home range, it is difficult to pinpoint the origin of the introduction. However, it is likely that it was brought over via an international vessel because it was originally found in Honolulu Harbor, a main hub for international commerce, tourism, recreational boating, and shipping (Martinez et al. 2012, Wade et al. 2018). The invasive algae *Acanthophora spicifera* was brought via hull fouling to Pearl Harbor from Guam in 1950 (Doty 1961; Weijerman et al., 2008) and it could be a possibility that *A. erecta* was transported in a similar fashion. *A. erecta* is not reported to have hull fouling qualities; however, one other theory is that it is transported via anchors that are deployed in psammophytic habitats. A foreign vessel that had pulled up anchor from an *A. erecta* algal meadow could bring rhizoids attached to the anchor or chain and kept in the anchor hold as viable fragments, then redeployed near Honolulu Harbor as the vessel waits to enter. This last theory helps explain the pattern of interisland spread of *A. erecta* to Maui and Hawai'i island, both sightings were located near main harbors. Following Conklin et al. 2004, successful introductions may take many "attempts". It might not have been the first time that *A. erecta* has been brought to Hawai'i, but it was the first time that all the situational events lined up to have a viable introduction that led to establishment and spread.

Commented [KP31]: Better to say: this species has not been recorded as a hull fouler previously. Something along these lines.

There are a lot more questions coming from this study than when we started. The overarching goal was met by testing novel removal strategies that could help managers control *A. erecta* populations, to be further discussed in Chapter 3. The ecological strategies of *A. erecta* support the label of “an invasive species”, and given time, appears to have strong competitive traits against our native psammophytic community.

LITERATURE CITED

- Beach, K., Walters, L., Vroom, P., Smith, C., Coyer, J., & Hunter, C. (2003). Variability in the ecophysiology of *Halimeda* spp. (Chlorophyta, ~~Bryopsidales~~) ~~On~~ ~~Bryopsidales~~ ~~On~~ ~~ChenichConch~~ Reef Florida Keys, USA. *Journal of Phycology*, 39(4), 633–643
- Bulmer, R. H., Townsend, M., Drylie, T., & Lohrer, A. M. (2018). Elevated turbidity and the nutrient removal capacity of seagrass. *Frontiers in Marine Science*, 5, 462. <https://doi.org/10.3389/fmars.2018.00462>
- Carlton, J. T. (1994). Patterns of transoceanic marine biological invasions in the Pacific Ocean. In E. A. Kay, *A Natural History of the Hawaiian Islands*, (pp. 504–518). University of Hawaii Press. <https://doi.org/10.1515/9780824844264-043>
- Carlton, J. T. (1996). Pattern, process, and prediction in marine invasion ecology. *Biol Cons*, 78. [https://doi.org/10.1016/0006-3207\(96\)00020-1](https://doi.org/10.1016/0006-3207(96)00020-1)
- Carlquist, S. (1966). The biota of long-distance dispersal. I. Principles of dispersal and evolution. *The quarterly review of biology*. 41(3), ~~pp~~ 247-270
- Coles, S. L., DeFelice, R. C., Eldredge, L. G., & Carlton, J. T. (1999). Historical and recent introductions of non-indigenous marine species into Pearl Harbor, Oahu, Hawaiian Islands. *Marine Biology*, 135. <https://doi.org/10.1007/s002270050612>
- Davidson, I.C., Scianni, C., Minton, M.S., & Ruiz G.M. (2018). A history of ship specialization and consequences for marine invasions, management and policy. *Journal of applied ecology*. 55(4), 1799-1811. <https://doi.org/10.1111/1365-2664.13114>
- Davidson, J. L., & Shoemaker, L. G. (2023). Resistance and resilience to invasion is stronger in synchronous than compensatory communities. *Ecology*, 104(11). e4162. <https://doi.org/10.1002/ecy.4162>
- Davis, B. C., & Fourqurean, J. W. (2001). Competition between the tropical alga, *Halimeda incrassata*, and the seagrass, *Thalassia testudinum*. *Aquatic Botany*, 71(3), 217–232. [https://doi.org/10.1016/S0304-3770\(01\)00179-6](https://doi.org/10.1016/S0304-3770(01)00179-6)

