

**STUDYING THE ECOPHYSIOLOGICAL RESPONSES OF NATIVE HAWAIIAN
MACROALGAE IN A CHANGING WORLD**

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“To navigate, you must be brave and you must remember.” – Mau Piailug

My academic voyage was not an easy one. Some days were sunny and some days were stormy. Some days were a mix of everything. In the end, I was able to reach my destination because of the endless love, patience and support from those who helped me build my canoe and sailed along with me: spiritually, physically, mentally and emotionally.

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ABSTRACT

In the Hawaiian flora, approximately 550 species of algae or *limu* are identified, yet little is known about their physiological ecology: growth rates, photosynthetic capacities, or other features of biology, even for common native species. Culturally, Native Hawaiians as with many Pacific Islanders gather marine plants for nourishment, use in medicine, hula and other traditional ceremonies. Ecologically, the *limu* are the primary producers of coastal reefs, provide refuge, and food for a wide range of herbivores and omnivores, and thus are of critical importance to nearshore marine food web. In this study, I 1) analyzed the biodiversity, species composition, and photosynthetic rates of a 2-year-old benthic plant community under future climate change conditions, 2) explored how irradiance and temperature affect the growth and photosynthesis of the native macroalga *Microdictyon setchellianum*, and 3) cultivated *Sargassum aquifolium* germlings on limestone tiles to characterize their early life stages and explored the effects of shading. Results reveal that 1) the biodiversity, species composition, and total photosynthetic rates did not dramatically change under predicted climate change conditions suggesting that our *limu* community may be resilient to changes in coastal acidification and increasing temperatures of coastal waters, 2) *M. setchellianum* fulfilled expectations for a broadly distributed alga in terms of depth as well as biogeography, had lower growth rate under shaded conditions, but still photoacclimated under both low and high irradiance levels in a short period of time which is consistent with the physiological adjustments for an alga that can be found in the intertidal to deep water communities, 3) and young *Sargassum* germlings successfully grew on tiles, had high rates of photosynthesis, and an ability to recover after being shaded with physiological expectations for germlings that grow under deep shade of dense, moving canopies of adult *Sargassum*. This research provides valuable insight into the

physiological capabilities of two abundant Native Hawaiian *limu*, as well as the community of 32 species that developed in experimental mesocosms. In sum, single species studies reveal remarkable abilities that sustain their abundance in current reefs and give insight into changes in our future reefs. Analysis of communities that develop under future scenarios reveals hope for the stability of primary producer communities, at least in the Hawaiian islands. Further research to provide other crucial data will help estimate the ecological success of our benthic community and build restoration tools to help rehabilitate native limu populations such as *Sargassum*, as interest is building to restore native species on our reefs. Overall, this dissertation fills in biological data gaps for important native Hawaiian algal species and describes a cultivation tool that serves as a step towards replenishing the population of *Sargassum* on the reefs.

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LIST OF ABBREVIATIONS

AFRC – Ānuenuue Fisheries Research Center

E_k – minimum saturating irradiance

ETR_m – electron transport rate maximum

Jr. PAM – Junior Pulse Amplitude Modulated Fluorometer

MLCD – Marine Life Conservation District

MHI – Main Hawaiian Islands

NWHI – Northwestern Hawaiian Islands

PAR – photosynthetically active radiation

SGR – Specific growth rate

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CHAPTER 1: GENERAL INTRODUCTION

Anthropogenic activities have contributed significantly to greenhouse gas emissions altering the biogeochemistry of our oceans at an alarming rate (Harley et al. 2006, Intergovernmental Panel on Climate Change 2021). Tropical coastal reefs have been experiencing a shift in sea surface temperature and ocean acidity affecting the overall health of the reef, diversity, and food web (Polovina 1984, Grigg et al. 1984, Doney et al. 2012). These changes will have a huge impact on tropical species that are sensitive to narrow changes leading to a shift in community structures that can result in many native species being at risk of extinction (Harley et al. 2006, Mora et al. 2013).

Abiotic and biotic factors that drive phase shifts are illustrated by the Relative Dominance Paradigm that predicts how nutrient availability and herbivory, or wave exposure highly influences which of the four groups (turf, crustose coralline algae, macroalgae and coral) would be dominant on a reef (Littler and Littler 1985, Smith et al. 2010, Lowe and Falter 2015). This study was replicated by Smith et al. (2001) on Puakō Reef on Big Island underscoring the influence of nutrient availability and herbivory on the alteration from coral to algal dominated reefs. Another factor is competition within and amongst species (Connell 1961, Bonin et al. 2015, Aschehoug et al. 2016). Paine's (1966) classic study on the removal of the starfish *Pisaster* showed that the absence of a "keystone species" from a community can have a huge impact on the species diversity, spatial distribution, and trophic levels as well (Paine 1969).

As of July 10th, 2022, the atmospheric CO₂ concentration was 417.68 ppm, a ~100 ppm increase in the past six decades (NOAA Global Monitoring Laboratory). Climate change driven by human activities is one of the leading causes of coral reef decline across the globe (Hoegh-Guldberg et al. 2007, Hughes et al. 2003). In general, increase in CO₂ levels are thought to have detrimental

effects on the seaweed community, but studies have shown that the growth and photosynthesis of freshwater algae (Yang and Gao 2003), seagrass and macroalgae (Koch et al. 2013) have increased with elevated CO₂ concentrations. Growth and photosynthetic responses may vary across species and may be species specific as shown in corals (Bahr et al. 2016). An increase in CO₂ concentrations may be beneficial for some algae while disadvantageous to others (Ji et al. 2016). This will lead to a shift in community structure and diversity. Increase in CO₂ and temperature can increase photoprotection and photosynthesis in intertidal macroalgae as well (Celis-Pla et al. 2017).

Global surface temperatures are predicted to increase by 1.0 - 1.8 2 °C by the year 2100 under low greenhouse gas scenarios versus a 3.3 – 5.7 °C increase under high greenhouse gas scenarios (IPCC 2021). Temperature fluctuations across the globe will affect the photosynthetic process of seaweeds, which is crucial to their growth and survival (Pakker et al. 1995; Davison 1991). Comparative studies on the photosynthetic acclimatization for tropical and temperate species demonstrated that the temperate species have a wider temperature tolerance range and can acclimatize to seasonal changes whereas the tropical species have a narrower temperature range and did not show any seasonal differences in photosynthesis (Dawes et al. 1998). This shows that temperate species are more susceptible to increased temperatures.

Significance of Macroalgae in Hawai‘i

Hawai‘i has over 550 species identified across the archipelago (Abbott and Huisman 2004, Abbott 1999, Huisman et al. 2007) yet the last decades of research have focused on the invasives (Smith et al. 2002, 2004, Conklin and Smith 2005, Wade et al. 2018, Veazey et al. 2019). Since the 1950’s, different algae have been introduced, intentionally and unintentionally, to the

Hawaiian Islands (Smith et al. 2002). An example of an alga that was introduced intentionally is the invasive red alga *Gracilaria salicornia* (C. Agardh) E. Y. Dawson. *G. salicornia* was brought in for agar production with little understanding of the basic biology of growth and reproduction (Smith et al. 2002). Unknowingly, *G. salicornia* had the ability to reproduce asexually via fragmentation allowing it to spread and outcompete native species over a short period of time through the formation of thick mats that smothered the reefs (Smith et al. 2004). Another mat forming red alga *Chondria tumulosa* has taken over reefs at Pearl and Hermes Atoll in the Papahānaumokuākea Marine National Monument (PMNM), but the researchers are still unsure of where it came from. This alga has not been identified as a native species, but it possesses invasive traits (Sherwood et al. 2020). Currently, studies are underway to understand the native *Chondria* that is found on O‘ahu (Celia Smith pers. comm.) highlighting the urgent need to understand our native species to gain insight on the invasive counterpart. Very little attention has been focused on our native species resulting in limited published information on their ecology or biology. Fundamental modern data such as growth rate and rates of photosynthesis are missing. These gaps limit our ability to anticipate how reef communities will change in the next 100 years as primary production changes.

Macroalgae or *limu* as they are called in the Hawaiian language has been an integral part of the Hawaiian culture, traditions, and identity. The Native Hawaiians placed great value on their limu knowing that the abundance and health of the limu community meant the people would thrive as well. They have woven limu into their daily lives by using the different varieties for food, medicine, hula, and traditional ceremonies (Huisman et al. 2007, McDermid et al. 2019). This year, Hawai‘i State Governor David Ige made a proclamation designating the year 2022 as the “Year of the Limu” (The Maui News 2022) as a way to recognize the ecological and cultural

significance the limu community holds in Hawai‘i. Ecologically, marine algae provide a wide range of services such as food for important herbivorous grazers that include invertebrates (Van Heukelem 2016), fish and larger animals like the Hawaiian green sea turtle (Odum and Odum 1955, Russell Balazs 2000). As primary producers, they oxygenate coastal waters (Ryther 1953), serve as a sanctuary for microorganisms (Egan et al. 2013), and their stature provides refuge for juveniles from several trophic levels (Parrish and Boland 2004) and a natural wave barrier for coastal communities against erosion (James et al. 2019). In addition, calcified macroalgae such as crustose coralline algae are significant to the expansion of the reef (Vroom and Braun 2010) and serve as recruitment for coral. These contributions are crucial to the structure of food webs as we know them (Odum and Odum 1955, Ryther 1953).

***Microdictyon setchellianum* Ecology**

The green algal genus *Microdictyon* (Empire Eukaryota, Kingdom Plantae, Phylum Chlorophyta, Class Ulvophyceae, Order Cladophorales, Family Anadyomenaceae, Guiry and Guiry 2022) has mesh-like blades that may anchor at a common location in the understory forming rosettes that are frequently covered with sand or turf species making it hard to locate and identify on the reef. Those in tide pools are exposed to frequent wave action making them less obscure and cleaner of turf species showing its bright green color. New younger blades have a brighter green color, while the older blades are darker in color. New cells grow from the inside and outwards. The tips of the blades are sometimes white which may indicate possible mortality or reproduction compared to the genus *Ulva* when cells are released or damaged. The two identified species in Hawai‘i are *M. umbilicatum* and *M. setchellianum* that are very similar in morphology but differ in cell size. *M. umbilicatum* has a more delicate net structure (<0.1 mm) compared to *M.*

setchellianum's coarse filaments (<0.5 mm) (Abbott and Huisman 2004, Huisman et al. 2007, Guiry and Guiry 2022).

Microdictyon is found in the Central Indo-Pacific region that includes the Federated States of Micronesia (FSM), French Polynesia, and the Hawaiian archipelago (Abbott and Huisman 2004, Howe 1934, Guiry and Guiry 2022). Species of *Microdictyon* are highly abundant in dense meadows covering >75% of the fore reefs in the Papahānaumokuākea Marine National Monument (Vroom and Braun 2010). This genus is also common on the reef flat and lagoon of Ant Atoll in Pohnpei forming dense free floating and anchored mats around and in between coral heads (Yamase pers. obs.). This genus has a wide range depth from rocky intertidal shores to 65 m depths in the mesophotic zones in the NWHI (Spalding 2012, Pyle et al. 2016). *Microdictyon* can form extensive meadows on a reef providing ecological services to a wide range of herbivores. This plant was also a common member of the understory of reef plants in Waikīkī in the 1960s (Doty 1969).

Currently, there are no published papers on the Hawaiian species of *Microdictyon* that specifically focuses on the simple biology such as reproduction, growth, and photosynthetic rates. This alga is one of the dominant species on reefs in Hawai'i and plays a major role in the marine community, yet crucial information for this alga is unavailable. This missing information is crucial in assisting resource managers and the local community in sustaining this meadow forming alga that supports the rich biodiversity across the Hawaiian archipelago. The alga's limited distribution across the Indo-Pacific is one reason why *Microdictyon* meadows and trophic structure of reefs are at risk (Mora et al. 2013).

Due to the lack of studies on the Hawaiian material, we do not know what is influencing the abundance and distribution of this alga across the islands as a successful meadow forming alga.

A comparison study between the populations in the deep waters in Papahānaumokuākea and the shallow waters in the Main Hawaiian Islands need to be conducted to better understand this dominant species that is supporting and sustaining life from the shallows to the deep and across the Hawaiian archipelago.

***Sargassum aquifolium* Ecology**

Sargassum (Empire Eukaryota, Kingdom Chromista, Phylum Ochrophyta, Class Phaeophyceae, Order Fucales, Family Sargassaceae, Guiry and Guiry 2022) is classified as a winter species that can be found on rocky intertidal shores that are exposed to high wave action (Huisman et al. 2007). This genus has long lateral branches that can grow up to 1 m long creating canopies when standing upright in the water column. This canopy forming alga can support a wide range of marine organisms that occupy the seafloor up to the water surface. In Hawai‘i, five species are identified: *S. aquifolium* (Turner) C. Agardh, *S. obtusifolium* J. Agardh, *S. polyphyllum* J. Agardh, *S. hawaiiensis* Doty and Newhouse and *S. muticum* (Yendo) Fensholt. The three most common species are *S. aquifolium*, *S. obtusifolium*, and *S. polyphyllum*, which are usually found in dense golden beds along rocky shorelines that are found in areas like Makapu‘u and Sand Island Beach Park. *Sargassum* species are very similar in morphology making it difficult to distinguish between them. For *S. polyphyllum*, it has a more distinct spiral and bushy-like blade morphology compared to the other two common species making it easier to identify. Whereas *S. aquifolium* and *S. obtusifolium* have closer morphological features that require a closer look at the receptacles, a specialized branch that houses gametes, to determine species. The receptacles for *S. aquifolium* are spiny and burr-like versus the more oblong and rounded receptacles of *S. obtusifolium* (Huisman et al. 2007). In addition to receptacles, they have structures called

vesicles, air-filled sacs that assist with buoyancy, allowing them to float and move along with the ocean current.

All three have different distributions with *S. aquifolium* being found almost across the whole tropical region, *S. obtusifolium* in the eastern Pacific basin, and *S. polyphyllum* in the central Pacific with some reported in the southern hemisphere (Guidry and Guidry 2022). None of these species are endemic to Hawai‘i but two have been first collected and identified in Hawai‘i.

Sargassum is also very common in the northernmost atolls in the NWHI (Vroom and Braun 2010) and in the Federated States of Micronesia (Tsuda 2002, Tsuda 2006).

Sargassum aquifolium is one of the most important, if not the most important, limu in Hawai‘i as it holds invaluable connections to the native Hawaiian community and the reefs as well. This alga is known as limu kala in Hawaiian and is used in a traditional ceremony, ho‘oponopono, to seek forgiveness when there is conflict between two parties. This alga is even used for medicinal purposes, dancing, food, and other ceremonies (Abbott and Huisman 2004, Huisman et al. 2007, McDermid et al. 2019).

Blue Carbon Initiatives

Due to the dramatic effects of climate change, coastal blue carbon initiatives are starting to gain interest to use our natural coastal and marine ecosystems to capture carbon (The Blue Carbon Initiative). The continuous emission of greenhouse gasses is affecting all aspects of our livelihoods (Mora et al. 2018) and so now we are looking to our natural ecosystems such as mangroves, marshes, seagrasses, and even macroalgal communities for help (Howard et al. 2017, Herr et al. 2017). Hill et al. (2015) demonstrated that macroalgal communities have the ability to contribute to the blue carbon initiative on a global scale. Gouvea et al. (2020) used models and

algorithms to quantify the amount of carbon that can be absorbed through the above-ground biomass (AGB) of *Sargassum*. *Sargassum*'s estimated AGB was 13.1 Pg C on a global level, which is significant compared to the amount that other ocean ecosystems can absorb. Our changing oceans will modify just about every parameter that regulates plant growth and photosynthesis affecting seaweed communities ranging from the kelp forests to the calcareous algae (Harley et al. 2012). Simple and crucial biological data for many of our native algal species in the Pacific remain unavailable or nonexistent even in the 21st century. Basic biological data such as growth and photosynthetic rates are nonexistent for most dominant species that play a major role in ecosystems. This is a major problem because these species serve as the base of the food web and foundation of the marine community in the NWHI. Just like golden forests found along the coast of California, limu kala provides the same significance by providing shelter, nursery grounds, protection, and food to many marine organisms. It also attaches to the substrate through a holdfast that allows it to remain anchored just like the roots of a tree. The blades or leaves of the limu kala forest provide shade and house many organisms that are hiding from predators. My research will fill in missing gaps in our current data and provide us information on how *Microdictyon setchellianum* is a keystone species along with the native brown alga *Sargassum aquifolium* that holds cultural and ecological significance to the Hawaiian community. These experiments will give us fundamental information to answer how will ocean changes impact key reef plants for the broader Pacific reef biota?

Research Focus

This dissertation focuses on filling in crucial ecophysiological data gaps that exist for the native Hawaiian macroalgae community including exploring cultivation tools to help restore native algal populations that have been replaced by the disruption of climate change and invasive

species. Chapter 1 explores how different environmental parameters such as seawater temperature, daylength, and seasonality between 2013-2021 influences the abundance, growth, and photosynthetic rates of the dominant native green alga *Microdictyon setchellianum*. Chapter 2 includes development of a cultivation process of rearing germlings of the culturally significant native brown alga *Sargassum aquifolium* or *limu kala*, examining how different irradiance levels affect the growth and photosynthesis of juvenile *S. aquifolium* and how they compare to their mature adult counterparts. Fieldwork for both species were conducted using an open outdoor seawater system that is located at the Ānuenuue Fisheries Research Center. Both chapters entail the first recorded growth and photosynthetic rates for these native species. Chapter 4 assesses the biodiversity, abundance, and community structure of a 2-year-old algal benthic community that has been growing under future climate change conditions. This highly collaborative experiment was conducted in an open mesocosm setup at the Hawai‘i Institute of Marine Biology. This climate change study provides realistic long-term results of what our reefs in Hawai‘i may look like in the next 100 years. Overall, this dissertation will provide valuable insight about our native plants, which will help island communities across the Pacific make better and informed decisions regarding management and sustainability of their reefs.

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CHAPTER 2: ASSESSING ALGAL DIVERSITY AND PHOTOSYNTHETIC RESPONSES IN A 22-MONTH MESOCOSM STUDY SIMULATING CLIMATE CHANGE, KĀNE‘OHE BAY, HAWAI‘I

ABSTRACT

Climate change is a global phenomenon that is impacting the primary productivity of major marine ecosystems including coral reefs and benthic algal communities. By the year 2100, the ocean is predicted to become more acidic and warmer, which is expected to be detrimental to the survival of many tropical organisms known to have narrow tolerance ranges. A broad multidisciplinary two-year study completed in 2019 tested these predictions. Outdoor mesocosms were populated by natural recruitment processes via unfiltered water from Kāne‘ohe Bay and were allowed to grow under conditions that simulated predicted ocean changes in four treatments: 1) ambient, 2) acidic (-0.2 pH units), 3) heated (+2 °C), and 4) a combination of acidic and heated treatments (-0.2 pH units and +2 °C). In this study, we measured the biodiversity, species composition, and total photosynthetic rates of the 2-year-old benthic plant community that developed in each mesocosm and across treatments. All mesocosms were photo documented, algal specimens were collected and identified, and photosynthetic rates were taken using pulse amplitude-modulated fluorometry. A total number of 32 species were identified in this experiment with only one being the invasive alga, *Acanthophora spicifera*. Species composition in terms of percent cover, and total electron transport rate maximum (ETR_m) and minimum light saturation (E_k) did not significantly differ across treatments. These results suggest that for this region, the algal biodiversity, community composition, and photosynthetic responses are not likely to decline under ocean conditions predicted for the 21st century. The plant community in Kāne‘ohe Bay appears well suited and will likely continue to recruit and grow, leading to reefs dominated by native species. Understanding patterns and diversity of benthic plants that settle and acclimate under these environmental parameters will help us better manage future reefs.

Keywords: *macroalgae, photosynthesis, temperature, ocean acidity*

INTRODUCTION

Climate change is an ongoing phenomenon impacting coral reefs across the globe (Hoegh-Guldberg et al. 2017, Smith et al. 2020). By the year 2100, it is projected that average global sea surface temperatures are very likely to increase by 3.3 °C to 5.7 °C compared to 1850-1900 records under the highest greenhouse gas emission scenario while global ocean pH will decrease by 0.4 to 0.5 units (IPCC 2021). These changes in ocean acidification and temperature can alter marine ecosystems by affecting the development, physiology, and distribution of tropical marine flora and fauna (Baker et al. 2008, Koch et al. 2012, Bahr et al. 2016, Mostofa et al. 2016, Des et al. 2020).

Because coral reefs are complex systems that support high levels of biodiversity, climate change impacts are expected to be severe, particularly for corals (Hoegh-Guldberg et al. 2017, Kaplanis et al. 2020, Peña et al. 2021). Major events such as coral bleaching are expected to progress and become more frequent (Hughes et al. 2018). The changes in ocean chemistry and temperature will increase the vulnerability of coral leading to the decline in biodiversity (Grottoli et al. 2014, Ainsworth et al. 2016) and the migration and distribution of fish populations will be altered due to environmental cues being prolonged or shortened (Langan et al. 2021, Hu et al. 2022). In addition, crustose coralline algae (CCA), a significant key player in building reefs, coral settlement, and recruitment, will decline due to poor cell wall development and sensitivity to temporal changes (Chan et al. 2020, Britton et al. 2021, Moore et al. 2021, Peña et al. 2021). These are a few examples of how marine ecosystems and communities across trophic levels will be impacted.