- Drew, E. A., & Abel, K. M. (1988). Studies on *Halimeda*: II. Reproduction, particularly the seasonality of gametangia formation, in a number of species from the Great Barrier Reef Province. *Coral Reefs*, 6(3–4), 207–218. <https://doi.org/10.1007/BF00302017>
- Eldredge, L.G. & Smith, C.M., (2001). A guidebook of introduced marine species in Hawaii. *ResearchGate. Bishop Museum archives*
- Fumo, J. T., Powell, B. S., Kosaki, R. K., & Sherwood, A. R. (2024). Modeling the dispersal of the cryptogenic alga *Chondria tumulosa* (Rhodophyta, Ceramiales) in the Papahānaumokuākea Marine National Monument. *Aquatic Invasions*, 19(3), 259–273. <https://doi.org/10.3391/ai.2024.19.3.135377>
- Gaither, M. R., Toonen, R. J., & Bowen, B. W. (2012). Coming out of the starting blocks: Extended lag time rearranges genetic diversity in introduced marine fishes of Hawai‘i. *Proceedings of the Royal Society B: Biological Sciences*, 279(1744), 3948–3957
- Giddens, J., Friedlander, A., Conklin, E., Wiggins, C., Stamoulis, K., & Donovan, M. (2014). Experimental removal of the invasive peacock hind (roi) *Cephalopholis argus*, in Puakō, Hawai‘i: Methods for assessing and managing marine invasive species. *Marine Ecology Progress Series*, 511, 209–221
- Glor, R.E., (2010). Phylogenetic insights on adaptive radiation. *Annual review of the ecology, evolution, and systematics* 41, 251-270
- Hillis-Colinvaux, L. (1974). Productivity of the coral reef alga *Halimeda* (Order Siphonales). *Proceedings of the second international coral reef symposium 1. Great barrier Reef Committee, Brisbane. Proceedings of the 6th International Coral Reef Symposium, Australia, 1988 (Vol. 3), pp. 35-42*
- Inglis, G. J., Hurren, H., Oldman, J., & Haskew, R. (2006). Using habitat suitability index and particle dispersion models for early detection of marine invaders. *Ecological Applications*, 16(4), 1377–1390. [https://doi.org/10.1890/1051-0761\(2006\)016\[1377:UHSIAP\]2.0.CO;2](https://doi.org/10.1890/1051-0761(2006)016[1377:UHSIAP]2.0.CO;2)
- Krause, G. H., & Weis, E. (1991). Chlorophyll Fluorescence and Photosynthesis: The Basics. Annual review plan Physiology. *Plant Molecular Biology* 42(313-49), 325-329
- Les, D. H., Cleland, M. A., & Waycott, M. (1997). Phylogenetic studies in Alismatidae, II: Evolution of marine angiosperms (Seagrasses) and hydrophily. *Systematic Botany*, 22(3), 443. <https://doi.org/10.2307/2419820>
- Midway, S. R., & Hodge, A.-M. C. (2012). Carlquist revisited: History, success, and applicability of a natural history model. *Biology & Philosophy*, 27(4), 497–520

<https://doi.org/10.1007/s10539-011-9296-9>

- Pereda-Briones, L., Tomas, F., & Terrados, J. (2018). Field transplantation of seagrass (*Posidonia oceanica*) seedlings: Effects of invasive algae and nutrients. *Marine Pollution Bulletin*, 134, 160–165. <https://doi.org/10.1016/j.marpolbul.2017.09.034>
- Price, J. P. (2004). Floristic biogeography of the Hawaiian Islands: Influences of area, environment and paleogeography. *Journal of Biogeography*, 31(3), 487–500. <https://doi.org/10.1046/j.0305-0270.2003.00990.x>
- Pyle, R. L., Boland, R., Bolick, H., Bowen, B. W., Bradley, C. J., Kane, C., Kosaki, R. K., Langston, R., Longenecker, K., Montgomery, A., Parrish, F. A., Popp, B. N., Rooney, J., Smith, C. M., Wagner, D., & Spalding, H. L. (2016). A comprehensive investigation of mesophotic coral ecosystems in the Hawaiian Archipelago. *PeerJ*, 4, e2475. <https://doi.org/10.7717/peerj.2475>
- Richardson, K. H., Wright, J. J., Šimėnas, M., Thiemann, J., Esteves, A. M., McGuire, G., Myers, W. K., Morton, J. J. L., Hippler, M., Nowaczyk, M. M., Hanke, G. T., & Roessler, M. M. (2021). Functional basis of electron transport within photosynthetic complex I. *Nature Communications*, 12(1), 5387. <https://doi.org/10.1038/s41467-021-25527-1>
- Sherwood, A. R., & Guiry, M. D. (2023). Inventory of the seaweeds and seagrasses of the Hawaiian Islands. *Biology*, 12(2), 215. <https://doi.org/10.3390/biology12020215>
- Smith, C. M., & Walters, L. J. (1999). Fragmentation as a strategy for *Caulerpa* species: Fates of fragments and implications for management of an invasive weed. *Marine Ecology*, 20(3–4), 307–319. <https://doi.org/10.1046/j.1439-0485.1999.2034079.x>
- Smith, J. E., Smith, C. M., & Hunter, C. L. (2001). An experimental analysis of the effect of herbivory and nutrient enticement on benthic community dynamics on Hawaiian reef. *Coral reef*, 19: 332-342, DOI 10.1007/s003380000124
- Smith, J. E., Hunter, C. L., Conklin, E. J., Most, R., Sauvage, T., & Squir, C., & Smith, C. M. (2004). Ecology of the invasive Red Alga *Gracilaria gracilaria salicornia* (Rhodophyta) on O'ahu, Hawai'i. *Pacific Science*. 58(2) 325-343
- Smith, J.E. Hunter, C.L., & Smith, C.M. (2010) The effect of top-down versus bottom-up control on benthic coral reef community structure. *Oecologia* 163, 497-507. DOI 10.1007/s00442-009-1546-z
- Tsuda R.T. (2015). New species records of marine benthic algae in the Pahānaumokuākea Marine National Monument (North Western Hawaiian Islands). *Records of the Hawaii Biological Survey for 2014 Part I: Articles*. Edited by Neal L. Evenhuis & Scott E. Miller. Bishop Museum Occasional Papers 116, 41–47