Despite Odum and Odum's (1955) early contribution of primary production to Eniwetok's trophic dynamics, few studies focus on the effects of climate change on benthic macroalgae, especially for taxa in the central Pacific. The macroalgal community provides a suite of services such as the foundation for the food web, primary production, oxygenation, and aiding as bioindicators for reef health that are linked to water quality, herbivory levels, and nutrient enrichment (Smith et al. 2000, 2005, Dailer et al. 2012, Amato et al. 2016, Cannon et al. 2019). Yet, macroalgae still remains overlooked and many climate change studies focus on corals (Hughes et al. 2018), invertebrates (Przeslawski et al. 2008) and seagrass (Short and Neckles 1999). Reefs can support long-lived, diverse assemblages of algae that successfully recruit and grow faster than local herbivory (Smith et al. 2010, Vroom and Braun 2010), and CCA that can possibly persist for years (Teichert et al. 2020). These diverse contributors are likely to yield distinctly different sets of responses to climate change variables. Hence, exploring the response of the benthic community through a holistic approach is highly encouraged to achieve more realistic interactions and responses between and across species.

Many studies have explored the responses of our reefs by focusing on specific genera within a short timeframe, but few studies have allowed full recruitment from the reef over a long-term period. Although these short-term studies provide valuable insight as to how these organisms respond to global change, long-term data are crucial to understanding and predicting the future health of our reefs as climate change continues to persist. This study is part of a larger climate change experiment that has taken a holistic approach to exploring the responses of the different benthic communities that were present in the mesocosms. The findings for the cryptobiota (Timmers et al. 2021) and coral community (Bahr et al. 2020, McLachlan et al. 2022) have already been reported. The crustose coralline algae are the focus of a separate investigation

(Cheryl Squair, pers comm). This broad assessment of major ecosystem components provides significant insight as to how our benthic community will respond on a community and species level providing ecological relevance to the natural processes that take place on the reef. Here, we report the results from examining the long-term effects of predicted future conditions on the species composition, diversity, and photosynthetic response of the macroalgal benthic community.

METHODS

Mesocosm Setup

This experiment was conducted at the Hawai'i Institute of Marine Biology, Coconut Island, in Kāne'ōhe Bay, Hawai'i, in an open (unshaded) region adjacent to surrounding reefs. An outdoor, open flow seawater system delivered coastal water from the adjacent reef slope located about 30 m from shore, 2 - 3 m off the reef slope with a ~ 3 - 4 m depth (Figure 2.1). The seawater was unfiltered allowing for natural recruitment and plankton to enter to feed the array of invertebrates that colonized the mesocosms. Growth of communities in 40 polypropylene mesocosms (Utilatub, Manufacture #19F, Item #44728, Volume = 0.075 m³) recruited and grew under ambient reef conditions in terms of day length, and solar intensities. This fully factorial design consisted of four treatments with ten replicates each: 1) control (temperature = ~24 °C and CO₂ = ~ 400 μatm), 2) acidic (~750 μatm), 3) heated (+2 °C) and 4) a combination of acidic and heated treatments (Figure 2.2) with a 1.2 L min⁻¹ flow rate (1 hr residence time). Details of environmental parameters (salinity, temperature, pH, and pCO₂) are provided in Bahr et al. (2020), Timmers et al. (2021), and McLachlan et al. (2022). Further, each mesocosm contained a powerhead (Maxi-Jet Pro) to produce water motion and an outflow pipe to keep the water in each

tank leveled. All treatments were randomized. Ambient irradiance between 10 am – 2 pm ranged from 1400 – 2,000 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$. Irradiance in the mesocosms decreased by ~25 - 30% from the shade cloth located above the system in addition to the sun position shifting throughout the day. Threadfin Butterflyfish (*Chaetodon auriga*) and Convict Tangs (*Acanthurus triostegus*) were placed in tanks to provide herbivory and rotated every two weeks to ensure equal herbivory across mesocosms. This setup is further described in Timmers et al. (2021). All algal data were collected in the final months of the two-year experiment when communities were well established.

Photo-documentation and Percent Cover of Mesocosm Wall Communities

All four sides of each mesocosm were photo documented using a Canon PowerShot G10 camera in an underwater housing. The bottom of the mesocosms were not photo documented due to the setup of another experiment. Two photos (4416 px X 2480 px) for each side were taken, documenting the four-side walls within each mesocosm. All photos were cropped to 2600 px X 2010 px (resolution: 2999.999 px/in or 118.11 px/cm) using Adobe Photoshop CS5 (Version 12.1 x64) and then uploaded to CoralNet – UCSD, a web-based program that analyzes coral reef images to estimate percent cover using an annotation point generator (Beijbom et al. 2015). A total number of 45 simple random points were generated, but only 25 points were used for percent cover. Random points that landed outside of usable view planes (e.g. water interference) were voided and the next point that landed on the wall was accepted until 25 points were identified. Percent cover included coral, crustose coralline algae (CCA), the chlorophyte, phaeophyte, and rhodophyte macroalgae by genus, cyanobacteria, and empty space (Appendix 2.S1). Turfs were not included as a category in this analysis because they are too small to

properly identify in the photos. After all photos were analyzed, the data were downloaded from CoralNet as an excel file and percent cover was calculated. A nonmetric multidimensional scaling (nMDS) plot was then constructed to examine the ordination of the benthic composition between treatments.

Photosynthetic Measurements - Jr. Pulse Amplitude Modulated (PAM) Fluorometer

Photosynthetic rates of all macroalgae and tufts that consisted of turf species were taken in July 2018, after photo documentation was complete. A Jr. Pulse Amplitude Modulated Fluorometer (Walz, Germany) was used to measure photosynthetic rates as rapid light curves calculating the maximum electron transport rate (ETR_m) and minimum amount of light saturation (E_k) (Ralph and Gademann 2005). The experimental design was set to have a single reading recorded for every collected taxon within each mesocosm as an initial community measure of photosynthesis. These measurements took place between 10 am - 2 pm during maximum daytime irradiance and photosynthesis activity hours for the plants. Photosynthetic readings were taken *in situ*. If plants were found at the lower parts of the mesocosm, a portion of the sample was carefully detached and brought up just below the water surface for readings. All species collected in each tank were averaged for a global, total number of readings for each treatment: Control: 28 readings, Acidic: 28 readings, Heated: 27 readings, Acidic + Heated: 22 readings; Table S2). The averages for each replicate (Control: $n = 10$, Acidic: $n = 9$, Heated: $n = 10$, Acidic + Heated: $n = 9$; two treatments only have 9 replicates because those tanks lacked macroalgal growth) were averaged again for each treatment.

Sample Collections

After photosynthetic measurements were taken, all frondose and turf samples were carefully collected by hand and placed in Ziploc bags that were labeled with a tentative species name, tank number and date. Each Ziploc contained seawater from the appropriate experimental mesocosm in which the sample was collected and placed in a cooler to be transported to the Botany Department, UH Mānoa for further identification. Aniline blue stained permanent slides of macroalgae and turf were made within 48 h of collection following Tsuda and Abbott (1985). All slides were identified using taxonomic keys found in Abbott (1999) and Abbott and Huisman (2004). Taxonomic synonyms were confirmed using AlgaeBase (Guiry and Guiry 2021). All CCA samples were dried and wrapped in bulbous paper (4x6 inches, Cat. 70086-01, Electron Microscopy Sciences) then placed in a snap cap vial (15 dram) that contained silica gel. Each vial was labeled with a short description, tank number and date. These samples were stored for analysis that is still underway (Squair pers comm).

Maragos et al. (2004) used a 5-point scale “DACOR” to determine which coral species were Dominant, Abundant, Common, Occasional, or Rare. A modified approach was used to classify the identified taxa into four groups instead of five: D (dominant): alga present in $\geq 50\%$ of the tanks, C (common): alga present in $\geq 25\%$ of the tanks, O (occasional): alga present in $< 25\%$ of the tanks, and R (rare): alga identified in only one tank.

Statistical Analysis

Abundance was calculated by the mean percent cover from all four sides of each mesocosm for each treatment. Diversity was measured by the total number of species identified in each treatment. A one-way ANOVA was used to compare the mean ETR_m and E_k across treatments

using JMP Pro (Version 16.1.0). The Bray-Curtis dissimilarity test was used to assess the differences of the benthic community composition across treatments. These differences were visualized through a non-metric multidimensional scaling (nMDS) plot using R (Version 1.1.463). A set of data points that are close in proximity to one another means they are similar to each other in contrast to those that are farther away. Stress values above 0.3 means the ordination is arbitrary, values below 0.2 is fair and values below 0.1 are considered robust (Clarke 1993, Quinn and Keough 2002). The function *ordispider* in vegan package was used to connect data points in each treatment to visualize scatter and overlapping of data.

RESULTS

Percent Cover Across Treatments

Percent cover across treatments showed CCA and cyanobacteria as the two dominant groups that covered the mesocosm vertical walls (Figure 2.3). CCA had the highest percent cover in the heated treatment (32.8%) followed closely by the control (31.7%) with the lowest cover in the acidic treatment (17.7%). With the one outlier in the acidic treatment, there are no significant differences between treatments ($P = 0.271$, $f = 1.36$). In contrast, when the outlier was removed, significant differences were detected between treatments ($P = 0.019$, $f = 3.81$). The results showed that the acidic percent cover was significantly lower compared to the control ($P = 0.049$) and heated treatments ($P = 0.024$). Cyanobacteria covered at least 49% of the walls in each treatment with the control and acidic treatment equally having the highest cover of 57.7%.

Benthic Composition Across Treatments

There were no distinct differences in communities across all treatments (Stress = 0.13, Figure 2.4). nMDS plots also reveal no benthic composition differences across side walls in each treatment. No differences were found (Control: Stress = 0.11, Acidic: Stress = 0.11, Heated: Stress = 0.15, Acidic + Heated: Stress = 0.10) based on the proximity of data points plotted and lack of clustered data. All stress values were below 0.2 indicating that the data are fair representations of the wall communities. The permutational ANOVA analysis showed no significance for communities across treatments ($P = 0.325$, $f = 1.16$, $R^2 = 0.09$).

Photosynthetic Responses

ETR_m ranged from a minimum of $6.2 \mu\text{mol electrons m}^{-2} \text{ s}^{-1}$ for *Agissea inamoena* and a maximum of $84.0 \mu\text{mol electrons m}^{-2} \text{ s}^{-1}$ for *Hypnea cervicornis*. E_k ranged from a minimum of $44.3 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ for *Hypnea cervicornis* and a maximum of $84.0 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ for *Jania adhaerens*. There were no significant differences between the mean ETR_m ($P = 0.640$, $f = 0.57$) and mean E_k ($P = 0.840$, $f = 0.28$) values across treatments (Figure 2.5 and Figure 2.6, respectively, Appendix 2.S3). Both ETR_m and E_k varied across species within treatments, but with no significant differences.

Algal Diversity and Reproductive Plants

After two years of growth under experimental conditions, a total of 32 macroalgal species were identified in this experiment (16 Rhodophyta, 12 Chlorophyta, 4 Ochrophyta); 97 percent were native species despite the water intake location being in a well-known region for invasive algae. The number of species found in each treatment: 1) Control = 18 (10 Rhodophyta, 6 Chlorophyta,

2 Ochrophyta), 2) Acidic = 19 (9 Rhodophyta, 6 Chlorophyta, 4 Ochrophyta), 3) Heated = 14 (7 Rhodophyta, 5 Chlorophyta, 2 Ochrophyta), and 4) Acidic + Heated = 17 (8 Rhodophyta, 7 Chlorophyta, 2 Ochrophyta) (Figure 2.7). Algae that were found in all treatments include the red algae *Acrochaetium* sp., *Hypnea cervicornis* and the red crusting alga, *Agissea inamoena*, the green alga *Dictyosphaeria versluysii*, and the brown algae *Dictyota friabilis* and *Feldmannia mitchelliae*. The taxa that were exclusively in the control treatment were *Champia vieillardii*, *Gelidium crinale* and *Valonia aegragropila*. The taxa that were only found in the acidic treatment were *Chamaebotrys boergesenii*, *Dictyota acutiloba*, *Dictyota ceylanica*, and a *Gelidiella* sp. The taxa that were only present in the heated treatment were *Acanthophora spicifera* (2 tanks out of 10) and *Gelidiella antipai* (1 tank out of 10). Finally, the taxa that were present in the combined acidic and heated treatment were *Champia parvula* and *Phyllocladion anastomosans* (Appendix 2.S4).

Hypnea cervicornis was the only alga that was classified as dominant having presence in $\geq 50\%$ of the tanks. The common algae were *Dictyota friabilis*, *Feldmannia mitchelliae*, and *Jania adhaerens* with presence in $\geq 25\%$ of the tanks. Most of the algae were in the occasional and rare categories (Appendix 2.S5).

A total of eight species displayed reproductive structures during the identification process. The eight species are *Champia parvula* (Acidic + Heated, tetraspores), *Gelidium pusillum* (Acidic, cystocarps), *Chamaebotrys boergesenii* (Acidic, tetrasporangia), *Gelidium crinale* (Control, spermatangia sori), *Pterocladia caerulescens* (Heated, cystocarps), *Hypnea cervicornis* (Acidic, tetrasporangia), *Dictyota friabilis* (Control, oogonia sori), and *Feldmannia mitchelliae* (Acidic, plurilocular structures) (Figure 2.8). All identified taxa have a life history of sporic meiosis also known as alternation of generations.

DISCUSSION

Climate change in our oceans will lead to thermal stress and acidification causing detrimental effects to many animal species in our coral reefs (Baker et al. 2008, Peña et al. 2021). Studies have shown that organisms with narrow tolerance ranges and that depend on carbonate to build their skeletal structures will be negatively affected (Fabricius et al. 2015, Britton 2021).

However, this study showed that the benthic algal community remained stable in terms of species composition, diversity, and photosynthetic responses. The preconceived notion that climate change will be harmful for our oceans does not garner support for this particular marine plant community. The ecophysiological responses need to be further explored on a species-specific level and yet also explored at ocean basin scales, because of species diversity. We need to identify those “winners” and “losers” to understand the real shifts that may take place on our reefs.

The data collected for the algal community was taken near the end of the 2-year study when species were well settled and mature. The absence of an alga in a tank does not mean it was never present during the experiment. What emerges as important is the presence of each taxon under experimental conditions, indicating that these algae were able to successfully settle, compete for space, continue to grow in such conditions and in a surprising number, and reproduce in their respective treatments. This community of taxa, broadly speaking, represents those that are considered to be dominant such as *Hypnea cervicornis*, common such as *Feldmannia mitchelliae*, and *Dictyota friabilis* and uncommon or rare such as *Chamaeobotrys boergesenii*, *Champia parvula*, *Phyllocladion anastomosans*, and *Valonia aegragropila*. This was quite surprising given that the intake for the water system was from a reef that would generally not possess these species. The plant communities represent natives that are naturally

recruited from the water column. The prolific production of reproductive cells by these plants with an alternation of generations successfully settled and grew on the mesocosm walls outcompeting other life histories for limited space. As with the reef, there is also limited space and a combination of biotic and abiotic factors – irradiance, herbivory, temperature, water motion, seasonality, and time of reproduction – that determine if a plant can be successful. CCA and cyanobacteria were the two groups that dominated the mesocosm walls across all treatments compared to the other functional groups, such as corals, sponges, and macroalgae. CCA along with many other calcifying marine organisms are highly sensitive to ocean acidification resulting in the lowest coverage in the acidic treatment. Many studies have shown that ocean acidification will inhibit the development, calcification rates, abundance, and photosynthetic capacity of these important reef builders (Jokiel et al. 2008, Kuffner et al. 2008, Fabricius et al. 2015, Britton et al. 2021). This will negatively impact coral larvae settlement (Webster et al. 2012) including its own microbiome communities (Webster et al. 2011). Another factor that affects CCA growth is temperature. Siboni et al. (2015) showed that two populations of *Porolithon onkodes* with different optimal temperatures (28 °C and 30 °C) performed better close to their optimal temperature and recruited similar levels of coral larvae. This shows that responses may also differ within species. Macroalgae comprised less than 15% of the mesocosm walls across all treatments, which may have been due to the herbivorous fish that were rotated throughout the experiment and simply being outcompeted for space by CCA. Despite percent cover being low, a total number of 32 species were identified representing high diversity. Overall, mean photosynthetic parameters did not differ across treatments showing that the plant communities possibly successfully acclimated over the 2-year timeframe. Photosynthetic rates differ across species which may explain the wide variations across all treatments resulting in no

significant differences for both averaged parameters. The exposure to irradiance in the tanks also varied depending on the time of day and the side of the wall the algal samples grew on. Ocean acidification can influence the rate of photosynthesis with more carbon dioxide available for use. This portion of the photosynthetic capacity is being explored in a related paper (Kawachi, in prep). This response is also species dependent as some micro- and macroalgae in the genera *Nannochloropsis*, *Isochrysis*, *Sargassum*, *Gracilaria*, and *Ulva* can sequester carbon at different levels (Kaladharan et al. 2009). In Germany, the photosynthesis and growth of *U. lactuca* increased in elevated CO₂ conditions (Olischläger et al. 2013). Kim et al. (2016) showed that the growth and photosynthesis of five macroalgal species responded differently to elevated CO₂ concentrations in a mesocosm study. In comparison of the photosynthetic parameters of the acidic treatment to the control treatment, mean ETR_m are similar and mean E_k was slightly higher in the acidic treatment.

Results reported here show that algal diversity did not decline with heat and acidity as single or combined effects. The only invasive *Acanthophora spicifera* was found in two tanks in the heated treatments. *A. spicifera* is found across the tropics and subtropical areas across the globe (Global Invasive Species Database 2021) and was accidentally introduced to Hawai‘i through a military fuel barge from Guam (Doty 1961). Because of its ability to grow and spread rapidly via fragmentation, *A. spicifera* remains one of the top invasive algae in Hawai‘i (Kilar and McLachlan 1986, Smith et al. 2002). Based on its Pacific-wide distribution, this alga may favor warmer waters which may be why it was only found in the heated treatment and may continue to persist on the reefs with future water conditions. It is striking that no other known invasive successfully recruited to any treatment including the control tanks.

The native algae that were found in most of the elevated temperature tanks were *Dictyosphaeria versluysii* and *Hypnea cervicornis*. An example of a strong correlation between increasing biomass and water temperature was demonstrated by the alga *Sargassum polyphyllum* in Glenn et al.'s (1990) study. Temperature alone explained 65% of the variability in standing crop for Waikīkī, but this alga was not present in the two-year-old community. It is possible that no native population of *Sargassum* was in the vicinity of the intake pipe as suggested by biodiversity surveys for the Bay (Smith et al. 1973). As for the other native species that were present in only one of the treatments, there is a possibility that they were present in multiple treatments, but then disappeared over time with increased competition for space or possible over-grazing by the herbivores.

In addition to high diversity, all the species identified in this experiment have a sporic meiosis life history whereas the gametic meiosis life histories are not represented at all. Some of the collected samples portrayed reproductive structures indicating that the environmental parameters were ideal for the plants to become fertile. These results demonstrate that future ocean conditions are favorable for some taxa, and the plant community may persist. Environmental factors such as tidal and lunar cycles are known to trigger reproduction in some algae such as *Ulva* (Lüning and Kadel 2008, Carl et al. 2014) and *Sargassum* (Monteiro et al. 2009), with the underlying presumption that most algae exhibiting sporic and gametic meiosis life histories reproduce sexually and asexually when growth conditions are optimal. The reproductive plants were found across all treatments suggesting that some species may thrive under future ocean conditions. Reproduction, a fundamental and crucial part of the continued success of generations for all living organisms, was evident in eight species. This demonstrates that some species such as *Champia parvula*, *Gelidium pusillum*, *Chamaebotrys boergesenii*, *Gelidium crinale*,

Pterocladia caerulescens, *Hypnea cervicornis*, *Dictyota friabilis*, and *Feldmannia mitchelliae* will be able to continue to be productive under predicted ocean scenarios. These plants exhibited signs of health through the development of reproductive structures. In contrast, Alfonso et al. (2022) looked at herbaria specimens to determine the effects of irradiance and temperature on the thallus size and reproduction. Two out of the three species they studied showed that the shortening of the thallus and decrease in reproductive structures were linked to increase in temperature, irradiance, and desiccation exposure. Plants such as *Hypnea cervicornis*, *Dictyosphaeria versluysii*, *Dictyota friabilis*, *Feldmannia mitchelliae*, and the red crust alga *Agissea inamoena* were found in all four treatments showing that they will be able to be successful in our changing ocean. These results are also supported by the overall photosynthetic parameters, ETR_m and E_k , being consistent across the four treatments. This reinforces the urgency for research on our fleshy macroalgae to better understand the success of our reefs. Furthermore, this provides new insight into which marine plants will remain on our reefs (winners) and which marine plants may decline (losers). If our fleshy algae may continue to thrive well into the future, then our herbivore community may also continue to thrive as well since their food source will remain. For the “losers” who are not as readily adapted to climate change, the ecophysiological responses of these macroalgae will be affected and so will their palatability, which influences herbivory levels (Poore et al. 2013, Phelps et al. 2017). This will have a ripple effect in all directions throughout the food web. In the future, we may see a more algae-dominated reef, which will still be able to support a diverse ecosystem such as those in the NWHI (Vroom et al. 2006, Vroom and Braun 2010).

This 22-month mesocosm study is the longest climate change study to date that offers a broad examination of the different communities providing ecological relevance to the reef. Mesocosms

play an integral part of research that allow us to closely examine complex systems such as coral reefs (Fordham 2015). This algal-focused paper is a piece of the bigger story that revolves around the different communities that make up a coral reef ecosystem. The published results for the other community groups show signs of resiliency against the future climate change parameters (Bahr et al. 2020, Timmers et al. 2021, McLachlan et al. 2022). This was the same for the benthic algal community in this mesocosm study. In sum, all these results should play a major role in the decision-making process as we continue to develop conservation strategies to protect our reefs and the livelihoods of coastal communities (Cinner et al. 2011, Adam et al. 2014, Harvey et al. 2017).

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FIGURES



Figure 2.1. Aerial view of the Hawai‘i Institute of Marine Biology on Moku O Lo‘e, Kāne‘ohe Bay, Hawai‘i (21.433 °N latitude, 157.7863 °W longitude). Red arrows are pointing to the intake pipe (IP) and the location of the mesocosms (M).



Figure 2.2. Forty mesocosms in position during the two year study at Hawai‘i Institute of Marine Biology.

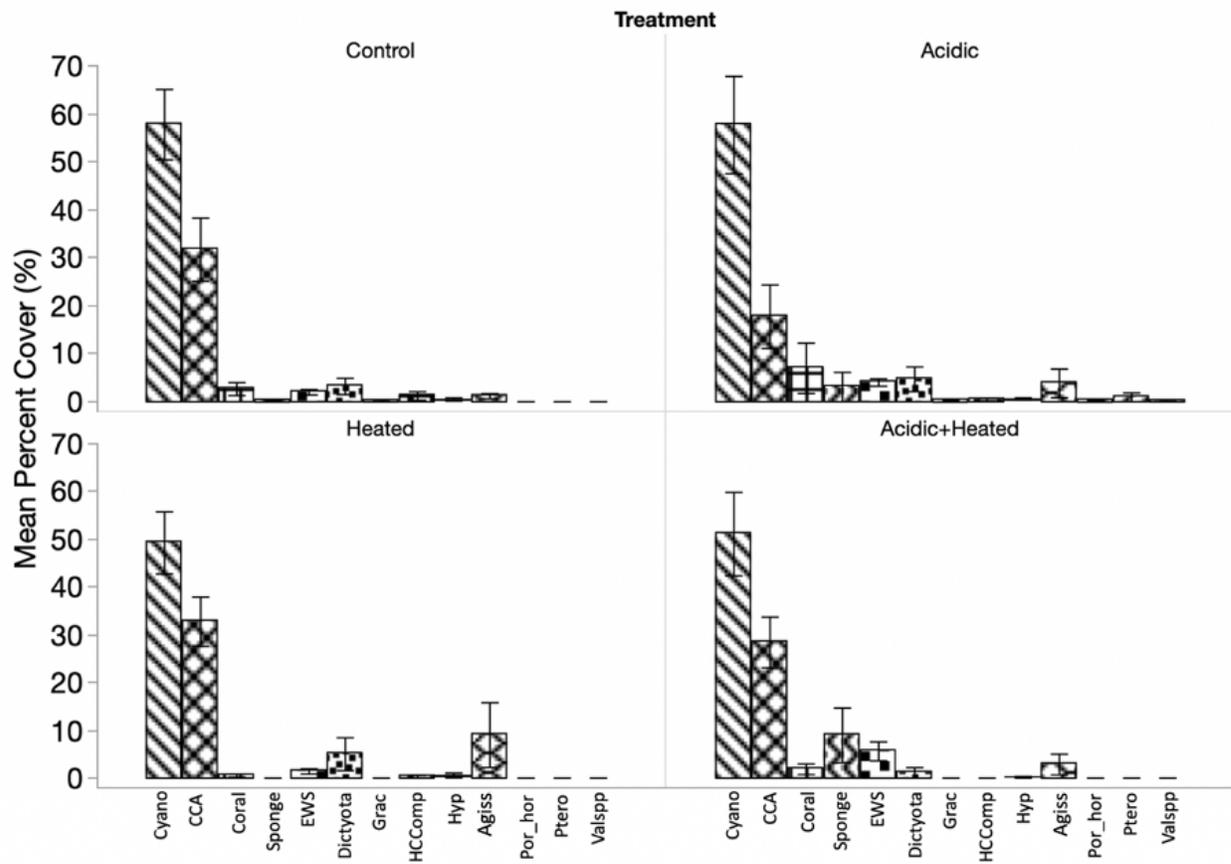


Figure 2.3. Mean percent cover for each major taxon in each treatment (mean \pm Std Err). Each taxon is represented by an identification code: Cyanobacteria = Cyano, Crustose coralline algae = CCA, Coral = Coral, Sponge = Sponge, Empty White Space = EWS, *Dictyota* = Dictyota, *Gracilaria* = Grac, *Hypnea*/Cyanobacteria competition = HCComp, *Hypnea* = Hyp, *Agissea* = Agiss, *Portieria hornemannii* = Por_hor, *Pterocliadiella* = Ptero, and *Valonia* = Valspp.

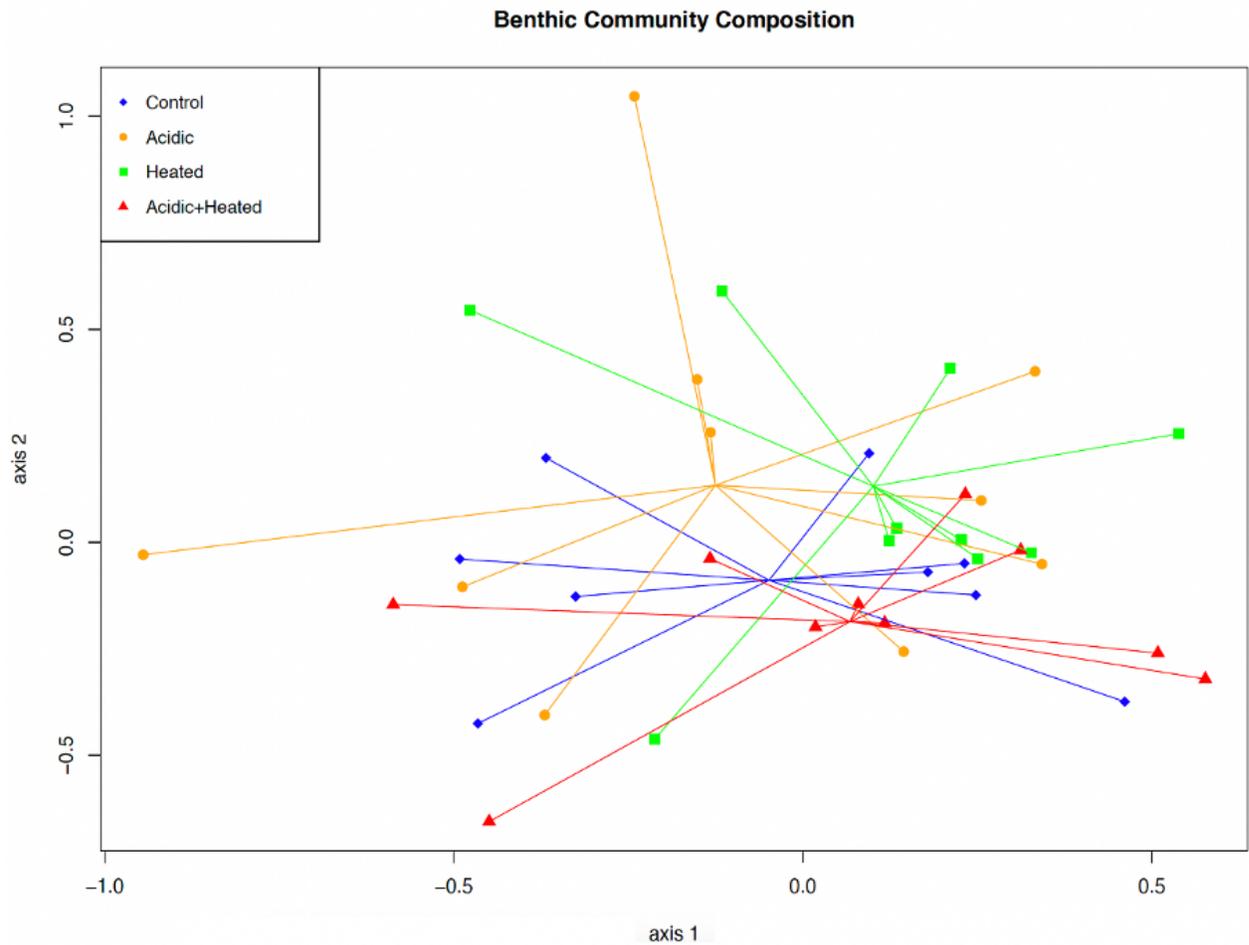


Figure 2.4. Nonparametric multidimensional scaling plot (nMDS) plot of the benthic composition for each replicate held under the four treatments (control = blue diamonds, acidic = orange circles, heated = green squares, acidic + heated = red triangles). Stress = 0.13. Function ordispider (package: vegan) was used to connect data to points to visualize variation.

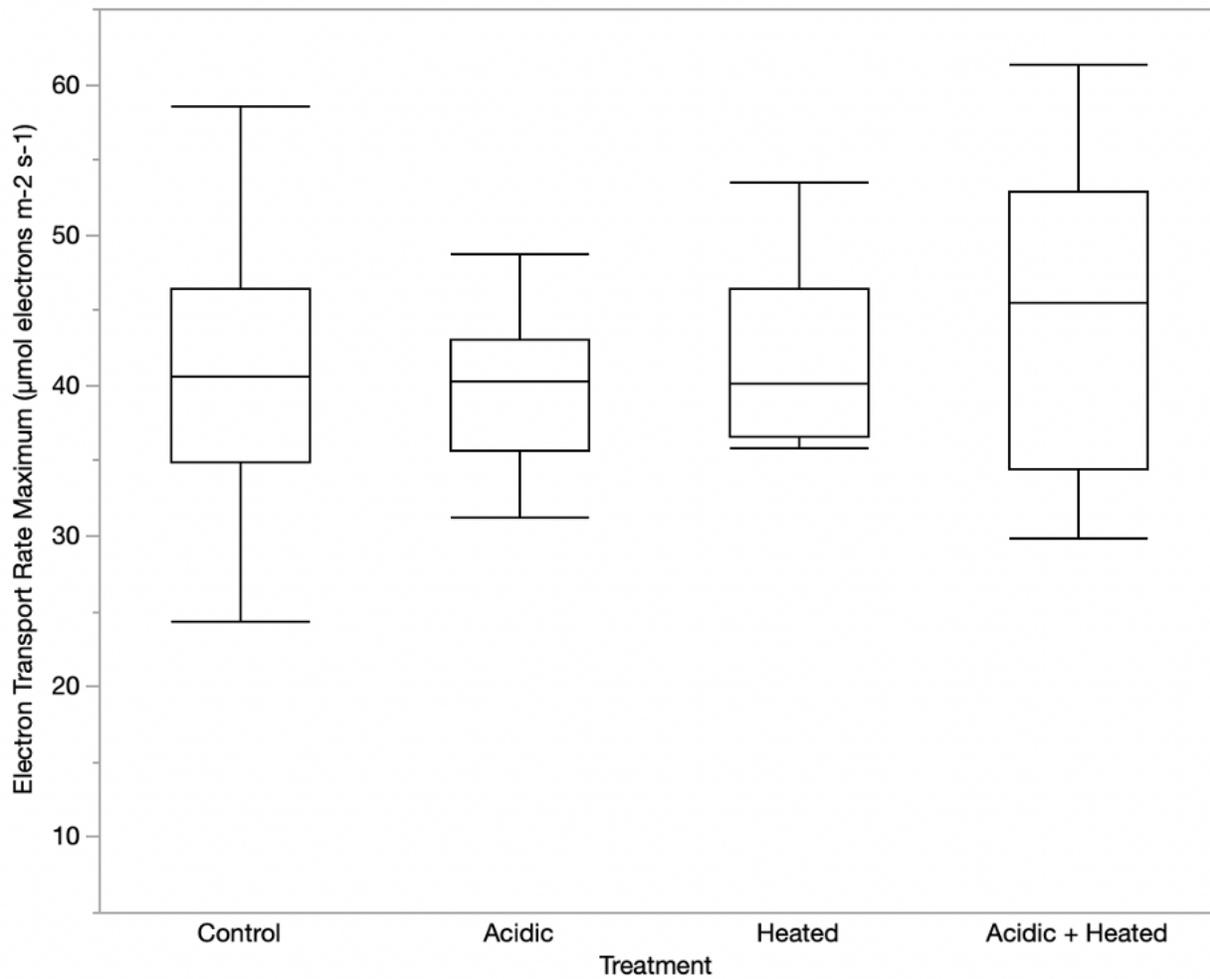


Figure 2.5. Variation in mean ETR_m values ($n = 9-10$ samples) for algae community in each treatment. There are no significant differences between the mean values for each treatment (ANOVA $P = 0.6402$). The center line represents the median, box limits represent the quartiles, and whiskers are 1.5x interquartile range.

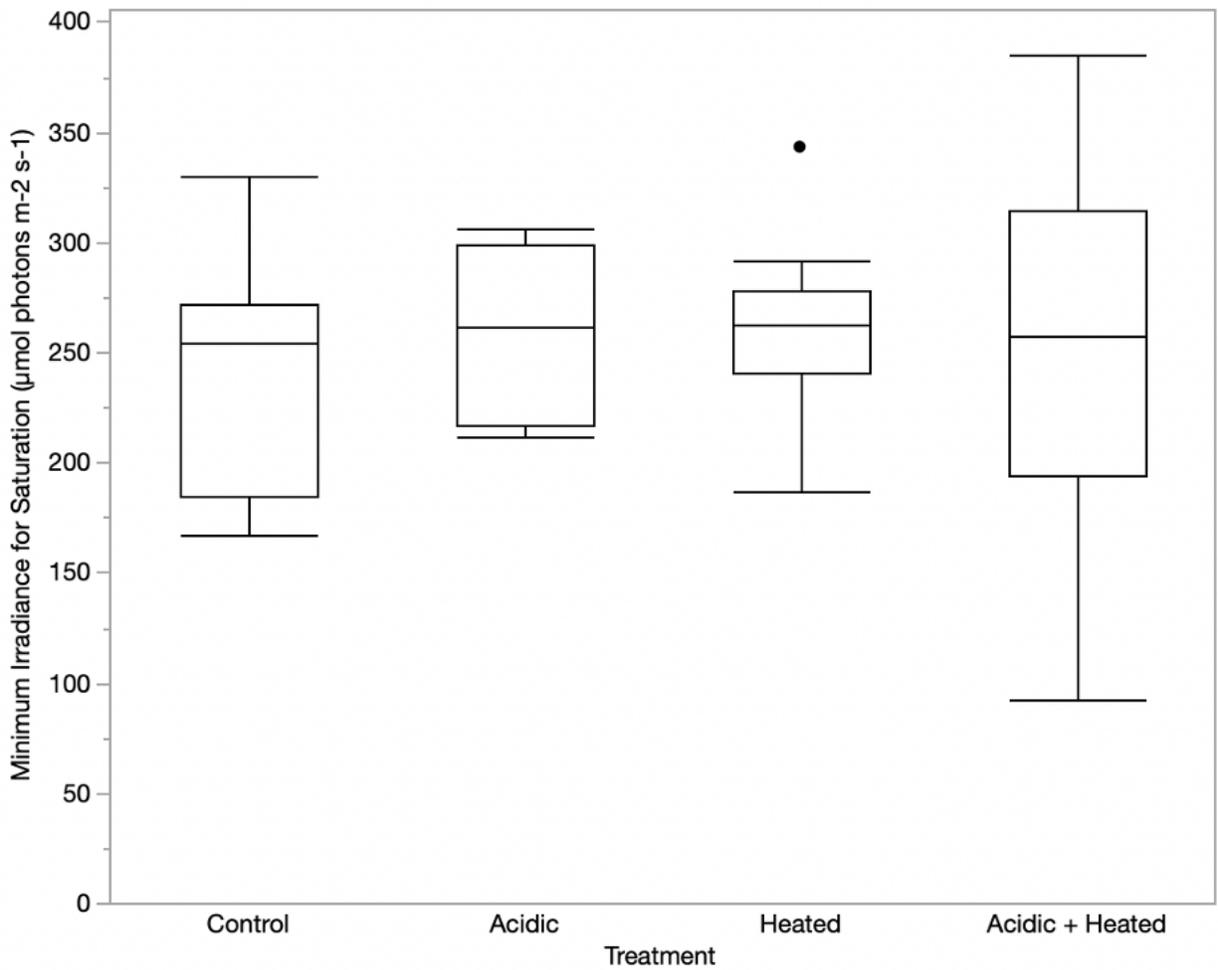


Figure 2.6. Boxplot illustrates the variation in mean E_k values ($n= 9-10$ samples) for plants from each treatment. There are no significant differences between the mean values for each treatment (ANOVA $P = 0.8402$). The center line represents the median, box limits represent the quartiles, whiskers are 1.5x interquartile range, and closed circles as outliers.

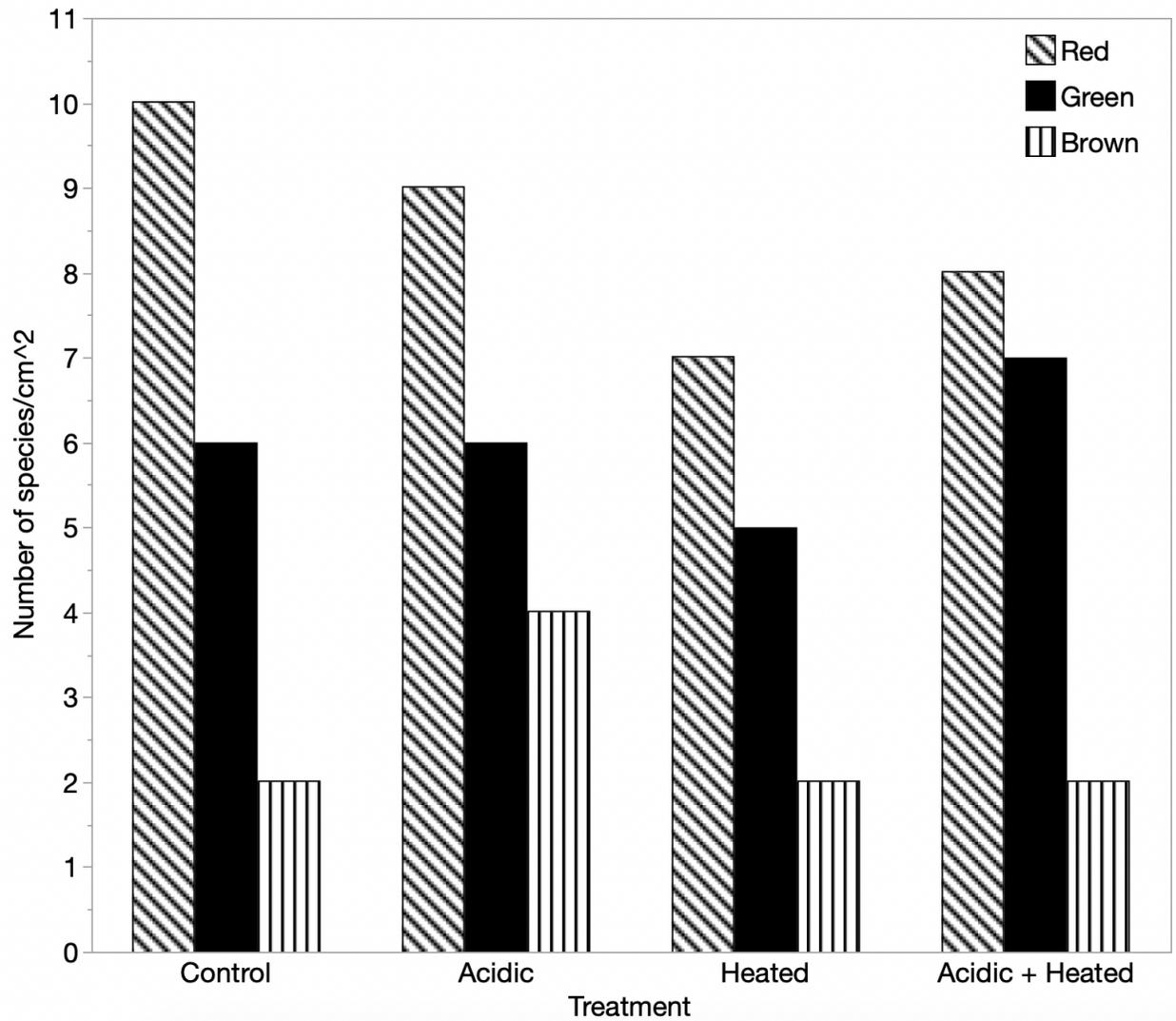


Figure 2.7. Examination of number of species in three phylogenetic clades in each treatment.