- Tussenbroek, B. I. van, Vonk, J. A., Stapel, J., Erftemeijer, P. L. A., Middelburg, J. J., & Zieman, J. C. (2006). The biology of *Thalassia*: Paradigms and recent advances in research. In *Seagrasses: Biology, Ecology and Conservation* (pp. 409–439). Springer Netherlands. https://doi.org/10.1007/978-1-4020-2983-7_18
- Van De Verg, S. E., & Smith, C. M. (2022). Protocol to control the invasive alga *Avrainvillea lacerata* in a shallow Hawaiian reef flat. *Applications in Plant Sciences*, *10*(4), e11490. <https://doi.org/10.1002/aps3.11490>
- Verbruggen, H., De Clerk, O., N'yert, A.D.R., Spalding, H. & Vroom, P (2006). Phylogeny and taxonomy of *Halimeda incrassata*, including descriptions of *H. kanaloana* and *H. heteromorpha* spp. Nov (Bryopsidales, Chlorophyta). *European Journal of Phycology* *41*(3): 337-362
- Vroom, P. S., & Smith, C. M. (2001). The challenge of siphonous algae. *American Scientist*, pp.525 - 521
- Vroom, P. S., & Smith, C. M. (2003). Life without cells. *Biologist* *50*(5) 223-226.
- Wade, R., Spalding, H., Peyton, K., Foster, K., Sauvage, T., Ross, M., & Sherwood, A. (2018). A new record of *Avrainvillea cf. erecta* (Berkeley) A. Gepp & E. S. Gepp (Bryopsidales, Chlorophyta) from urbanized estuaries in the Hawaiian Islands. *Biodiversity Data Journal*, *6*, e21617. <https://doi.org/10.3897/BDJ.6.e21617>
- Waycott, M., Duarte, C. M., Carruthers, T. J. B., Orth, R. J., Dennison, W. C., Olyarnik, S., Calladine, A., Fourqurean, J. W., Heck, K. L., Hughes, A. R., Kendrick, G. A., Kenworthy, W. J., Short, F. T., & Williams, S. L. (2009). Accelerating loss of seagrasses across the globe threatens coastal ecosystems. *Proceedings of the National Academy of Sciences*, *106*(30), 12377–12381. <https://doi.org/10.1073/pnas.0905620106>
- Weijerman, M., Most, R., Wong, K., & Beavers, S. (2008). Attempt to control the invasive red alga *Acanthophora spicifera* (Rhodophyta: Ceramiales) in a Hawaiian fishpond: An assessment of removal techniques and management options. *Pacific Science*, *62*(4), 517–532. [https://doi.org/10.2984/1534-6188\(2008\)62\[517:ATCTIR\]2.0.CO;2](https://doi.org/10.2984/1534-6188(2008)62[517:ATCTIR]2.0.CO;2)
- Williams, S. L. (1990). Experimental studies of Caribbean seagrass bed development. *Ecological Monographs*, *60*(4), 449–469. <https://doi.org/10.2307/1943015>
- Williams, S. L., & Smith, J.E. (2007). A global review of the distribution, taxonomy, and impacts of introduced seaweeds. *Annual review of ecology, evolution and systematics*. *38*(1) 327-359. DOI 10.1007/s00442-009-1546-z

Several recommendations emerge from this study:

1. **Increase surveillance for *A. erecta* in vulnerable areas** within the main Hawaiian Islands such as quiet water locations within Maui Nui and nearby harbors as well as heavily anchored areas. DAR has an Aquatic Invasive Species team (AIS) that directly focuses on early detection and rapid response.

2. In areas of high density (>20 plants m⁻²), I recommend using 30 ml of hydrogen peroxide diluted to 3% solution to treat *A. erecta* plants. Based on further research that was not part of this study, greater volumes have been shown to have greater success in reducing *A. erecta* density within a meadow. Preliminary injections should ~~occur~~reoccur with monthly to semi-monthly follow up injections until the population is below 4 plant/ m².