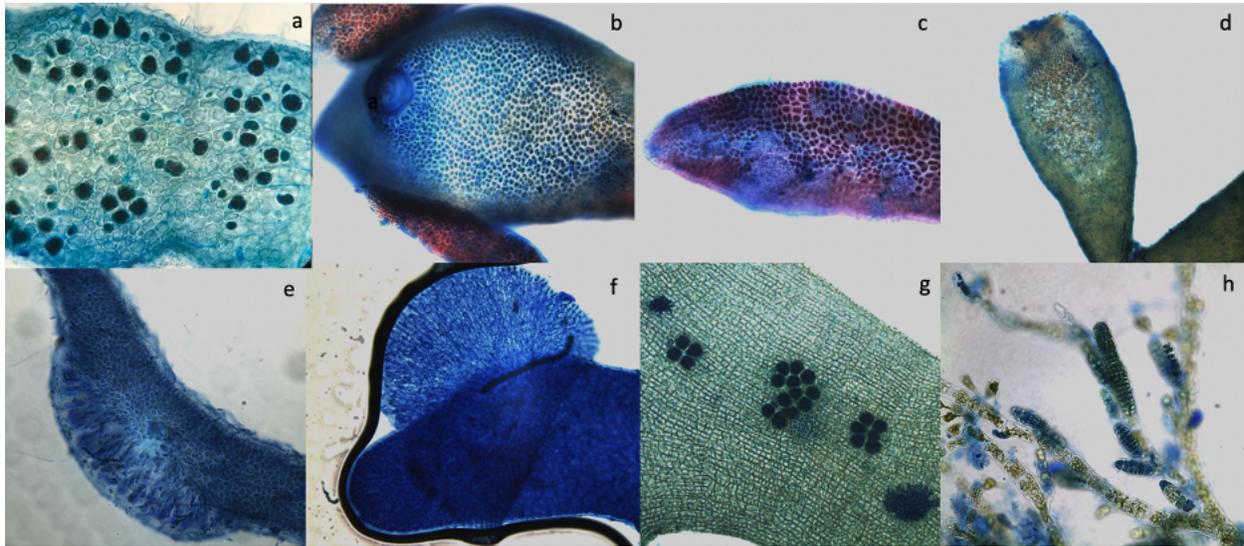


Figure 2.8. Algal reproductive structures observed during identification. (a) *Champia parvula* – tetraspores dispersed throughout thalli. (b) *Gelidium pusillum* – cystocarp with pit plug. (c) *Gelidium crinale* – thallus with spermatangia sori. (d) *Pterocladia caerulescens* – thallus with cystocarp. (e) *Hypnea cervicornis* – tetrasporangia on branch. (f) *Chamaeobotrys boegersenii* – tetrasporangia on branch. (g) *Dictyota friabilis* – different stages of oogonia sori. (h) *Feldmannia mitchelliae* – plurilocular structures on branches.

SUPPLEMENTAL MATERIALS

Appendix 2.S1. Labels to identify major groups for the analysis via percent cover of mesocosm walls on CoralNet.

Name	Code	Group label	Description
Coral	Coral	Hard coral	Any coral sp.
Sponges	Sponge	Other invertebrates	Purple/green sponge
Cyanobacteria	Cyan	Other	Black in color and had bubbles present
Empty Space	EWS	Other	White space, no growth visible
None	None	Other	Other 20 points not included in analysis (not included in percent cover analysis)
Crustose coralline algae	CCA	Rhodophyta	Ranged from pink, red, purple, and green; had some cyano growth
<i>Dictyota</i>	Dictyota	Ochrophyta	Any <i>Dictyota</i> sp.
<i>Gracilaria/Rhodymenia</i>	Grac	Rhodophyta	Any <i>Gracilaria</i> sp.
Hyp/Cyano	HCComp	Rhodophyta	<i>Hypnea</i> sp. completely covered with cyanobacteria (black with bubbles)
<i>Hypnea</i>	Hyp	Rhodophyta	Any <i>Hypnea</i> sp.
<i>Lobophora</i> spp.	Lob	Ochrophyta	Any <i>Lobophora</i> sp.
<i>Portieria hornemannii</i>	Por_hor	Rhodophyta	Any <i>Portieria</i> sp.
<i>Pterocliadiella</i> spp	Ptero	Rhodophyta	Any <i>Pterocliadiella</i> sp.
<i>Valonia</i> spp	Valspp	Chlorophyta	Any <i>Valonia</i> sp.

Appendix 2.S2. Numbers of photosynthetic readings taken in each tank and the number of readings for each treatment.

Tank	Treatment	Number of Photosynthetic Readings / Tank	Treatment	Total Numbers of Photosynthetic Readings/ Treatment
1	Acidic	2	Control	28
2	Control	2	Acidic	28
3	Heated	4	Heated	27
4	Acidic + Heated	2	Acidic + Heated	22
5	Heated	2		
6	Acidic + Heated	3		
7	Acidic	6		
8	Heated	3		
9	Control	5		
10	Control	3		
11	Control	2		
12	Acidic	2		
13	Acidic + Heated	0		
14	Acidic	4		
15	Acidic	2		
16	Acidic + Heated	3		
17	Control	2		
18	Heated	3		
19	Heated	2		
20	Acidic + Heated	1		
21	Acidic + Heated	4		
22	Heated	4		

23	Control	4
24	Acidic + Heated	1
25	Acidic	3
26	Control	2
27	Heated	2
28	Acidic	3
29	Acidic + Heated	2
30	Acidic	3
31	Heated	3
32	Control	2
33	Acidic + Heated	5
34	Heated	2
35	Control	4
36	Acidic	3
37	Control	2
38	Acidic + Heated	1
39	Acidic	0
40	Heated	2

Appendix 2.S3. Results of a One-way ANOVA results for the average ETR_m and E_k across treatments.

One-Way ANOVA	Source	DF	Sum of Squares	Mean Sq.	F Ratio	P-value
ETR_m	Treatments	3	110.1784	36.7261	0.5676	0.6402
	Error	34	2199.980	64.7053		
	C. Total	37	2310.158			
E_k	Treatments	3	2710.22	903.41	0.2789	0.8402
	Error	34	110118.2	3238.77		
	C. Total	37	112828.4			

Appendix 2.S4. List of species collected and identified by experimental treatment. Each X represents the number of tanks the alga was found in each treatment.

Species	Control	Acidic	Heated	Acidic + Heated	Reproductive
<i>Acanthophora spicifera</i> (Vahl) Borgesen 1910			XX		No
<i>Acrochaetium</i> spp.	XX	XX	X	X	No
<i>Bryopsis pennata</i> J. V. Lamouroux 1809	X	X		X	No
<i>Chamaebotrys boergesenii</i> (Weber Bosse) Huisman 1996		X			Yes
<i>Champia parvula</i> (C. Agardh) Harvey 1853				X	Yes
<i>Champia vieillardii</i> Kützing	X				No
<i>Cladophora capillaris</i> (Montagne) Rabenhorst 1864		X	X	X	No
<i>Cladophora dotyana</i> (W. J. Gilbert 1965)			X		No
<i>Cladophora flexuosa</i> (O. F. Müller) Kützing		X		X	No
<i>Cladophora socialis</i> (Kützing)	X	XXX		X	No
<i>Cladophora</i> sp.	XX		X	XX	No
<i>Dictyosphaeria cavernosa</i> (Forsskål) Børgesen 1932			X		No
<i>Dictyosphaeria versluysii</i> (Weber Bosse 1905)	X	X	XXXX	XX	No
<i>Dictyota acutiloba</i> J. Agardh		X			No
<i>Dictyota ceylanica</i> Kützing		X			No
<i>Dictyota friabilis</i> Setchell 1926	XXXX	XXX	XXX	X	Yes
<i>Feldmannia mitchelliae</i> (Harvey) H. -S. Kim 2010	XXXX XX	XXXX XXX	XX	XXXX	Yes

<i>Gelidiella antipai</i> Celan 1938			X		No
<i>Gelidium crinale</i> (Hare ex Turner) Gaillon 1828	X				Yes
<i>Gelidiella</i> sp.		X			No
<i>Gelidium pusillum</i> (Stackhouse) Le Jollies 1863	X	X		X	Yes
<i>Gracilaria parvispora</i> I. A. Abbott 1985	XX	XXXX		X	No
<i>Hypnea cervicornis</i> J. Agardh	XXXX XXXXX	XXXX XXX	XXXX XXXX	XXXX XX	Yes
<i>Jania adhaerens</i> J. V. Lamouroux	XXXX		XXX	XXX	No
<i>Malaconema minimum</i> Hollenberg 1963	X			X	No
<i>Phyllocladion anastomosans</i> (Harvey) Kraft & M. J. Wynne 1996				X	No
<i>Portieria hornemannii</i> (Lyngbye) P. C. Silva in P. C. Silva, Meñez & Moe 1987		X	X		No
<i>Pterocladia caerulescens</i> (Kützing) Santelices & Hommersand 1997		X			Yes
<i>Agissea inamoena</i> (Pilger) Pestana, Lyra, Cassano & J. M. C. Nunes 2021	X	XX	XX	XXXX	No
<i>Ulva flexuosa</i> (Collins & Hervey) M. J. Wynne	XX	XX			No
<i>Valonia aegagropila</i> J. Agardh	X				No
<i>Valonia ventricosa</i> J. Agardh 1877		XX	X		No

Appendix 2.S5. The commonality is measured by the number of mesocosms (total = 40) each taxon was found in. This is symbolized by the letters D = Dominant ($\geq 50\%$ of the tanks), C = Common ($\geq 25\%$ of the tanks), O = Occasional ($< 25\%$ of the tanks), and R = Rare (found in only one mesocosm).

Species	Dominant ($\geq 50\%$)	Common ($\geq 25\%$)	Occasional ($< 25\%$)	Rare (Only 1 mesocosm)
<i>Acanthophora spicifera</i> (Vahl) Borgesen 1910			X	
<i>Acrochaetium</i> spp.			X	
<i>Bryopsis pennata</i> J. V. Lamouroux 1809			X	
<i>Chamaebotrys boergesenii</i> (Weber Bosse) Huisman 1996				X
<i>Champia parvula</i> (C. Agardh) Harvey 1853				X
<i>Champia vieillardii</i> Kützing				X
<i>Cladophora capillaris</i> (Montagne) Rabenhorst 1864			X	
<i>Cladophora dotyana</i> (W. J. Gilbert 1965)				X
<i>Cladophora flexuosa</i> (O. F. Müller) Kützing			X	
<i>Cladophora socialis</i> (Kützing)			X	
<i>Cladophora</i> sp.			X	
<i>Dictyosphaeria cavernosa</i> (Forsskål) Børgesen 1932				X
<i>Dictyosphaeria versluysii</i> (Weber Bosse 1905)			X	
<i>Dictyota acutiloba</i> J. Agardh				X
<i>Dictyota ceylanica</i> Kützing				X
<i>Dictyota friabilis</i> Setchell 1926		X		

<i>Feldmannia mitchelliae</i> (Harvey) H. -S. Kim		X		
<i>Gelidiella antipai</i> Celan 1938				X
<i>Gelidiella</i> sp.				X
<i>Gelidium crinale</i> (Hare ex Turner) Gaillon 1828				X
<i>Gelidium pusillum</i> (Stackhouse) Le Jolis 1863			X	
<i>Gracilaria parvispora</i> I. A. Abbott 1985			X	
<i>Hypnea cervicornis</i> J. Agardh	X			
<i>Jania adhaerens</i> J. V. Lamouroux		X		
<i>Malaconema minimum</i> Hollenberg 1963			X	
<i>Phyllocladion anastomosans</i> (Harvey) Kraft & M. J. Wynne 1996				X
<i>Portieria hornemannii</i> (Lyngbye) P. C. Silva in P. C. Silva, Meñez & Moe 1987			X	
<i>Pterocladia caerulescens</i> (Kützting) Santelices & Hommersand 1997				X
Red algal crust (possibly <i>Agissea inamoena</i> (Pilger) Pestana, Lyra, Cassano & J. M. C. Nunes 2021			X	
<i>Ulva flexuosa</i> (Collins & Hervey) M. J. Wynne			X	
<i>Valonia aegagropila</i> J. Agardh				X
<i>Valonia ventricosa</i> J. Agardh 1877			X	

**CHAPTER 3: EFFECTS OF LIGHT REGIMES ON THE GROWTH AND
PHOTOSYNTHESIS OF THE NATIVE GREEN ALGA *Microdictyon setchellianum*
HOWE (CHLOROPHYTA:CLADOPHORALES)**

ABSTRACT

Understanding how physical factors such as irradiance affect the growth and photosynthesis of macroalgae is crucial to estimating their ecological success and health of the reef community. The native macroalga *Microdictyon setchellianum* Howe is a common plant on many shallow to deep reefs in the Main Hawaiian Islands as a component in the understory or as a principal element of reef cover as in deeper waters of Pearl and Hermes Atoll, Papahānaumokuākea Marine National Monument. This native has one of the widest depth distributions on record for a green alga (intertidal to 106 m). Despite its abundance across the Hawaiian archipelago, growth and photosynthetic rates for this alga are unstudied. Its distributions suggest some insight into the physiological ecology of this important species. Here, we examined 1) the influence of temperature, warm and cool seasons, and day length on initial photosynthetic capacities of wild-collected *Microdictyon setchellianum* and 2) the extent to which this species can physiologically acclimate to full sun or shaded light fields via changes in growth and photosynthesis in flow-through out-door mesocosms. Between 2013-2021, samples were collected, cleaned, and allowed to recover from collecting for 48 h before initial photosynthetic measurements were taken. Experimentally, plants were then examined for potential irradiance acclimation, by randomly placing individuals in flowing seawater outdoor mesocosms simulating shaded and full sun conditions for one week. The shade treatment of $350 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ was generated by imposing one layer of shade cloth, while a second mesocosm received full irradiance, above levels needed to saturate photosynthesis ($\sim 700 - 1,000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$). Results revealed that day length and mean temperature as single and combined factors did not affect values of ETR_m but did affect values of E_k . The mean ETR_m during the cold-water months was only slightly significantly higher than the average values for warm months. The mean E_k values between the cold and warm months did not differ. For plants in the sun and shade experiment, mean initial ETR_m significantly decreased from 1 d to 7 d for both treatments in December, but not in June. After 7d, mean E_k significantly decreased in June for both treatments and only under full sun in December. Specific growth rates (SGR) for the samples that received full sun were significantly higher than their control counterparts during the month of December only. Overall, SGR for the samples that received full irradiance were higher than the samples that received shade. The ability of *M. setchellianum* to acclimate under both shaded and full irradiance under short periods meets expectations for an alga that is an abundant species in both the shallow reef or intertidal community in O‘ahu and deeper waters across the archipelago.

Keywords: Macroalgae, photosynthesis, growth, climate change, *Microdictyon*

INTRODUCTION

Macroalgae are vital reef plants that sustain marine ecosystems from the intertidal to deep mesophotic reefs in the Hawaiian archipelago (e.g. Glenn et al 1990; Pyle et al. 2016). These plants play crucial roles in the food chain as primary producers that provide food for herbivores (Odum and Odum 1955; Polovina 1984), shelter and protection for many organisms (Wilson et al. 1990, Egan et al. 2013, Umanzor et al. 2017) and are evolutionarily ancient competitors for reef space (Vroom 2010). In addition, macroalgae oxygenate waters via photosynthetic generation of oxygen as a by-product and condition coastal water, by uptake of nutrients; both processes are crucial ecosystem services for survival of reef invertebrates and fishes (Ryther 1953).

In the Hawaiian flora, approximately 550 species of marine plants are recognized (Abbott 1999, Abbott and Huisman 2004, Huisman et al. 2007), yet little is known about the biology, growth rates or primary production by most of the native Hawaiian marine plant community. In this era of dynamic changes in our oceans (Hughes et al. 2018, IPCC 2021), characterizing overall community responses (Yamase, Chapter 2), as well as dominant biomass producing algae, become important to resource management. Among the understudied taxa in Hawai'i are species in the green algal genus *Microdictyon* (Chlorophyta, Anadyomenaceae) (Guiry and Guiry 2022). A recent Google Scholar search for “*Microdictyon* Hawaii” on 6/3/21 yielded 0/639 papers that did not specifically focus on the biology of *Microdictyon setchellianum*. Instead, these studies asked other field-biology questions, explored herbivore diets, and conducted reef assessments in the Hawaiian archipelago that included *Microdictyon*. Four results specifically focused on *Microdictyon*, but in other locations: Korea (*M. japonicum* - morphology descriptions, Oh et al. 2001), Australia (*M. umbilicatum* - bloom biomass for compost, Winberg et al. 2013; *M.*

umbilicatum - protoplast regeneration, Kim et al. 2013), and the Cayman Islands (*M. marinum*; further identification needed – seasonal abundance, Dell et al. 2020), not the Central Pacific region where *M. setchellianum* is known to be distributed (Guiry and Guiry 2022). Any new distributions *M. setchellianum* are at odds with Abbott and Huisman (2004) and await species validation as was recently pursued for other green algae (O’Kelly et al. 2010, Spalding et al. 2016, Verbruggen et al. 2006).

A framework for relating physiological traits of native species have been inferred by Glenn et al. (1990) where species in Waikīkī reef were sorted into warm and cold water preferences, based on changes in biomass for that time frame and suggesting biogeographic origins for these native species. Different taxa were also identified as long- or short-day plants, based on changes in irradiance, as day-length over the year. In contrast, those species native to the Central Pacific, are likely to have biomass that remains largely unchanged throughout the year and are likely to have uniform growth, and rates of photosynthesis across the seasons. Examining the genus *Microdictyon* provides a test case for evaluating the physiological ecology of native species across the annual cycles found in the mid-range of the Hawaiian archipelago, on O‘ahu.

The genus *Microdictyon* has a mesh-like morphology that creates vertically standing sheets of tissues, nestled into the uneven texture of the reef substrate (Oh et al. 2001, Guiry and Guiry 2022). Sheets are rarely more than 10 cm tall when growing in shallow water and may anchor many other algae on reefs (Doty 1969; Abbott and Huisman 2004). In a very early sampling of the mesophotic realm from 10 - 165 m depths, *M. setchellianum* was reported to be second in abundance for green algae after *Halimeda discoidea* at depths of 106 m of the 101 species identified from the Main Hawaiian Islands (Doty et al. 1974). Additionally, *M. setchellianum* forms dense near mono-specific meadows on fore reefs on the Pearl and Hermes Atoll and French Frigate Shoals, the

Papahānaumokuākea Marine National Monument (PMNM; Vroom and Page 2006; Vroom and Braun 2010). A study at Gardner Pinnacles, PMNM, reports abundances in the range of 30 to 60 % cover for *M. setchellianum* (Vroom and Timmers 2009). Parrish and Boland (2004) reported *Microdictyon setchellianum* was the primary alga on all eight banks at depths between 20 m – 40 m they studied in the NWHI. A recent synopsis of mesophotic findings (Pyle et al. 2016) states that *Microdictyon* beds are a distinct habitat type from 50 to more than 125 m depths. Thus, *M. setchellianum* is a common constituent of reefs across the Hawaiian Archipelago, from shallow to deep regions and is recognized as one of the ten most abundant green algae in Hawai'i (James et al. 2004).

Additionally, evidence of grazing underscores the role that *M. setchellianum* plays in sustaining a diversity of herbivores. For example, cells of *M. setchellianum* have been found in the gut content of native herbivores including the Hawaiian green sea turtle *Chelonia midas* (Russell and Balazs 2000), have been grazed upon by the native collector sea urchin, *Tripneustes gratilla* (Van Heukelem 2015) and the sailfin tang, *Zebrasoma veliferum* (Chulakote 2022). Many organisms such as sea slugs, sea snails, brittle star fish, crabs, shrimp, worms, lobsters, crustose coralline algae (CCA), turf and juvenile forms of other macroalgae grow in between the layers and on the surface of *M. setchellianum* blades (Abbott and Huisman 2004; Yamase, pers. obs.). This is the first of a planned series of studies to apply methods that document the physiological performance of Hawaiian native algae in response to environmental parameters, with the goal of providing fundamental, valuable insight into mechanisms governing growth and photosynthetic capacities of native algae. This study examines 1) seasonal effects on photosynthesis and 2) intensity influences for photosynthetically active radiation (PAR) on both the growth and photosynthesis of *M. setchellianum*. Two photosynthetic parameters are focused on in this study,

the maximum electron transport rate (ETR_m) and minimum irradiance needed to saturate photosynthesis (E_k). Although this alga is highly abundant and has a wide depth distribution across the entire Hawaiian archipelago, this research begins with initial characterizations of physiological responses for the shallow population of *M. setchellianum* on O‘ahu.

METHODS

Collection Sites Between 2013-2021

Waikīkī Marine Life Conservation District. The Waikīkī Marine Life Conservation District (MLCD) is located on the south shore of O‘ahu and extends from the groin on Kapahulu Avenue to the reef on the ocean side of the Waikīkī War Memorial Natatorium. The reef of interest is approximately 20-30 m from the Natatorium seawall (21.26478 °N latitude, 157.82303 °W longitude) at a depth of 0.9 to 1.5 m. This reef flat once flourished with reef plants and served as a research location in the late 1960’s to early 1970’s by Doty (1969) and Allender (1971) where *M. setchellianum* was a common member of the understory. Collections were made in “open” seasons.

Makapu‘u Beach. Makapu‘u Beach (21.313597 °N latitude, 157.660050 °W longitude) is located on the southeast side of O‘ahu. This rocky shoreline is lined with black basalt lava rock with tidepools along the coast. Small clusters of *M. setchellianum* are found inside crevices and exposed to fluctuating irradiance levels and wave action depending on the tides and time of the year.

Ka‘a‘awa Beach. Ka‘a‘awa Beach (21.549869 °N latitude, 157.845759 °W longitude) is located on the north shore of O‘ahu. This reef flat is mostly rubble with a sandy bottom that extends ~ 25-30 m out before larger coral heads are seen covered with CCA and macroalgae. *Microdictyon*

setchellianum is found growing in the crevices and sides of these coral heads. Specimens are mostly covered with epiphytes and sand making it hard to identify in the field.

All three locations served as collection sites (Figure 3.1) between the years 2013 – 2021 to minimize impact of collecting experimental materials.

Environmental Parameters

Average Hourly Water Temperature. Temperature data from the year 2010 – 2020 was downloaded from the National Oceanic and Atmospheric Administration (NOAA) Tides and Currents website for Honolulu Harbor (Station ID: 1612430) and Moku o Lo‘e or the Hawai‘i Institute of Marine Biology (HIMB, Station ID: 1612480). Data for the Waikīkī MLCD (Nearshore Sensor 04) were retrieved from the Pacific Islands Ocean Observing System website that is part of the U.S. Integrated Ocean Observing System. Temperature data from HIMB were used for Makapu‘u and Ka‘a‘awa because there were no data available and HIMB is located in between both field sites. Average water temperature was plotted against initial photosynthetic parameters, E_k and ETR_m , for all *M. setchellianum* samples that were collected.

Warm and Cold Seasons. The average hourly water temperatures from each site were plotted and divided into two seasons, cooler and warmer months. The months with an average temperature above 26 °C were considered warm and average temperatures below 26 °C were cooler (Figure 3.2). This temperature was based on the combined annual average water temperature for all three collection sites between 2010 – 2020.

Average Day Length. The sunrise and sunset times for 2019 were calculated using the NOAA’s Earth System Research Laboratories website. The average day length for each month was plotted (Figure 3.3), as representative of this typical yearly pattern.

Experimental Design

Collection and mesocosm setup. Wild plants of *M. setchellianum* were collected between 2013 – 2021 from the three sites described above for initial photosynthetic measurements (Figure 3.4). All samples were carefully collected, placed in Ziploc bags with seawater and transported in a cooler to avoid fragmentation associated with the plant's fragile thallus. Samples were placed in mesocosms of filtered seawater in a flow through system of open-air tanks at the Ānuenuue Fisheries Research Center (AFRC), Sand Island, O'ahu (Kawachi 2012, Appendix 3.S1). This setup consisted of six 1 m³ volume water baths that received filtered Honolulu Harbor water from six m subsurface, but only two water baths were used for the sun and shade experiments. Water was filtered through a sand filter (Hayward Pro Series Top Mount Sand Pool Filter, Model: S310T), a three-stage cartridge filter (25.4 cm x 6.35 cm, two 20 µm and one 5 µm pore size) and finally delivered via PVC pipes to 5 L aquaria located in each water bath. The system was set to deliver 300-350 ml m⁻¹ of highly filtered seawater to each aquarium.

Photosynthesis Measurements. All samples were cleaned from epiphytes and placed in individual aquaria for a two-day wound-healing period. After recovery, samples were placed in a covered multicompartiment container that had seawater from the mesocosms and transported to the lab (1 min walk from the outdoor seawater system). Samples were placed in diffuse sunlight for 20 minutes to minimize high light stress. Initial photosynthetic rates, specifically the maximum electron transport rate (ETR_m) and the minimum irradiance needed to reach saturation (E_k), were measured immediately thereafter by using a Junior Pulse Amplitude Modulated Fluorometer (Jr. PAM, Walz, Germany). Rapid light response curves were examined to measure photosynthetic activity as described in Beer et al. (1998) and Ralph and Gademann (2005).

Full Sun vs Shade Experiment. In 2013, plants were collected during the months of June (summer) and December (winter) from the Waikīkī Natatorium War Memorial seawall. Both experiments had a total of 20 samples each (10 = control, 10 = experimental). The collection and seawater setup described above were followed. The removal of excess water immediately followed after photosynthetic measurements were taken. Each alga was spun 10 times in a salad spinner and then placed on a balance (Ohaus Scout Pro Portable Balance) for initial wet weight measurements. Initial wet weights for each month varied with the availability of plant material on the reef. Finally, each sample was placed in an artificial holdfast that consisted of weighted polyethylene tubing, where the alga was held upright on the bottom of each aquarium, approximating their field position. All samples were then transported back to the seawater system and randomly placed in a designated aquarium. Each sample took about four minutes to process all measurements. This process was performed at the beginning and end of each one-week experiment.

Two mesocosms were used throughout these experiments. One mesocosm received full sun (between 700 – 1,000 $\mu\text{M photons m}^{-2} \text{ s}^{-1}$) while the second mesocosm was covered with one layer of shade cloth to simulate *in situ* depth irradiance ($\sim 350 \mu\text{M photons m}^{-2} \text{ s}^{-1}$) that is found at the collection site in Waikīkī (1.5 m depth at solar noon). All aquaria were positioned on cement blocks within the water bath to avoid full submersion under water. This allowed the aquaria to maintain a constant temperature. Each aquarium received aeration and flow through seawater.

Photosynthetically active radiation (PAR) Measurements. Immediate PAR was measured in both mesocosms closest to the algal sample using a LICOR 4π sensor (LI-COR Biosciences, Lincoln, NE). Onset HOBO pendant data loggers (Onset Part#: UA-002-64, MA, USA) were also placed in one aquarium in each mesocosm to record temperature and irradiance every 15 minutes. The

temperatures and irradiance from 10 am – 2 pm were specifically selected to calculate the mean max irradiance and temperature in each mesocosm.

Photosynthesis and Growth Measurements for *M. setchellianum*. Photosynthetic rates, using the Jr. PAM, were measured at the beginning (1 d) and end (7 d) of each experiment between 9 am – 11 am. All photosynthetic measurements (~90 seconds per reading per sample) took place after samples were transported to the lab and placed in indirect sunlight for 20-minutes to adjust to equal light levels.

The specific growth rate (SGR) for each sample was calculated after 7 d using the following formula:

$$\text{SGR (g g}^{-1} \text{ d}^{-1}) = ((\text{Final wet weight} - \text{Initial wet weight})/\text{Initial wet weight})/ 7 \text{ d}$$

Statistical Analysis

All statistical analysis was conducted using JMP Pro (Version 16.1.0). The distribution of the initial photosynthetic data for ETR_m and E_k were explored using histograms and normal quantile plots. The response variables were log transformed to meet normality and decrease outlier influence when necessary. Equal variances were tested using Levene's test and the appropriate t-test was used to determine significant differences between the cold and warm seasons for both photosynthetic parameters. A regression analysis was used to examine the relationship between continuous day length and mean temperature on initial ETR_m and E_k separately. Pairwise t-tests were used to compare initial to final photosynthetic responses between treatments by month. T-tests were used to test differences between the shaded and unshaded samples by month for specific growth rates.

RESULTS

Effects of Mean Day Length, Seasons (Cool vs Warm), and Mean Temperature on Initial Measurements of Photosynthesis

Initial Maximum Electron Transport Rate for Wild Collection Plants. The overall mean initial ETR_m readings between 2013 – 2021 was $37.45 \mu\text{mol electrons m}^{-2} \text{s}^{-1}$ ($n = 317$), which ranged from $28.1 \pm 0.94 \mu\text{mol electrons m}^{-2} \text{s}^{-1}$ (January) to $50.3 \pm 3.32 \mu\text{mol electrons m}^{-2} \text{s}^{-1}$ (December; Figure 3.4). Variance did not differ between the cool and warm months (Levene $P = 0.6827$). A pooled t-test showed that the mean ETR_m for the cooler months was only slightly significantly higher than the warmer months ($P = 0.0426$, Figure 3.5). The cooler months had a mean of $38.1 \mu\text{mol electrons m}^{-2} \text{s}^{-1}$ and the warmer months had a mean of $34.7 \mu\text{mol electrons m}^{-2} \text{s}^{-1}$. A quadratic curve was fitted to the initial ETR_m data but did not attain acceptable P values nor reveal strong correlations with day length ($F_{2,314}=0.35$, $P = 0.7050$) and mean temperature ($F_{2,314} = 0.41$, $P = 0.6637$; Figure 3.6).

Initial Minimum Irradiance to Saturate Photosynthesis Among Wild Collection Plants. The mean initial E_k values calculated for Rapid Light Curves between 2013 - 2021 ranged from 153.3 ± 4.55 (January) to $355.2 \pm 16.88 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ (June; Figure 3.4) with some notable outliers. The overall annual mean is $272.16 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ($n = 277$). Variance did not differ between the cooler and warmer months (Levene $P = 0.4365$). There was no significant difference between the mean E_k for warmer months ($276.7 \pm 9.73 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) and the cooler months ($261.7 \pm 9.98 \mu\text{mol photons m}^{-2} \text{s}^{-1}$; Pooled t-test, $P = 0.1291$). A quadratic regression showed weak correlations with day length ($F_{2,274} = 9.96$, $P = <0.0001$) and mean temperature ($F_{2,274} = 10.42$, $P = <0.0001$) with initial E_k (Figure 3.5). A slight increasing trend in E_k was visible as day length increases and the peak of E_k was found between $25.5 - 26.5 \text{ }^\circ\text{C}$.

Effect of Different Irradiance Levels on Photosynthesis

Maximum Electron Transport Rate for Experimentally Treated Plants. After one week of treatments, ETR_m for *M. setchellianum* significantly decreased under both irradiance levels (full sun: $P = 0.0047$ and shade: $P = 0.0048$) for the month of December. Initial ETR_m under shaded conditions decreased from $45.4 \mu\text{mol electrons m}^{-2} \text{s}^{-1}$ to $34.1 \mu\text{mol electrons m}^{-2} \text{s}^{-1}$ while it decreased from $55.2 \mu\text{mol electrons m}^{-2} \text{s}^{-1}$ to $31.8 \mu\text{mol electrons m}^{-2} \text{s}^{-1}$ under full sun (Figure 3.7). There were no such differences in June under both sun ($P = 0.5603$) and shaded ($P = 0.2638$) conditions.

Minimum saturation irradiance for experimental plants. After 7 d, the mean initial E_k readings significantly decreased from $401 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ to $261.3 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ under full sun ($P = 0.0214$) and $379.1 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ to $282.0 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ under shaded ($P = 0.0666$) conditions in June. The same results were found for the month of December where mean initial readings decreased from $357.8 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ to $243.7 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ under full sun ($P = 0.0172$). The decrease from $275.8 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ to $212.9 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ under partial irradiance was almost significant ($P = 0.0586$, Figure 3.8).

Specific Growth Rates of M. setchellianum Under Different Irradiance Conditions

The samples that received full sun in December attained a mean SGR of $0.057 \text{ g g}^{-1} \text{ d}^{-1}$, which was significantly higher than their shaded counterparts ($P = 0.0013$; $\text{SGR} = 0.027 \text{ g g}^{-1} \text{ d}^{-1}$). The samples that received full sun during the months of June also had a higher mean SGR but was not significantly different. Overall, SGR was higher for the samples that received full irradiance (Figure 3.9).

DISCUSSION

The understudied ecophysiology of *Microdictyon* left a wide gap in our understanding of environmental factors that drive the growth and biomass accumulation of this alga. Given the broad Central Pacific distribution reported for *Microdictyon setchellianum*, the data presented here show a similarly broad photosynthetic capacity from two measures of photosynthesis, the maximal rates of photosynthesis, ETR_m , and the irradiance levels needed to saturate photosynthesis, the E_k values. Long term photosynthesis readings between 2013 – 2021 fluctuated monthly and did not display any clear trends, except a weak significant difference between the cool ($n = 112$) and warm ($n = 155$) months, despite having a large sample size. As for saturation levels, no significant difference was found between the cool and warm seasons but was observed when plotted across day length and temperature. Generally, this important primary producer remains photosynthetic and productive year-round allowing it to better compete for resources over ephemeral algae that fluctuate in abundance. The ability for this alga to photoacclimate under both sun and shade conditions also allow this alga to have wide depth distribution as seen across the Main Hawaiian Islands and the Papahānaumokuākea Marine National Monument.

The reproduction of *Microdictyon setchellianum* has not been observed, even by Lois Egerod who specialized on this order of green algae in Hawai‘i for her dissertation (Egerod 1952). A member of the Order Cladophorales possesses an isomorphic alternation of generations where diploid and haploid phases are morphologically and expected to be physiologically similar (Van Den Hoek et al. 1995, Hurd et al. 2014). Algal growth can be underestimated by release of tissue biomass in the form of swimming cells, spores and gametes, with the ultimate disintegration of reproductive cells and probable loss of weight. On the contrary, *Bryopsis plumosa* have

demonstrated the rapid regeneration of protoplasts from cells that have been damaged within 15 minutes (Kim et al. 2001). *Microdictyon umbilicatum* has also shown healing response within 10 minutes and through two pathways: 1) protoplasts becoming reproductive cells within two weeks of damage as well as 2) protoplasts going through cell division and then becoming reproductive (Kim et al. 2002). The ability to rapidly repair cell damage is a major advantage for *Microdictyon* as it can heal within a time frame that allows its abundance to remain on reefs as a common food source for many marine organisms and a reproduction distribution strategy.

After seven days, the final electron transport rates of the samples that were shaded had comparable photosynthetic rates to their full sun counterparts except for the short day, low angle month of December. Although the shaded samples were covered with shade cloth, the amount of irradiance was comparable to the *in situ* irradiance in Waikīkī and other collection sites at about high tide. This demonstrates that irradiance as low as $350 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ is sufficient for *M. setchellianum* to reach its maximum photosynthetic capacity. In December, there is a significant decline in ETR_m after one week under both light levels yet that decline falls in a similar range to those in the other months. Photoacclimation is evident through the significant decrease in E_k after seven days under both light levels yet achieving comparable ETR_m readings. Although not significant, similar trends with ETR_m being higher in lower irradiances was also reported for two brown algae, the Hawaiian species *Padina sanctae-crucis* (Cox and Smith 2015) and an Asian species, *Hizikia fusiformis*, now recognized as *Sargassum fusiforme* (Zou and Gao 2010). The ability to adapt to low and high light environments over a short amount of time is crucial to the survival of organisms especially those that are found in the intertidal areas. These areas are exposed to a wide range of irradiance levels as the tide changes. The ability to be productive under different light regimes is highly beneficial for distribution and survival during

these daily changes (Doty 1946, Henley and Ramus 1989, Zou and Gao 2010, Cox and Smith 2015).

M. setchellianum's layered and mesh-like morphology provide self-shading, which acts as a natural light filter from the top of the plant to the inside layer (Ramus 1978). Gomez et al. (2004) measured photosynthesis of 18 different algal species with different morphologies and found that algae with sheet-like features had the lowest light absorptances. Although *Microdictyon* may fall into the "low absorptance" category, it is still able to reach comparable photosynthetic rates at low saturation showing the ability to use minimum irradiance efficiently. After measuring photosynthetic rates for 37 tropical macroalgae in Florida, Mathieson, and Dawes (1986) stated the Chlorophyceae had the widest range of photosynthesis compared to the other algal classes, which is consistent with *Microdictyon*'s depth range across the Hawaiian archipelago.

The coolest water temperatures in Hawai'i are between December and March, with December having one of the lowest hours of sunshine, and the most precipitation and probably associated nutrient run-off, during the year (U.S. Climate Data). This may have resulted in the significant decrease in ETR_m and E_k during the month of December. In contrast, June was able to reach higher ETR_m with lower E_k after 7 d. Plants can be grouped as sun or shade – tolerant based on their morphological features and physiological responses to different light regimes (Ramus 1978, Cox and Smith 2015, Schumann et al. 2017), a finding that underscores the importance of rapid light response curves (Ralph and Gademann 2005). Rapid light curves show that as irradiance increases photosynthesis increases as well until maximum saturation is met. This relationship has been evident for all photosynthetic organisms (Ramus 1978, Stirk et al. 1995, Beer et al. 1998, Gomez et al. 2004) but shifts in E_k indicate the ability to optimize photosynthesis for current, available irradiances.

In the 1960's, frondose algae were collected in front of the Waikīkī Natatorium Seawall to the reef edge. *Microdictyon setchellianum* had the 12th highest biomass wet weight out of ~116 species that were collected between 1966-1969. This was the first study to focus on the productivity of reef plants through space and time (Doty 1969). Allender (1971, unpublished) continued this study allowing continuous data of wet weights for *M. setchellianum* showing fluctuations in wet weight throughout the years from 1966-1971 (Appendix 3.S2).

Overall, samples that received full sun had higher SGR compared to the samples that received ambient PAR. SGR varied widely between months. Although SGR was higher in plants that received full irradiance, final ETR_m values were slightly higher for the shaded samples. Turgor pressure being an inexpensive process for cell expansion and growth promotion could have led to higher specific growth rates (Cleland 1971, Cosgrove 1981) despite lower photosynthetic readings for the samples that received ambient irradiance.

Based on the data for the samples that received full irradiance, *M. setchellianum* is the second fastest-growing species (0.032 g g⁻¹ d⁻¹) compared to other native and, surprisingly, invasive plants that have been studied by Larned (1998) and Kawachi (2012) in similar mesocosm conditions (Appendix 3.S3). This alga along with another native *Ulva lactuca* (0.08 g g⁻¹ d⁻¹), have the potential to outcompete invasive species such as *Gracilaria salicornia* (0.016 g g⁻¹ d⁻¹), *Hypnea musciformis* (0.008 g g⁻¹ d⁻¹), and *Kappaphycus alvarezii* (0.001 g g⁻¹ d⁻¹), which have overgrown some Hawaiian reefs (Smith et al. 2002, Smith et al. 2004, Conklin and Smith 2005, Dailer et al. 2012).

There are no published data such as SGR and photosynthetic rates for this alga so baseline information for all samples that were exposed to full sun conditions were averaged to characterize the mean SGR, ETR_m, and E_k of *M. setchellianum* (Appendix 3.S4 and 3.S5).

Studying the ecophysiological factors that affect the growth and photosynthesis of the common alga *M. setchellianum* is one of the first steps to finding a native species that can outcompete fast growing invasives. Continued efforts toward understanding the growth rates of our native species are needed to find an alga that can be used towards out planting efforts as a way to mitigate impacts of invasive species that are not able to grow as fast on the reefs.

It is important to model future distributions of invasive algae such as *A. amadelpha* (Veazy et al. 2019). This study identified potential hotspots around the Main Hawaiian Islands with bottom current and increased water temperature as the driving factors. Des et al. (2020) also used modeled predictions to determine how climate change will influence community shifts of habitat forming macroalgae. The newly recognized and potent cryptogenic alga *Chondria tumulosa* was reported in 2019 to be growing in thick mats in the shallows to 50 ft reefs of Pearl and Hermes (Sherwood et al. 2020). Competitive abilities of *M. setchellianum* that has dominated deep portions of the Pearl and Hermes reef and is a common component in shallow waters will rely in part on this alga's ability to continue to grow faster than *C. tumulosa*, in the face of differential grazing pressures (Sherwood et al 2020). The overgrowth and dominance of *C. tumulosa* will pose great threats to the productivity of the reef if *C. tumulosa* overtakes *Microdictyon setchellianum*, losing refuge and palatable foods for native herbivores.