3.2. Pairing the injection method with natural high wave energy could increase effectiveness and reduce human effort. In areas of quiet water locations, it may not be possible to time injections with seasonal high wave energy. However, using storm surges or periods of elevated wind could also prove to have a similar effect, though the timing may be less predictable; PaclOOS site may prove to be a good source for this information for south shore O'ahu. For application and follow up, it is best to inject four to seven days before the swell hits, and then seven to 14 days after.

4.3. If chemical removal is not immediately available or other constraints exist, then physical removal would work temporarily until chemical removal became available. I recommend treatment 3 (holdfast removal), over the other two physical removal strategies. It is very important to retain or capture all algal material to reduce potential spread to other locations through fragmentation. Biomass that is collected can be disposed of via green waste, or other available composting options.

Capacity building in the longer term:

1. **Continue to collaborate with the Limu Lab** to acquire / modify / build out injection kits or other methods and secure concentrations of hydrogen peroxide to help the AIS team to be prepared to initiate surveys and be ready to respond to any sightings of *A. erecta*.

2. Develop a collaborative dive plan that could provide more dive support for locations on neighbor islands. With newly detected populations, chemical removal would be preferred using the technique and methods described in Chapter 2.

These strategies require follow up treatments. Optimal follow ups would occur monthly, but if the meadows are being reduced in density, then revisits could extend to two to three months. It would be prudent to continue site visits and applications every six months or until *A. erecta* is either undetectable or at low densities (< 4 plants/ m²).

Further research is needed in areas of reproduction for *A. erecta*. No reproductive structures were found as part of this study, and it would be important to understand this critical life stage. Algae that ~~have~~ moved into a new area that has not yet been colonized has been known to revert to asexual reproduction. This allows the plant to establish a population when viable mates are a limiting factor (Thornton 2024), also known as Bakers Law (Baker 1955). -The fragmentation ability would also be a very important aspect to investigate as an escape from limited sexual reproduction.

This study looked superficially at the prospect of fragmentation with no significant result, but a more in-depth study to look at how if this plant is using fission, budding, or if it is capable of parentheses would help elucidate how this plant is spreading up and down the coasts of O'ahu. Additionally, the distance that one plant's rhizoids can extend and its connectivity to other plants would be important to investigate based off the Perennation Hypotheses from Littler and Littler (2004). Core samples using e-DNA could assist in detecting how far the rhizoids can reach horizontally under the sand and if individuals are connected through them and can stream cytoplasm to one another.

Many new questions have come from what has been learned through this research including two related projects. At a larger ecological scale, the energy exchange between the psammophytic habitat and adjacent coral reef would be extremely informative to assess how they are integrated. Looking at the species found in the meadow and comparing them to the reef would help to determine which animals are using both habitats. Additionally, looking at the fish and invertebrates that only inhabit the psammophytic meadow and their status as native or non-native would also be beneficial to determine the ecosystem that is being supported by the novel community. This may shed new light on how we can help support biodiversity in our coastal habitats and build resilience within our ecosystems. The long-term goal is to protect the biodiversity of Hawaiian marine ecosystems, we must ~~continued~~ continue to explore, question, and learn.

LITERATURE CITED

Baker, G. H., 1955. Self-compatibility and establishment after "long distance" dispersal.

Commented [KP32]: Here you can talk about and cite the very limited observations of reproduction for this genus worldwide. Whatever is happening its cryptic.

Evolution, ~~Vol. 9, No. (-3) (Sep., 1955), pp.~~ 347-349. <https://doi.org/10.2307/2405656>

Thornton, M.B., 2024. Characterizing the reproductive system of *Avrainvillea* in Hawai'i. ~~MS?~~
~~MS, PhD?~~ The University of Alabama at Birmingham. ProQuest Dissertations & Theses,
2024. # 31139929

Appendix 1. Additional Data from Physical Removal [and Chemical](#) Experiments.