Many common factors such as herbivory and nutrients influence the distribution of macroalgae, but this was not the case for *Microdictyon marinum* in the Caribbean. Wave exposure was the only factor that affected the distribution due to high impact leading to mortality (Dell et al. 2020). These results are similar to the distribution of *M. setchellianum* on O'ahu. During the winter months, the population at Makapu'u tends to decrease with "high wave" exposure, suggesting that some quantitative metric such as wave height is needed. The scattered trimmed

clusters grow in a tidepool in between crevices and closest to the rock edge where incoming waves crash against, making it difficult for herbivory to take place. Although present, the plant blades remain short throughout the winter months and become bigger in the spring, which was when collection occurred (February to April). At the Waikīkī MLCD, collection became difficult overtime. This may arise from overgrowth of invasive algae in the area, which has outcompeted native algae populations such as *Sargassum* as well as *Microdictyon*. The last collection site at Ka‘a‘awa Beach seems to be the most reliable over the years. Lush clusters of *M. setchellianum* grow on coral heads or are on the reef covered with sand and epiphytes. They are found at a depth of 1.5 – 2 m and about 50 m from shore perpendicular to the public beach restroom, which may be contributing runoff or nutrients to the area. All biotic and abiotic factors in areas where *Microdictyon* is present needs to be studied to determine what is influencing the abundance and distribution.

Overall, based on the ecophysiological responses of the shallow reef population of *Microdictyon*, this native green alga is characterized as being efficient and well adapted to both low and high irradiance levels, which is beneficial during the changing tides and seasons, as typical of the Central Pacific habitats. This alga has a broad photosynthetic range allowing it to occupy spaces found in depths where irradiance may be limited. Resource managers should use *Microdictyon* beds to recruit the natural herbivore populations on the shallow reefs as they provide the necessities needed for a thriving community as evident on the pristine algal dominated reefs in the NWHI. Many of the reefs in O‘ahu have been overrun by invasive algae so rehabilitating them back with a native species such as *Microdictyon setchellianum* can assist in reviving the biodiversity and health in the area. A comparison study between the populations in the deep waters in the NWHI and the shallow waters in the MHI need to be conducted to better

understand this dominant species that is supporting and sustaining life from the shallows to the deep and across the Hawaiian archipelago.

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FIGURES



Figure 3.1. A) Ānuenuue Fisheries Research Center with the Honolulu Harbor (Station ID 1612340) located across and sensor at 1.5 m depth. B) PacIOOS nearshore sensor 04 located at the Waikīkī Aquarium, O‘ahu (21. 2659 °N latitude, 157.8228 °W longitude). C) Ka‘a‘awa Beach with collection site ~20-25 m offshore at 1.2 m depth. D) Makapu‘u Beach with collection site in tidepools. E) Moku o Lo‘e, HIMB (Station ID: 1612480) sensor at 1.2 m depth.

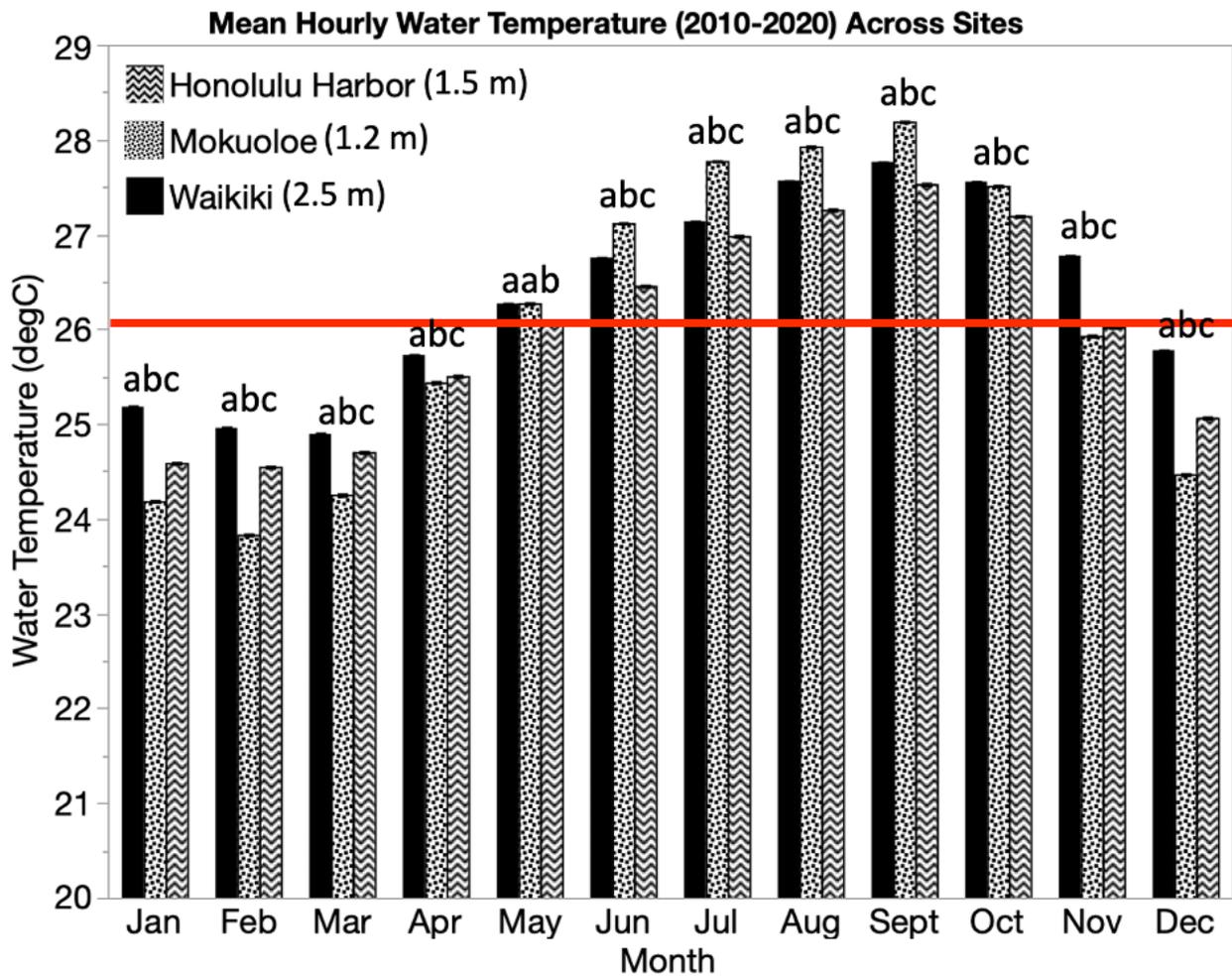


Figure 3.2. Average (\pm SE) hourly water temperature for Honolulu Harbor, Moku o Lo'e and Waikiki for the years 2010-2020. Months with average hourly temperatures below the red line are considered cooler months and months above the red line are considered warmer months. Sites for each month that are not connected by the same letter are significantly different.

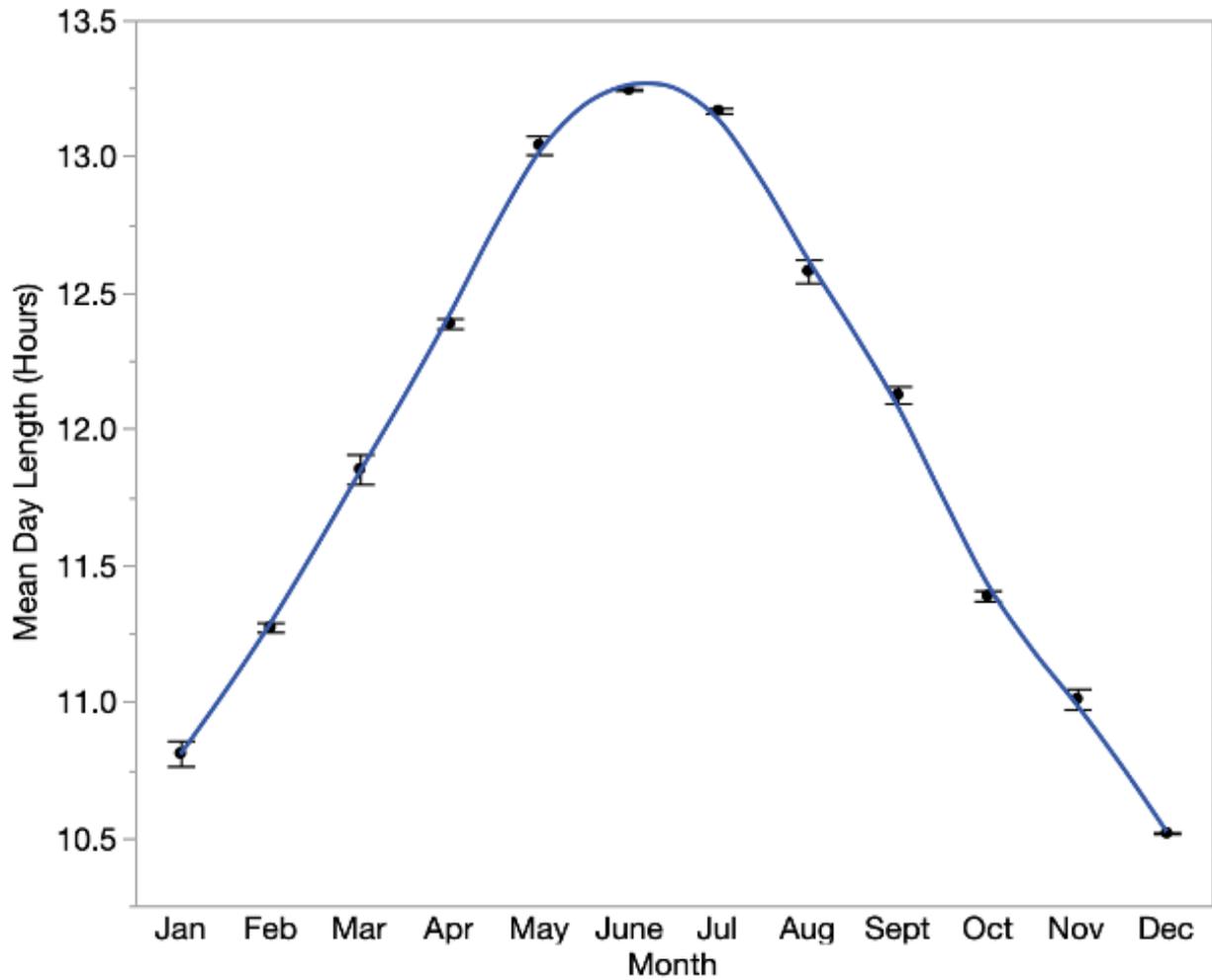


Figure 3.3. Average (\pm SE) hourly day length for the year 2019 illustrates the amount of irradiance varying across the months.

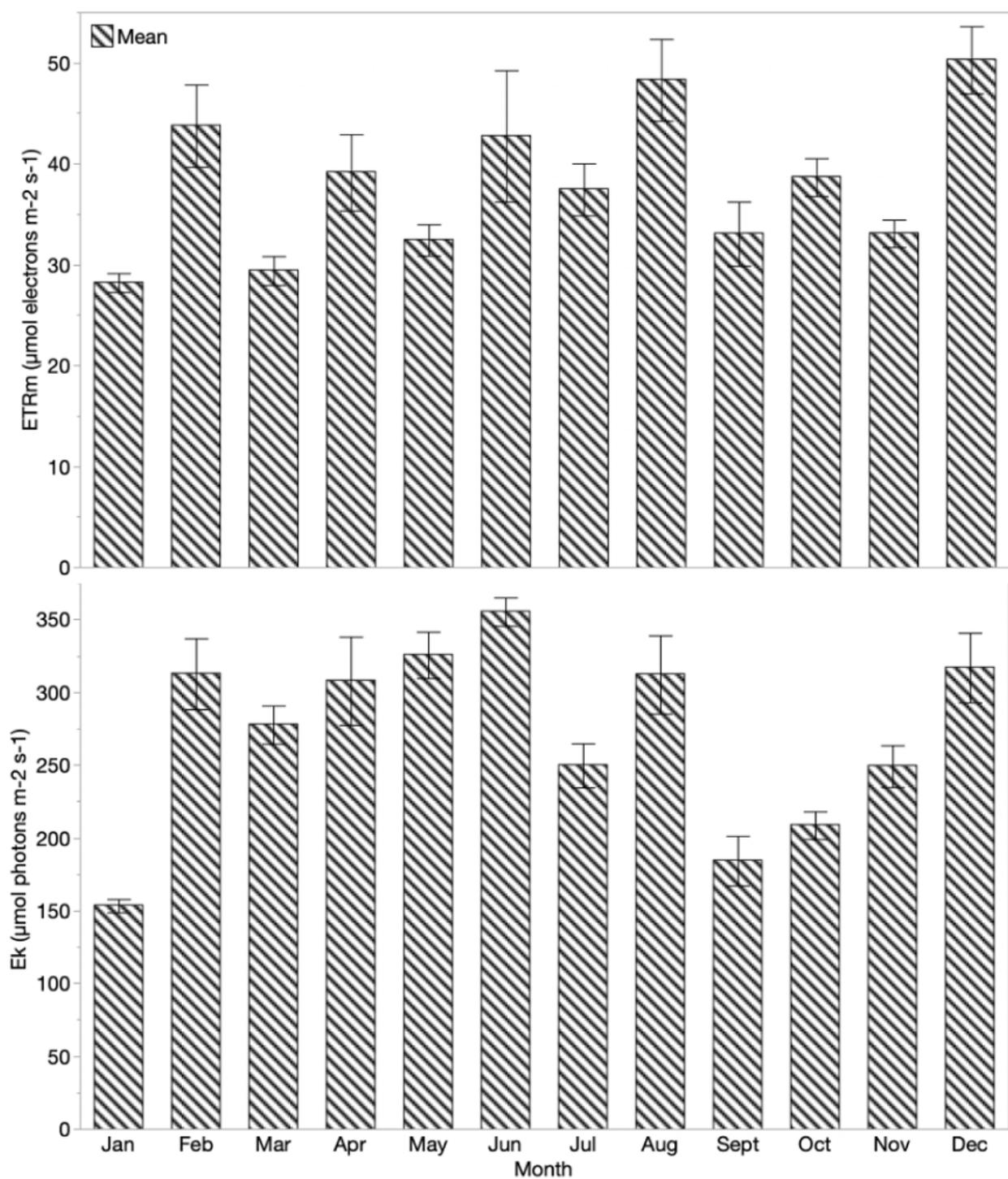


Figure 3.4. Mean initial ETR_m (top) and E_k (bottom) for wild collected *M. setchellianum*, across months between 2013 - 2021. Error bars represent Standard Error.

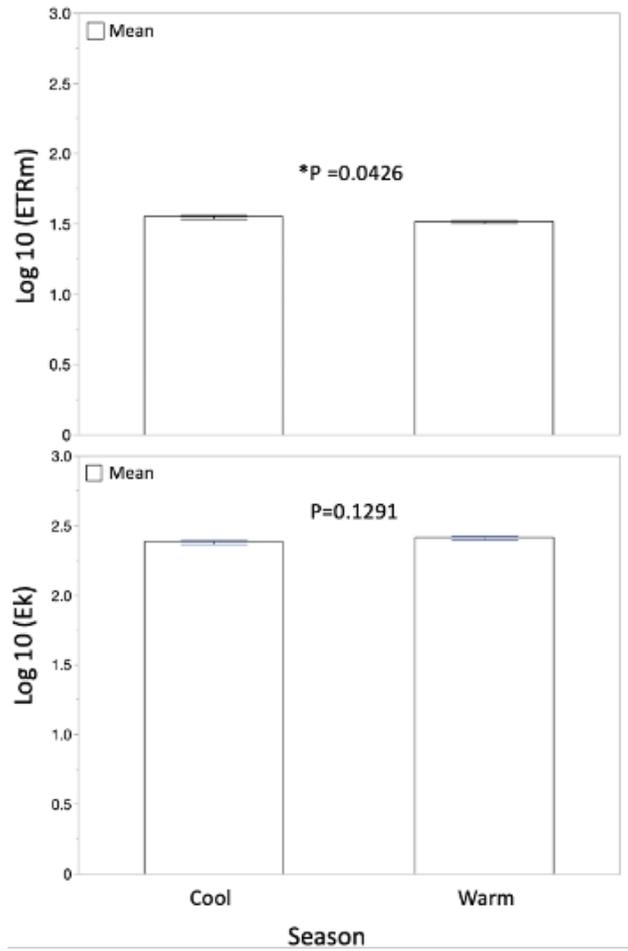


Figure 3.5. The log transformation of ETR_m and E_k readings plotted against the cool and warm seasons between 2013 – 2021. * marks a significant difference (P<0.05). Error bars represent standard error from means.

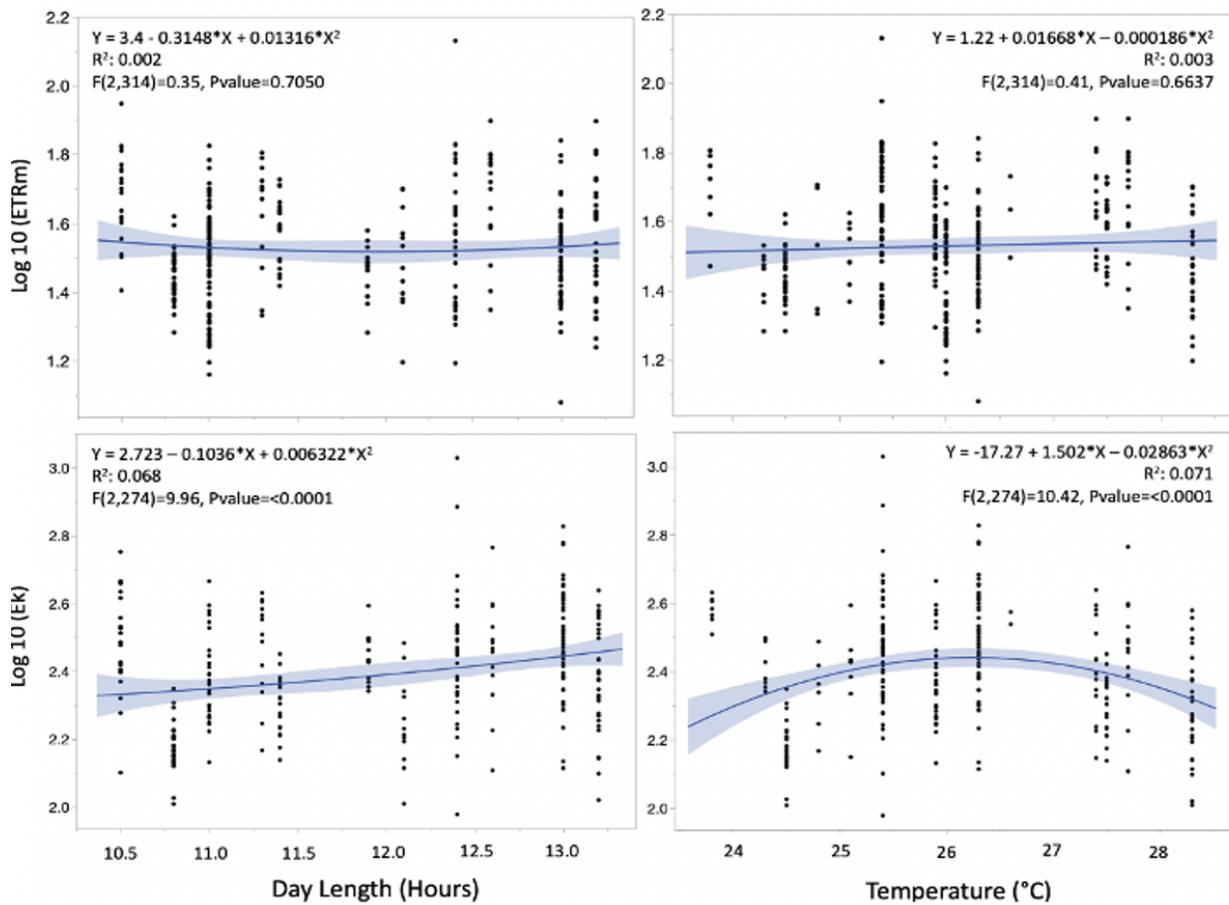


Figure 3.6. The log transformation of ETR_m and E_k readings plotted against day length and mean water temperatures between 2013 – 2021. R-square value and F-test are reported in the top left (day length) or top right (temperature) corner.