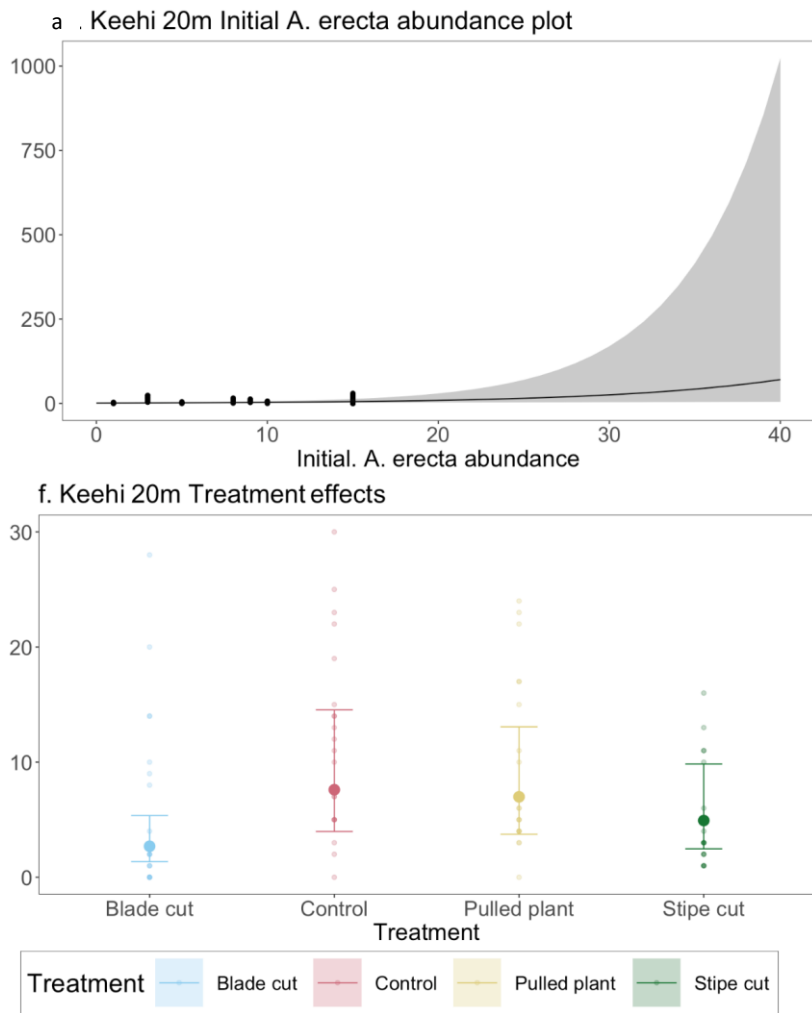


Fig aA. A graphical representation of the Quasi Poisson General Linear Mix Model results. The upper graph is the model's predicted distribution in gray and the actual distribution with black dots and a black line. The lower graph is the variance of each treatment at that sight.

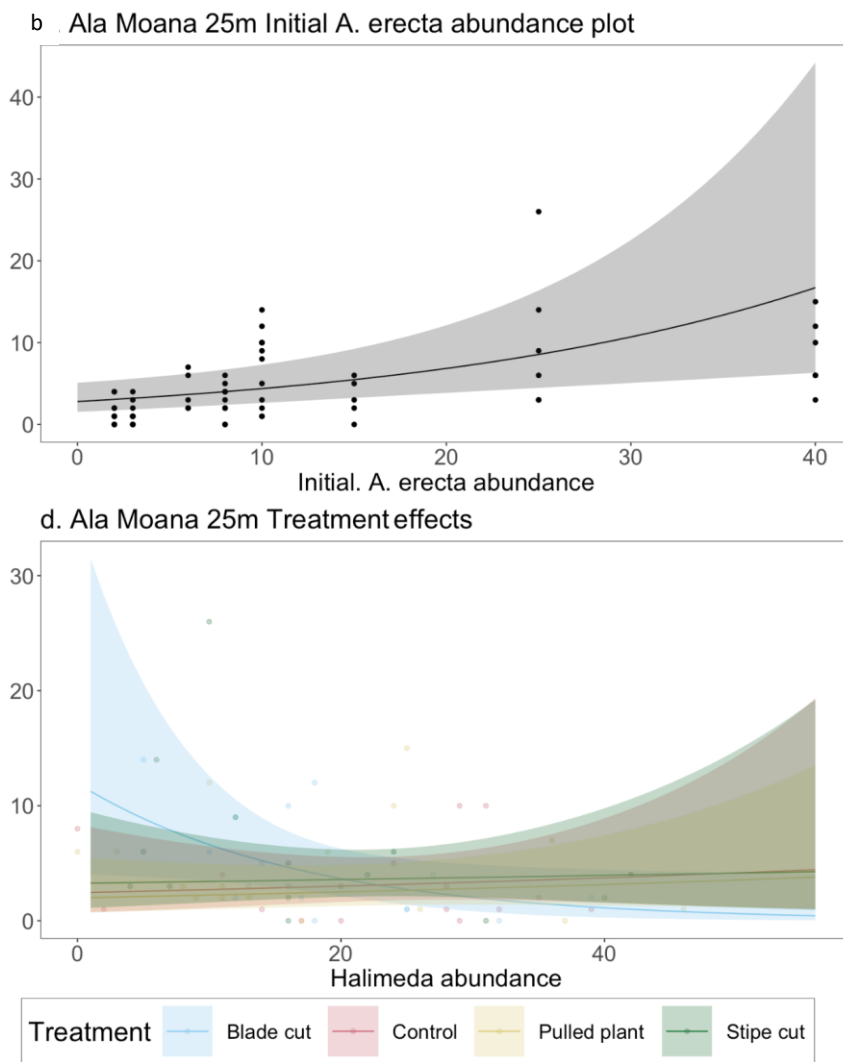


Fig 4B. A graphical representation for the Quasi Poisson General Linear Mix Model applied to data from the Ala Moana 25m site. The upper graph shows the predicted abundance of *A. erecta* in gray and the actual abundance in black dots with a black line fitted. The lower graph is the treatment effects of *A. erecta* on *H. kanaloana* abundance. With model predictions in solid color and actual trends in colored lines.



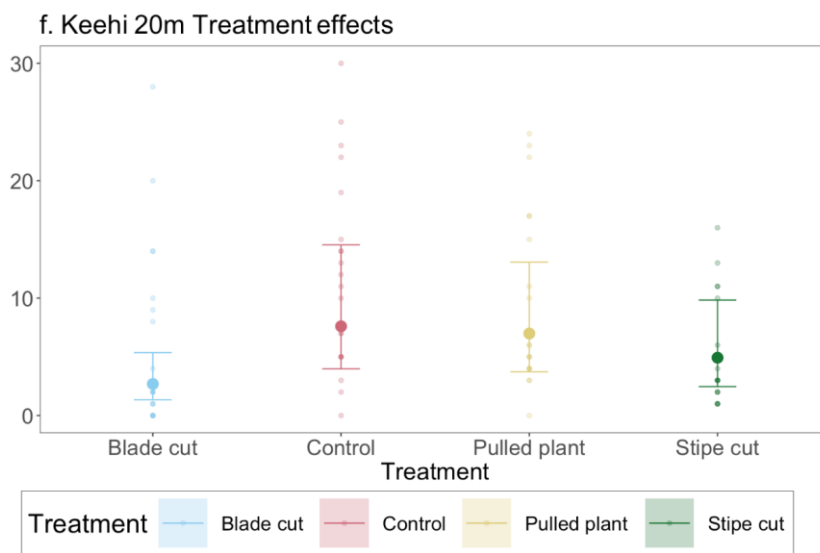
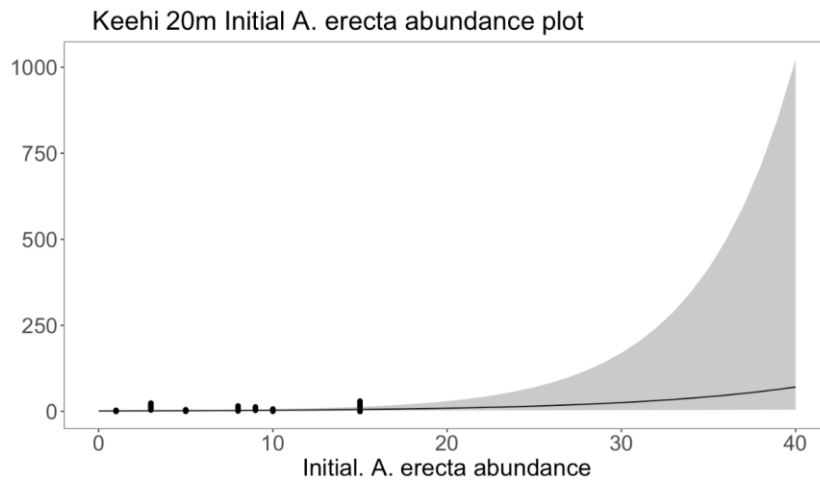


Fig eC. A graphical representation for the Negative Binomial General Linear Mix Model applied to data from the Ke'ehi 20 m site. The upper graph shows the model predictions in gray and the actual densities in black dots with a black line fitted. Lower graph shows the treatment effects variance on *A. erecta* densities.