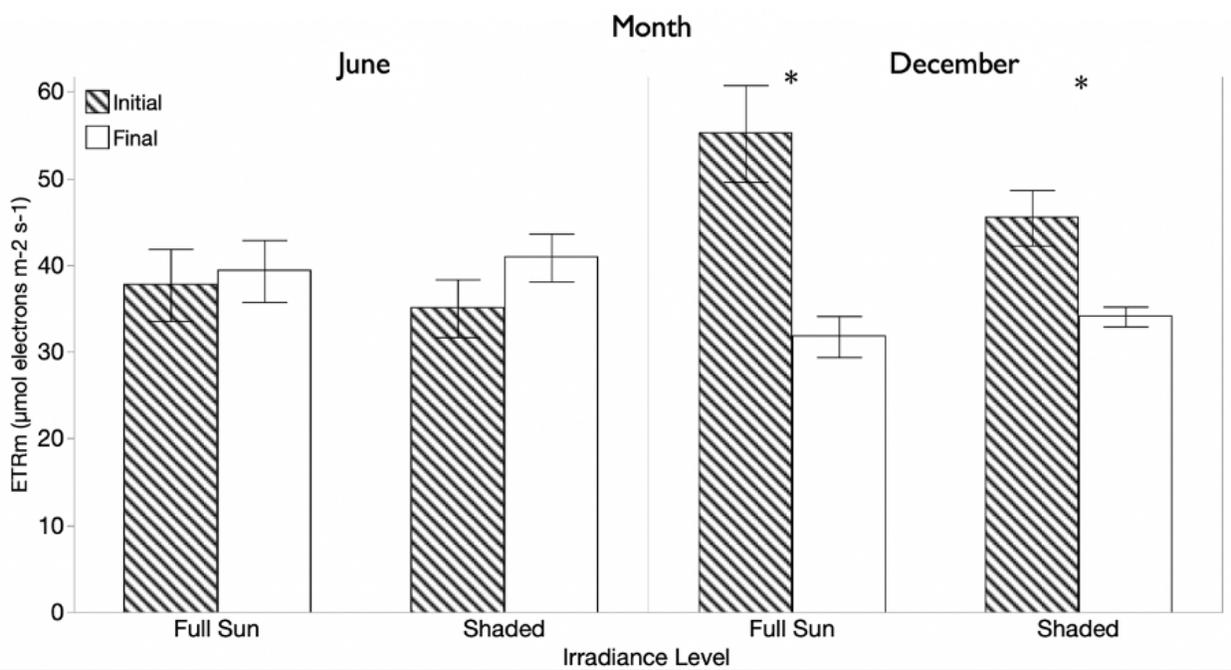


Figure 3.7. Mean initial and final electron transport rates (ETR_m) plotted against the two irradiance levels and months. * above bars symbolize a significant difference ($P < 0.05$) between the means for initial (1 d) and final (7 d) ETR_m. Error bars represent standard error from means.

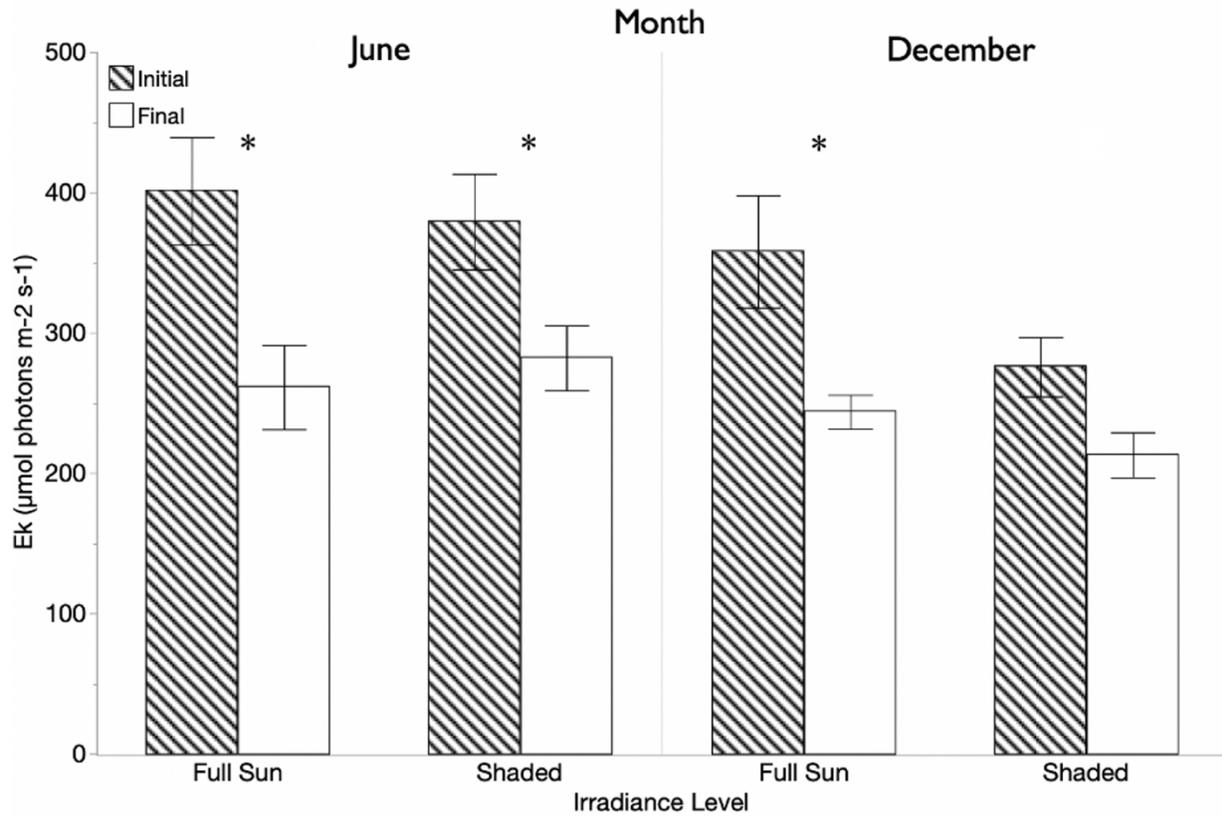


Figure 3.8. Means of the initial and final E_k were plotted against the two irradiance levels by month. * above bars symbolize a significant difference ($P < 0.05$) between the means of the initial (1 d) and final (7 d) E_k . Error bars represent standard error from means.

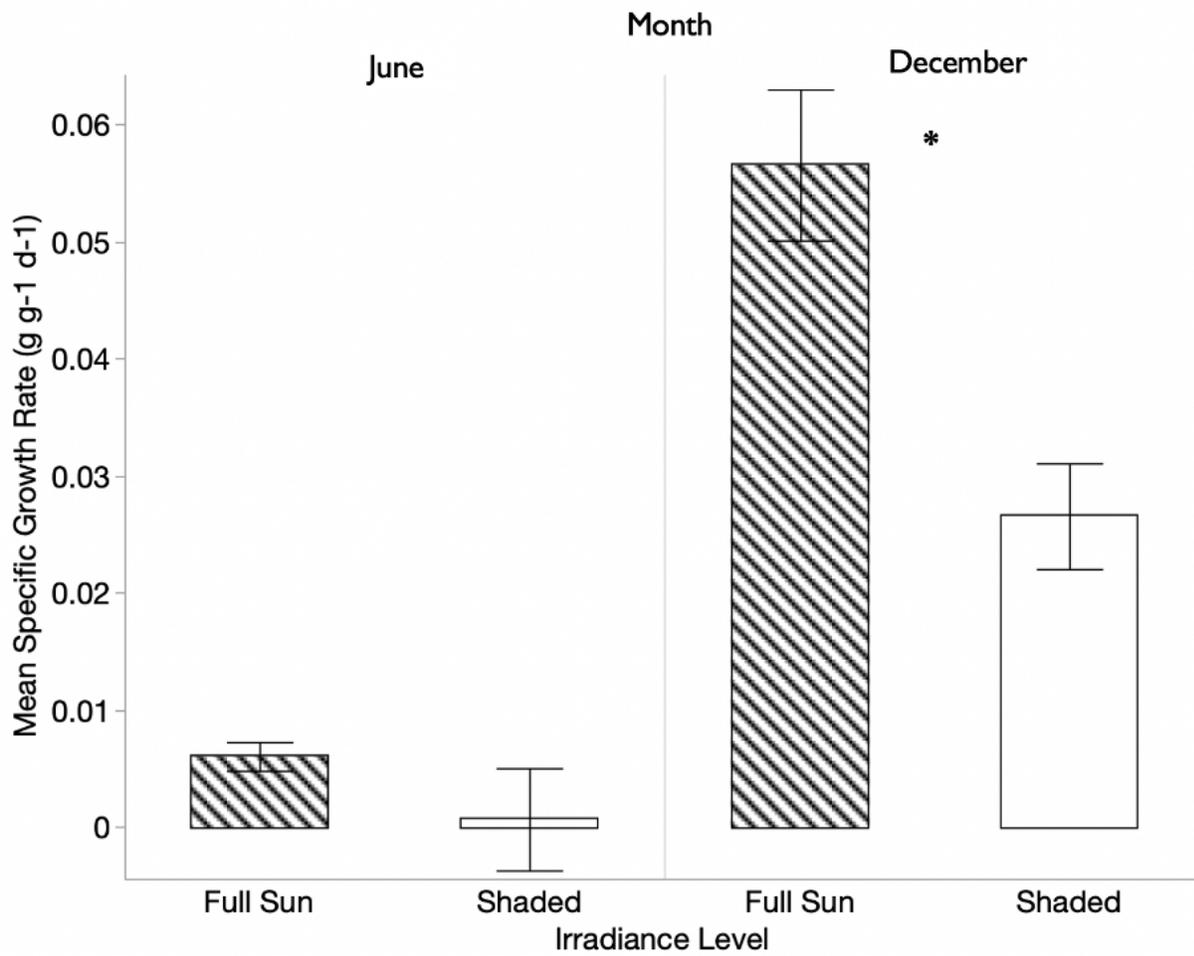
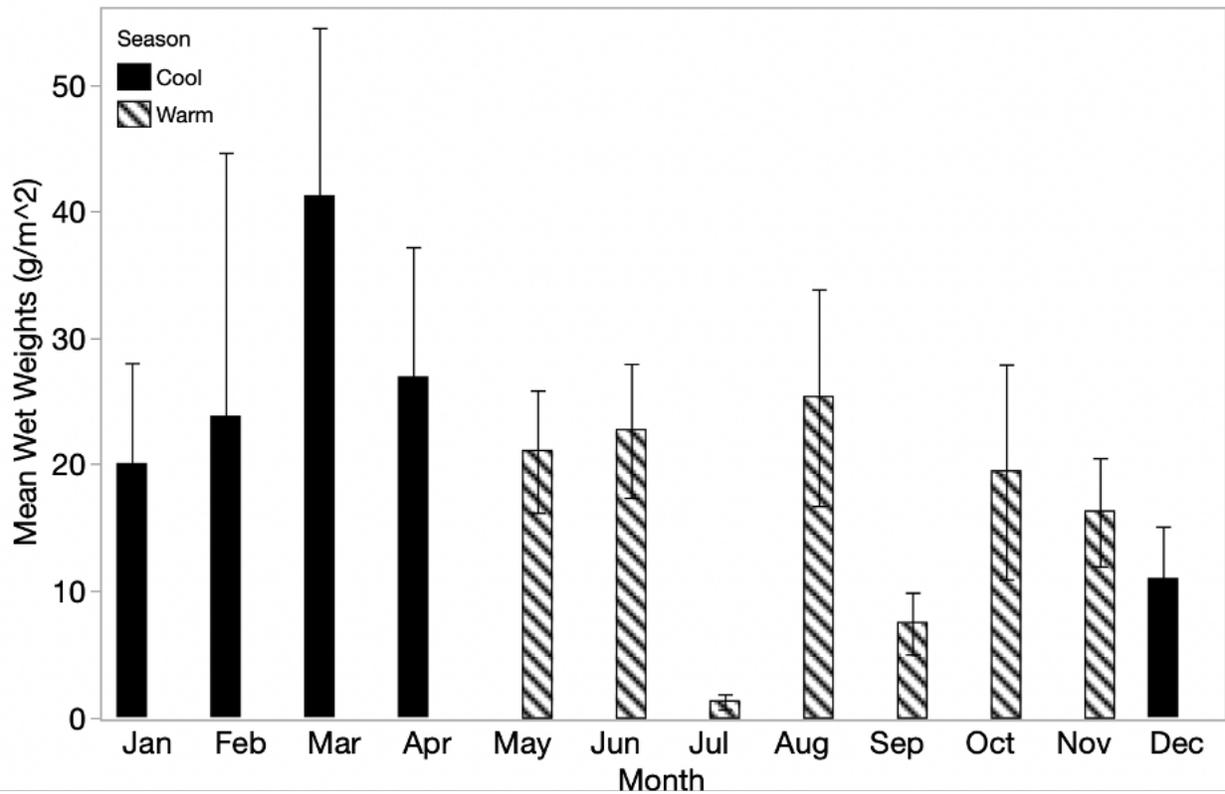


Figure 3.9. Mean specific growth rates were plotted against irradiance levels by month. * above bars symbolize a significant difference ($P < 0.05$) between the irradiance levels and a student's t-test was used. Error bars represent standard error from means.

APPENDIX



Appendix 3.S1 Outdoor seawater system at the Ānuenuue Fisheries Research Center, Sand Island, O'ahu that consists of six water baths.



Appendix 3.S2 Total wet weights of *Microdictyon* that were collected in front of the Waikīkī Natatorium from 1966 – 1973. Data were extracted from M.S. Doty (1966-1967 Frondose algae of Waikīkī) and Bruce Allender’s (1972-73 unpublished data) Waikīkī data sets. Error bars represent standard errors from the means.

Appendix 3.S3 Specific growth rate for reef algae in Hawai'i that were grown in open flow mesocosm systems under full sun irradiances by Larned (1998), Kawachi (2012), and Yamase, this study.

Alga	SGR (g g ⁻¹ d ⁻¹)	Phylum	Study
<i>Acanthophora spicifera</i>	0.110	Rhodophyta	Kawachi (2012)
<i>Ulva lactuca</i> as (<i>U. fasciata</i>)	0.080	Chlorophyta	Larned (1998)
<i>Microdictyon setchellianum</i>	0.032	Chlorophyta	This study (2021)
<i>Pterocliadiella capillacea</i>	0.031	Rhodophyta	Kawachi
<i>Codium edule</i>	0.017	Chlorophyta	Larned (1998)
<i>Gracilaria salicornia</i>	0.016	Rhodophyta	Larned (1998)
<i>Amansia glomerata</i>	0.015	Rhodophyta	Kawachi (2012)
<i>Hypnea musciformis</i>	0.008	Rhodophyta	Kawachi (2012)
<i>Anadyomene stellata</i>	0.008	Chlorophyta	Yamase unpublished data
<i>Sargassum echinocarpum</i>	0.003	Ochrophyta	Larned (1998)
<i>Dictyosphaeria versluysii</i>	0.003	Chlorophyta	Larned (1998)
<i>Kappaphycus alvarezii</i>	0.001	Rhodophyta	Larned (1998)
<i>Padina japonica</i>	-0.006	Ochrophyta	Larned (1998)
<i>Caulerpa racemosa</i>	-0.009	Chlorophyta	Larned (1998)
<i>Caulerpa sertularioides</i>	-0.014	Chlorophyta	Larned (1998)

Appendix 3.S4 Mean specific growth rate and photosynthetic rates for *M. setchellianum* under full sun conditions from 1 d (initial) and 7 d (final).

Month	Mean ETR _m ($\mu\text{mol electrons m}^{-2} \text{ s}^{-1}$)				Mean E _k ($\mu\text{mol photons m}^{-2} \text{ s}^{-1}$)				Mean SGR ($\text{g g}^{-1} \text{ d}^{-1}$)	
	N	Initial	N	Final	N	Initial	N	Final	N	7 d
June	10	37.7	10	39.31	10	401.0	10	261.3	10	0.006
Dec	10	55.2	10	31.75	10	357.8	10	243.7	10	0.057
Avg	20	46.5	20	35.5	20	379.4	20	252.5	20	0.032

Appendix 3.S5 Mean initial (after 24-48 h recovery, Day 0) wet weight, ETR_m and E_k values for all *Microdictyon setchellianum* experiments conducted from 2013 -2021. “Open” to collection for MLCD, Waikīkī are even numbered years. A permit was obtained to collect samples during the “closed” years.

Month/Year	Collection Site	N	Mean Wet Weight	N	Mean ETR_m ($\mu\text{mol electrons m}^{-2} \text{s}^{-1}$)	N	Mean E_k ($\mu\text{mol photons m}^{-2} \text{s}^{-1}$)
June 2013	MLCD, Waikīkī	40	2.43	23	37.19	23	385.5
Oct 2013	MLCD, Waikīkī	18	0.86	-	NA	-	NA
Nov 2013	MLCD, Waikīkī	40	1.93	40	28.10	-	NA
Dec 2013	MLCD, Waikīkī	20	1.16	20	50.28	20	316.8
Mar 2014	MLCD, Waikīkī	5	2.67	5	32.03	5	297.6
Apr 2014	MLCD, Waikīkī	3	3.06	3	46.21	3	294.6
July 2014	MLCD, Waikīkī	16	2.37	16	46.36	16	270.6
Aug 2014	MLCD, Waikīkī	16	3.07	16	46.18	16	294.4
Sept 2014	MLCD, Waikīkī	12	1.10	12	33.04	12	184.2
Feb 2015	MLCD, Waikīkī	6	2.04	6	33.42	6	223.3
Apr 2016	Makapu‘u Beach Park	32	1.88	32	39.53	32	318.0
May 2016	Makapu‘u Beach Park	32	1.63	32	29.97	32	285.2
Feb 2018	Makapu‘u Beach Park	8	1.23	8	51.50	8	379.5
Mar 2018	Makapu‘u Beach Park	8	1.85	8	27.74	8	265.2
July 2019	Ka‘a‘awa Beach Park, North Shore	16	2.57	16	28.51	16	228.7
Jan 2021	Ka‘a‘awa Beach Park, North Shore	30	NA	30	28.20	30	153.3

Oct 2021	Ka'a'awa Beach Park,	20	NA	20	38.65	30	208.53
Nov 2021	Ka'a'awa Beach Park,	30	NA	30	39.68	30	249.16
AVERAGE					37.45		272.16

CHAPTER 4: DEVELOPING A PLACE-BASED TOOL FOR REEF REPLENISHMENT IN HAWAI'I: MEASURING GROWTH AND PHOTOSYNTHESIS OF JUVENILE *Sargassum aquifolium* (TURNER) C. AGARDH 1820 (FUCALES)

ABSTRACT

Historically, native species of *Sargassum* or *limu kala* were Fuclean dominants on many reef flats in Hawai'i but are now nearly absent on O'ahu. Efforts to understand growth and photosynthesis by species in this genus are motivated by the need for new tools in reef restoration efforts as well as Blue Carbon initiatives. In this research, outdoor mesocosms were used to 1) cultivate *Sargassum aquifolium* germlings to study early life stages through settlement on limestone tiles, 2) investigate the effects of full ($\sim 959 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$) versus shaded ($\sim 224 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$) irradiance on the growth and photosynthetic rates of juveniles, and 3) compare photosynthetic rates of blades at the base, mid, and tip sections of adult plants to germlings as first steps to outplanting. Pulse Amplitude Modulated fluorometry was used to measure the maximum rate of photosynthesis (ETR_m) and minimum saturation irradiance level (E_k), and growth was measured by increase in vertical height. Germlings were visually observed on tiles within three days of hanging fertile adults in the mesocosm. At four weeks, germling density was 22.8 germlings/cm² and average growth rate was 0.014 mm/d. For 13-week-old juveniles, average daily growth did not significantly differ between light treatments but did across time (full sun $P = 0.0154$, shade $P = 0.0026$). Under full sun, mean ETR_m values significantly increased from 47.6 $\mu\text{mol electrons m}^{-2} \text{ s}^{-1}$ to 61.9 $\mu\text{mol electrons m}^{-2} \text{ s}^{-1}$ after four weeks. In contrast, mean ETR_m under partial irradiance significantly decreased to 29.1 photons $\text{m}^{-2} \text{ s}^{-1}$ in week 2, and then recovered to 44.6 photons $\text{m}^{-2} \text{ s}^{-1}$ in week 4. Mean E_k under full sun remained stable, but significantly declined from 526.3 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ to 331.4 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ after one week under shaded conditions ($P = 0.0013$). Similar measurements for adult plants revealed mean ETR_m was significantly lower at the base compared to the apical sections. In comparison to 11-week-old germlings, mean ETR_m was statistically lower for the germlings compared to the tip ($P < 0.0001$) and mid ($P = 0.0461$) sections of the adult plants. However, mean values of E_k for germlings were significantly higher than all three adult sections (All $P < 0.01$). This research demonstrates that *Sargassum* germlings are readily photosynthetic during early development and under different light regimes making them competitive on the reef. The successful cultivation of this alga via external fertilization can be used as an initial step to reestablish native populations. In the long-term, this will encourage the return of native herbivores seeking refuge in vertical growth of *Sargassum*'s golden forest. Understanding the ecophysiology during the early life stages of macroalgae remains crucial in development of restoration techniques for foundational species that were once dominant on reefs.