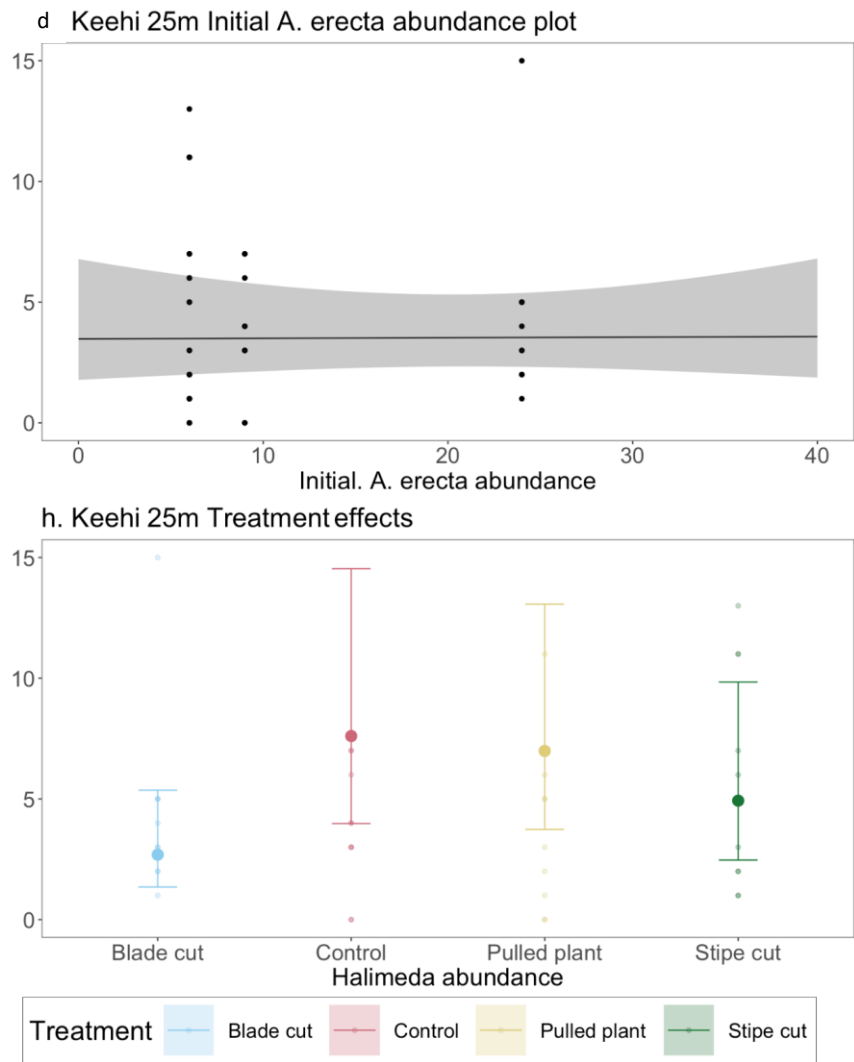


Fig eD. A graphical representation of the Quassi Poisson General Linear Mix Model applied to data from the Ke'ehi 25 m site. The upper graph shows the models predicted distribution of *A. erecta* abundance in the gray, and the actual abundance represented by the black dots fitted to a black line. The lower graph is the models' Treatment effects from *A. erecta* on the number of *H. kanaloana* numbers with the variance included on either side of the colored dot.

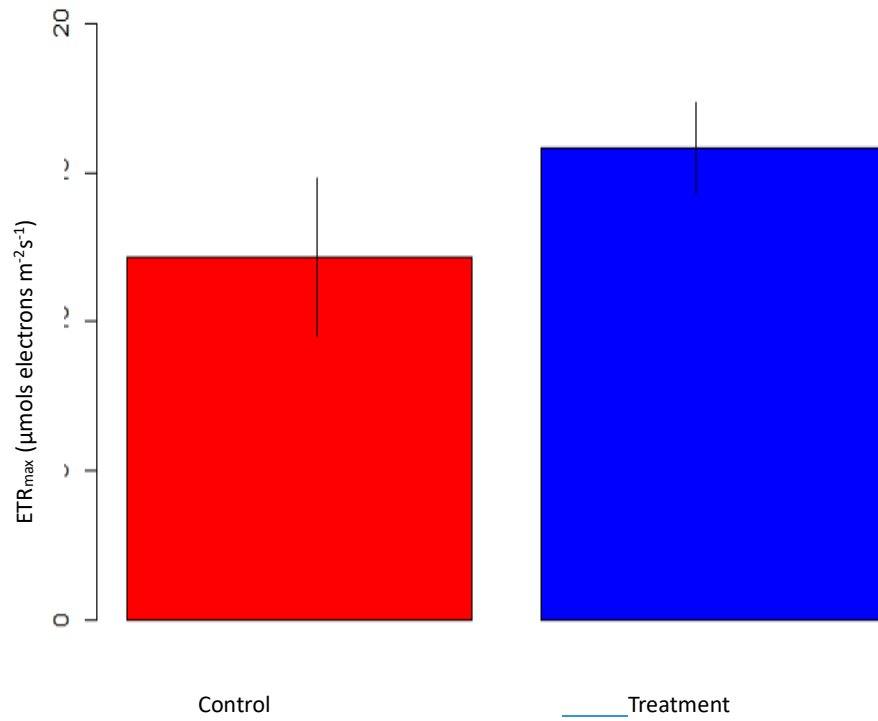


Figure 9E. Hydrogen peroxide impacts compare to saltwater impacts on *A. erecta* photosystems as revealed by measures of ETR_{max} ($\mu\text{mols electrons m}^{-2}\text{s}^{-1}$) before the injections were

applied and 4, 7, ~~11, and 11, and 15 days~~ 15 days after the injections. No significant difference between ~~the~~ treatment and control ~~was/were~~ detected

Appendix 2. PRELIMINARY BIOMECHANICAL TESTS FOR *Avrainvillea erecta* (BOT 682 lab work)

This Lab was undertaken to examine some of the physical properties of this species. Understanding how some of the dynamic forces of wave energy, herbivory, and potential spread through fragmentation will help describe how this plant survives and competes with other native plants in our psammophytic habitats.



Figure e. Biomechanical experiment (From left to right) Plant Flexure of the blade, Fragment viability tests, Penetrometer used to estimate thallus integrity and specimens used.

Estimates of plant flexure: A plant's flexure is an adaptive strategy for hydrodynamic conditions. If a plant is adapted to high amounts of water energy that species should be able to flex or bend. For many species the flexure is proportional to the amount of water energy the species can tolerate before the plant is damaged. To test this idea, the plant blade will be bent around rods of decreasing diameter (eg- 8.0, 6.5, 5.0, 3.5, 2.0, 1.0, 0.5 mm) to quantify the extent the plant can bend back on itself self. If tissue is intact after the smallest diameter rod, then the algae is scored as fully flexible. Flexure = fully flexible. This outcome supports that *A. erecta* is adapted to highly dynamic environments that includes high wave energy and surge. 360 degrees in all samples

Fragment sinking rate: Plants were cut to specific diameter sizes using the same rods that were used in *Plant Fragments*. Each fragment was patted dry and weighed by size class. A 500 mL graduated cylinder was filled with sea water. Using a stopwatch with second hand, the rate at which each fragment sank to the bottom was timed from the top of the water column which was approximately 13 cm tall. The height of the water column was measured and with each sink time, the rate of sinking was calculated for each fragment size. Each fragment size was tested three times and an average sinking rate \pm SD was calculated. The larger the fragment the longer the it took for it to sink to the bottom sink time, with the hypothesis concept that larger fragments could potentially be carried slower sink time could allow for longer farther before sinking to the benthos. If fragmentation is viable than this could increase the distance for spread. propagation if fragmentation was viable.

Table s1. Is the three trial runs and the average of those sink times for different sized fragments to sink from a 500 ml graduated cylinder beaker. ~~The larger the fragment the longer the sink time, with the concept that slower sink time could allow for longer propagation if fragmentation was viable.~~

Algae name	frag diam cm	time 1 sec	time 2 sec	time 3 sec	Av sink tm
Q	2	6.8	5.1	6.5	6.1
R	1.5	4.3	5	4.8	4.7
S	1.3	6.5	5.6	6.1	6.0
T	1	5.5	6.2	5.1	5.6

Plant Fragments: Different sized fragments were cut from the growing edge of *A. erecta* blades with a razor-edged circular rod that had diameters of decreasing size (2.1, 1.5, 1.3, 0.9 cm). Each fragment was patted dry and then placed in a 10 ml beaker with sea water to measure each fragments volume. The fragments were all placed in ice cube trays with filtered sea water from AFRC. Each fragment size was labeled and placed in the greenhouse under shade. Follow up volumes were taken over 10 days to see if new tissue was produced or lost. All fragments lost weight which suggests that the blade fragments of this size are not viable.

Table s2. ~~Is the~~ fragmentation trial that took different sized fragments of *A. erecta* blades and measured wet weight before and after 10 days to see if the plants were viable fragments. Results showed that all fragments decreased in weight.

Formatted: Font: Italic

Formatted: Font: Italic

<u>Algae name</u>	<u>frag diam cm</u>	<u>wet wt.gm</u>	<u>dry vol start</u>	<u>dry vol after 10 days</u>
Q	2	0.309	0.2	0.1
R	1.5	0.198	0.1	0.05
S	1.3	0.158	0.05	0.02
I	1	0.0477	0.01	0.001

Formatted Table

Appendix 3. FISH AND INVERTEBRATE COMMUNITY SUPPORTED BY THE MEADOW.

During this study, divers have seen a diversity of invertebrates found on the blades of *A. erecta*. The groups observed were annelids, mollusks, crustacean and egg sacs (Wheeler pers. obs.). Different fish species have also been observed foraging or dwelling within the co-occurring meadows. Some of the families regularly observed were Blenny, Box Fish, Chubs, Flounder, Goatfish, Goby, Trevally, Parrotfish, Porcupinefish, Pufferfish, Sand perch, Sting Ray's, Shark, Surgeonfish, Unicorn fish, Trigger fish, and Razor Wrasse. Some other groups were Octopus, Monk seal and Green Sea Turtles (LW, pers. Wheeler pers. obs). This amount of diversity found within the habitat indicates there is an energy exchange between the coral reef and the psammophytic meadows. The forces that drive this energy exchange were not a part of this study. However, it is an important part of both habitats' ecology and could inform the level of management needed for *A. erecta*.