Keywords: Macroalgae, *Sargassum*, juvenile growth, photosynthesis, pulse amplitude modulated fluorometry, reef replenishment

INTRODUCTION

Golden forests of the giant kelps *Macrocystis pyrifera* (Linnaeus) C. Agardh and *Nereocystis leutkeana* (K. Mertens) Postels and Ruprecht 1840 line the Pacific coasts providing a wide range of ecosystem services via their diverse understory to the dense canopies (Abbott and Hollenberg 1976, Estes and Steinberg 1988). These underwater forests slow water flow and provide food, shelter, and nurseries to organisms extending from the small invertebrates along the seafloor to the large apex predators in the upper trophic levels (Estes and Steinberg 1988, Steneck et al. 2002, Port et al. 2016). These forests are highly productive and can grow up to 30 cm a day (Vergés and Campbell 2020) altering irradiance, nutrient accessibility, and water flow throughout the water column (Stewart et al. 2009). On a broader scale, these forests aid in nutrient recycling, carbon sequestration, and serves as a natural storm barrier for coastal communities. In ecosystem services, the Hawaiian Islands have competitive dominants in three *Sargassum* species that are smaller in stature but hold major ecological value and cultural importance to the Native Hawaiian community (McDermid et al. 2019) - as expansive golden Fucalean forests (Gouvêia et al. 2020).

The genus *Sargassum* C. Agardh entails roughly 400 species that are distributed across the tropical and subtropical regions (Abbott and Huisman 2004, Guiry and Guiry 2022). In Hawai‘i, *Sargassum aquifolium* (Turner) C. Agardh (formerly known as *Sargassum echinocarpum*; Sargassaceae, Fucales), along with three other species, *S. polyphyllum* J. Agardh, *S. obtusifolium* J. Agardh, and *S. hawaiiensis* Doty & Newhouse, are responsible for some of the highest biomass production by native species across the Hawaiian archipelago (Doty 1969, Abbott and Huisman 2003). The three most common species that are usually observed co-occurring along the shorelines are *S. aquifolium*, *S. polyphyllum*, and *S. obtusifolium*. The fifth

species, *S. muticum* (Yendo) Fensholt, was an unintended hull-fouling introduction on a dry dock from southern California (Abbott and Huisman 2003, Huisman et al. 2007), and is a highly invasive seaweed in its non-native areas of the Pacific Coast of North America and Europe (Global Invasive Species Database 2022).

In Hawai‘i, native *Sargassum* species are usually found in the intertidal or along rocky shorelines that are exposed to mid to high wave action (De Wreede 1976, Huisman et al. 2007, McDermid et al. 2019). In other subtropical regions such as the Sargasso Sea, *Sargassum natans* (Linnaeus) Gaillon is a permanently pelagic species, completely detached from the substratum and freely floating in the open ocean (Guiry and Guiry 2022). The Great Atlantic *Sargassum* Belt spans from West Africa to the Gulf of Mexico with accumulation and blooms influenced by ocean currents and nutrient input from the Amazon River (Wang et al. 2019). Gouvêa et al. (2020) used algorithm models that calculated a global estimate of 13.1 Pg C being fixed by *Sargassum* as fast-growing anchored biomass or free-floating, pelagic ecosystems. Buoyancy is provided for most species of *Sargassum*, by the numerous small air bladders or pneumatocysts. These natural flotation structures also serve to keep benthic plants upright as well as support floating in the open ocean (Redmond et al. 2014). Both anchored and floating communities provide multiple ecoservices and support an array of marine life from the shallow nearshore to the deep offshore (Pendleton et al. 2014).

During the winter months, fertility and reproduction of these species in Hawai‘i are highest resulting in the upper portion of the plant to shed leaving the perennial holdfast behind (De Wreede 1976). In the spring, regeneration of a new adult upright axes from the holdfast will follow. The plants that grow over the summer, are sexually mature in the fall, and then become fertile and reproduce once again in the winter (De Wreede 1976, Norton 1977). The peak of

reproduction and standing crop differs across species and is also influenced by environmental factors such as seawater temperature (De Wreede 1976, McCourt 1984, Glenn et al 1992) and the spring tides that happen after a new or full moon (Inoh 1937). The mature and reproductive plants possess bushy finger-like structures or receptacles that house numerous conceptacles (cavities) that develop either oogonia (female egg) or antheridia (male sperm) from fertile cells. The shape of the receptacles as well as the blade morphology varies across species (Inoh 1937, Huisman et al. 2007). Plants may also be either monoecious or dioecious (Redmond et al. 2014).

Based on geographical location, species of *Sargassum* are considered either a nuisance or an important reef component for economic, biological, and cultural purposes. For example, in the five years, in the Caribbean, changes in currents across the Gulf of Mexico have stranded piles of otherwise floating *Sargassum* biomass on beaches negatively affecting the economy, marine life and created public health concerns from the release of toxic gases (Dabor et al. 2018, Langin 2018, Rodriguez-Martinez et al. 2020). In other regions, *Sargassum* is a very valuable plant that is cultivated in high quantities in many Asian countries including Japan (Ito 2013), the Philippines (Largo et al. 2020), South Korea (Ko et al. 2020), and China (Pan et al. 2019) to supply the demand for products such anti-inflammatory compounds, pharmaceuticals, and fertilizer. In Hawai‘i, *Sargassum* plays a culture role as a treasured plant, locally known as *limu kala* and serves a wide range of purposes for cultural and traditional events such as the forgiveness ceremony or *ho‘oponopono* in Hawaiian, local medicine, hula, and food (Abbott and Huisman 2004; Huisman et al. 2007, McDermid et al. 2019). The legend of the Hawaiian goddess, Hina Lau Limu Kala, tells of how fishermen ask her to keep them safe while out at sea. *Limu kala* is also used in the *huikala* ceremony to purify them before they set out (Handy and Pukui 2006). As for healing purposes, a *limu kala* lei was placed over individuals who were ill.

As the person entered the ocean, the lei would drift away symbolizing the illness being removed (Varez 2022). Currently, there are efforts to recognize *limu kala* as the State of Hawai‘i limu (Ito, pers. comm.) tied to the realization that this genus is arguably one of the most, if not the most, treasured limu in Hawai‘i. The year 2022 has been proclaimed by Governor Ige as the Year of the Limu acknowledging the utmost importance of this benthic genus to the Native Hawaiian people (Dennison 2022).

In the 1970’s, *Sargassum* spp. was the dominant genus for the reef flat located on the south shore of O‘ahu, in front of the Waikīkī’s War Memorial Natatorium. Stands stood as tall as one meter providing a visual refuge from predators and food source for juvenile fish and the local herbivore population in the area. Between 1971 -1973, monthly collections (22 total) of frondose algae were collected from this reef flat to measure wet and dry weights. Specifically, *S. polyphyllum* made up about 70% of the total biomass that was collected and had the highest wet (826.2 g m⁻²) and dry (187.70 g m⁻²) weight followed by *S. aquifolium* with wet weight of 128.5 g m⁻² and dry weight of 22.80 g m⁻² (Doty 1969, Glenn et al. 1990). Currently, there is a decline in *Sargassum* beds in Waikīkī due to multiple possible reasons that have yet to be explored. Some of those being the change in seasonality that influences productivity and the securing of the holdfast, overgrowth of adult plants that cast deep shade over new recruits, and the introduction of invasive algae such as *Gracilaria salicornia* (C. Agardh) E. Y. Dawson (Figure 4.1). Locations such as Japan and Korea are also working to restore *Sargassum* beds by outplanting germlings that have settled on concrete blocks to re-establish the community that once thrived on their reefs (Terawaki et al. 2003, Yoon et al. 2014).

The long-term goal of this study is to better understand the early life stages and ecophysiology of this very important alga, leading to reestablishment of local populations across

the state. In this study, the technique developed to settle and cultivate *S. aquifolium* germlings on limestone tiles is described. The settling density, daily growth rates, and the effects of different irradiance levels on the growth and photosynthesis of the young germlings were measured. In addition, photosynthetic comparisons between the adult plant blades and germlings were conducted. This research will help us determine how productive the germlings are during their early developing phase, which is crucial for their survival when transitioning into adult plants.

METHODS

Collection Site

Sand Island Beach Park, O‘ahu, Hawai‘i (21.301135 °N, 157.875844 °W) is located at the eastern end of the Honolulu Harbor that has a Pacific Islands Ocean Observing System node, and a shoreline hardened by basalt rock seawalls that are exposed to medium to high wave action. Along the seawall are golden beds of mixed *Sargassum aquifolium*, *Sargassum obtusifolium*, and *Sargassum polyphyllum* at high densities. Due to the similar morphological features of *S. aquifolium* and *S. obtusifolium*, the receptacle structures were further examined to properly identify and select uprights of *S. aquifolium*, the targeted species. Reproductive strands of *S. aquifolium* displaying mature fertile branches with receptacles and plants were collected on three different occasions: Round 1 (June 09, 2021), Round 2 (Sept. 07, 2021), and Round 3 (Oct. 05, 2021). Each round of collection was used for three separate experiments.

Algal Collection and Experimental Setup

Sand Island is in proximity (0.65 mi) to the Ānuenue Fisheries Research Center (AFRC) where the experiments took place. All samples were carefully placed in a ziploc bag with seawater and

transported to AFRC in a cooler. AFRC is equipped with six 0.15 m³ outdoor mesocosms that receive continuous filtered seawater, aeration, and full irradiance. Ambient low nutrient seawater is pumped from the Honolulu Harbor via an intake PVC pipe at a depth of 1.2 m on the ocean side of the harbor, and then filtered through a sand filter (Hayward Pro Series Top Mount Sand Pool Filter, Model: S310T). The seawater is then filtered again in a three-stage cartridge filter (first filter is 5 µm, and then two at 1 µm, 25.4 cm x 5.1 cm, Elmhurst, IL, USA). Filtered water is delivered to each water bath at a rate of 300 – 350 ml min⁻¹. The sand filter was backwashed three times a week to avoid clogging of the sand and cartridge filters.

Germling production and growth

One mesocosm was designated as the settling tank. The collected wild fertile *S. aquifolium* plants were zip tied to lines that spanned across the surface of the water in the mesocosm. This allowed the plants to float or dangle in the water. No modifications were made to the plants before they were placed in the mesocosm. Within three days, release of egg and sperm from fertile receptacles allowed for external fertilization in the water column. Germlings settled on 5 cm² limestone tiles that lined the bottom of the mesocosm (Figure 4.3). Water flow and aeration were provided to ensure oxygenated seawater recirculated within the mesocosm. When the next round of fertile plants was collected, all seeded tiles in the settling tank were transferred to another mesocosm for continued growth.

Environmental parameters

Photosynthetic Active Radiation (PAR) was measured using a LI-COR Spherical Underwater Quantum Sensor (LI-193SA, Lincoln, NE, USA) communicating with a data logger (LI-1400,

LI-COR, Lincoln, NE, USA). PAR was recorded every 15 min between 0630-1700 h. Onset HOBO Pendant Temperature/Light 64k Data Loggers (UA-002-64; Bourne, MA, USA) were placed in each mesocosm to record irradiance and temperature every 15 minutes (Appendix 4.S1).

Experiment I – Germling density and average growth on limestone tiles

The germlings that were cultivated from the adult plants on June 09, 2021 (Round 1) were used in this experiment to determine germling density and average growth rates for one month. After two and four weeks of growth in the mesocosm, top view photos and height measurements of 30 randomly selected germlings on individual tiles were taken. Each tile was marked with a pencil. Height measurements for growth were taken using an electronic digital caliper (Adoric, 0-6”) and recorded. Photos were taken using a pocket size smartphone microscope (A Apexel, 100x) that was attached to a 12-megapixel camera (Apple Inc.). The average daily growth rate was calculated by dividing the total average height of the germlings by 28 days and then dividing the total average height by four weeks for average weekly growth rate.

Each seedling was circled using the Preview app (Version 11.0) to estimate the average number of seedlings per cm². The following formula was used:

$$\frac{\text{Total \# of germlings counted}}{30 \text{ tiles}} = \frac{\text{\# of germlings}}{1 \text{ tile}} \times \frac{1 \text{ tile}}{5 \text{ cm}^2} = \frac{\text{\# of germlings}}{\text{cm}^2}$$

Experiment II - Sun versus shade responses

The 13-week-old germlings that were cultivated from the adult plants that were collected on Sept. 07, 2021(Round 2) were used in this experiment to measure how different irradiance levels affected growth and photosynthesis. The control water bath was exposed to full irradiance

receiving $\sim 959 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ and the experimental water bath was covered with one layer of shade cloth decreasing irradiance levels to an average of $\sim 224 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$. A drop in irradiance occurred between December 30, 2021 and January 04, 2022, associated with a multi-day winter storm. Both mesocosms received equal flow of seawater and aeration. These measurements were taken using a light sensor (LI-190R Quantum Sensor) that was attached to a LI-250 Light Meter (LI-COR Biosciences). Irradiance levels in the shaded mesocosm received similar levels to what is found under simulated *Sargassum* canopies (Appendix 4.S2; Nedlic 2019, unpublished). Each water bath housed 20 seeded tiles with numerous 13-week-old germlings. One germling on each tile was marked with a number and monitored to assess growth and photosynthetic rates over four weeks. All tiles were randomized and placement in each mesocosm was randomized as well.

Photosynthetic rates of *Sargassum* germlings were taken at Week 0 (baseline), 1, 2, and 4 using a Jr. Pulse Amplitude Modulated Fluorometer (Walz, Germany). The fiber optic (diameter = 1.50 mm) was placed in the center of each germling for maximum coverage. Rapid light curves were used to calculate the maximum electron transport rate (ETR_m) and minimum amount of light saturation (E_k) (Ralph and Gademann 2005).

Weekly photos were taken using an Olympus Tough T6-G 4K Camera to photodocument growth in terms of blade or thallus height. The camera was fixed on a microscope setting that allowed up to 4x magnification. A ruler was placed next to a germling, and a photo was taken. Average growth rate was calculated for each week.

Experiment III - Self-shading and photosynthetic consequences

A total of ten reproductive and equal in length strands of *S. aquifolium* were collected at Sand Island Beach Park on October 05, 2021 (Round 3) and transported to AFRC for immediate photosynthetic readings. Each strand was placed in individual aquaria that were situated in a mesocosm. One by one, each strand was carefully placed horizontally in a container with seawater and carried to the lab (~1 min. walk). Photosynthetic readings of individual blades at the base, mid, and top section of each plant was measured (Figure 4.4). These measurements were to determine if photosynthesis varied from the bottom to the top of the plant due to its upright stature underwater. These measurements were also compared to the photosynthetic readings of 11-week-old germlings from Round 2. 11-weeks was the earliest time the germlings were large enough to produce readings under the Jr. PAM fiber optic cable (1.5 mm diameter).

Statistical Analysis. All data analyses were conducted using JMP Pro (Version 16.1.0).

Distribution and variance for growth and photosynthetic parameters were explored using histograms, normal quantile plots, and Levene's test.

A nonparametric Kruskal Wallis test was used to test differences for growth across time for the sun treatment and shade treatment separately. If differences were detected, a Wilcoxon rank sum comparison paired method was used to determine those differences between weeks.

ETR_m was normally distributed, but E_k data were not. Those data were log transformed.

Transformed results are reported, but raw data was plotted in this paper (untransformed and transformed data had the same results). One-way ANOVA was used to determine differences across time for ETR_m and E_k for each treatment separately. If significant differences were present

between weeks, a Tukey's HSD test was used to identify them. A Welch's t-test was used to determine differences between treatments for photosynthesis and growth by week.

RESULTS

Experiment I – Germling density and average growth on limestone tiles

At two weeks, the total average number of seedlings that settled on each tile was 134 seedlings (max = 202, min = 63) with a density of 26.8 germlings/cm². Average daily and weekly growth rates are 0.020 mm and 0.275 mm, respectively. At four weeks, the total average decreased to 114 seedlings (max = 173, min = 51) per tile with a density of 22.8 germlings/cm² resulting in a ~15% decrease. The average daily and weekly growth rates are 0.014 mm and 0.406 mm, respectively. Zygotes settled and were visible on tiles within three days of hanging adults at the mesocosm's water surface. Branching by juveniles occurred as early as four weeks old.

Experiment II – Average growth and photosynthetic responses to different irradiances

Average daily growth rates between the sun and shaded treatments did not significantly differ during each week (Week 0: sun = 0.029 mm/d, shade = 0.028 mm/d; Week 1: sun = 0.024 mm/d, shade = 0.026 mm/d; Week 2: sun = 0.025 mm/d, shade = 0.023 mm/d; Week 4: sun = 0.024 mm/d, shade = 0.022 mm/d; All $P > 0.05$, Figure 4.5). On the contrary, average growth rates differed between weeks under the full sun ($P = 0.0154$, $H(3) = 10.4113$) and shaded ($P = 0.0226$, $H(3) = 9.5709$) treatments. Growth significantly decreased from a baseline of 0.028 mm/d in week 0 to 0.024 mm/d in full sun and 0.022 mm/d under partial shade after four weeks. Overall, a decreasing trend over time under both light regimes was evident.

Samples that received ambient irradiance had significantly higher mean ETR_m values compared to their shaded counterparts during weeks 1 (sun = 48.69 $\mu\text{mol electrons m}^{-2} \text{s}^{-1}$, shade = 35.88 $\mu\text{mol electrons m}^{-2} \text{s}^{-1}$), 2 (sun = 52.65 $\mu\text{mol electrons m}^{-2} \text{s}^{-1}$, shade = 29.11 $\mu\text{mol electrons m}^{-2} \text{s}^{-1}$), and 4 (sun = 61.93 $\mu\text{mol electrons m}^{-2} \text{s}^{-1}$, shade = 44.63 $\mu\text{mol electrons m}^{-2} \text{s}^{-1}$; All $P < 0.05$). The same significant trend was held for the mean E_k values, in weeks 1 (sun = 512.66 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, shade = 331.40 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$; $P = 0.0002$) and 2 (sun = 475.56 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, shade = 375.64 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$; $P = 0.0492$) except for week 4 (sun = 559 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, shade = 451.27 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$; $P = 0.0648$; Figure 4.6).

There were significant differences for mean ETR_m across time for the sun ($P = 0.0060$, $f = 4.5669$) and the shaded samples ($P = <0.0001$, $f = 11.1842$). The mean ETR_m for week 4 (61.93 $\mu\text{mol electrons m}^{-2} \text{s}^{-1}$) was significantly higher than weeks 0 (47.62 $\mu\text{mol electrons m}^{-2} \text{s}^{-1}$, $P = 0.0053$) and 1 (48.66 $\mu\text{mol electrons m}^{-2} \text{s}^{-1}$, $P = 0.0223$) under ambient conditions. Under shaded conditions, mean ETR_m values were significantly lower after weeks 1 ($P = 0.0166$) and 2 (52.64 $\mu\text{mol electrons m}^{-2} \text{s}^{-1}$, $P = < 0.0001$), but significantly increased at week 4 ($P = 0.0002$).

The calculated mean E_k from Jr PAM measurements for samples under ambient conditions remained steady for the sun treatment ($P = 0.4698$, $f = 0.8543$), but not for the shaded samples ($P = 0.0021$, $f = 5.4317$). Mean E_k significantly decreased from 526.28 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ in week 0 to 331.40 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ in week 1 ($P = 0.0013$). Mean E_k during week 2 (375.76 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) was lower than week 0 and trended towards significant differences but did not achieve that separation ($P = 0.0557$). Values were also lower during week 4 (451.27 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) but not significant ($P = 0.4661$).

Experiment III - PAM measurements for adult blade sections and germlings

Photosynthetic measurements were taken from the base, mid, and tip sections of ten fertile adult *S. aquifolium* plants. There were significant differences between the three sections for ETR_m ($P = 0.0004$, $f = 10.7256$). The base was significantly lower ($43.23 \mu\text{mol electrons m}^{-2} \text{ s}^{-1}$) compared to the mid ($58.58 \mu\text{mol electrons m}^{-2} \text{ s}^{-1}$, $P = 0.0226$) and tip ($68.08 \mu\text{mol electrons m}^{-2} \text{ s}^{-1}$, $P = 0.0003$) section. As for the mean E_k ($P = 0.0034$, $f = 7.0519$), the base was significantly lower with $239.94 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ compared to the tip with $324.59 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ ($P = 0.0026$). Photosynthetic rates increased from the bottom to the top of the plant.

There was a significant difference for mean ETR_m ($P < 0.0001$, $f = 9.7761$) and E_k ($P < 0.0001$, $f = 22.4628$) across the adult sections and germlings. Mean ETR_m at the tip section ($68.08 \mu\text{mol electrons m}^{-2} \text{ s}^{-1}$) was significantly higher compared to the germlings ($48.81 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$, $P = 0.0023$) and base ($43.23 \mu\text{mol electrons m}^{-2} \text{ s}^{-1}$; $P = < 0.0001$). The mid was also significantly higher ($58.58 \mu\text{mol electrons m}^{-2} \text{ s}^{-1}$) compared to base ($P = 0.0189$). The mean E_k for the germlings was significantly higher ($443.51 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$) than all three sections (base = $239.94 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$, $P = < 0.0001$; mid = $292.82 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$, $P = < 0.0001$; tip = $324.59 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$, $P = 0.0003$). The tip was also higher than the base ($P = 0.0115$, Figure 4.7 and Supp. Table 2).

DISCUSSION

The order Laminariales are the world's largest algae and create expansive underwater forests filled with diverse species. As significant as kelps are, there is still a great need for research as the environment continues to change. The same issues are at play with Hawai'i's native Fucalean algae *Sargassum* population that used to be prevalent on the reefs in Waikīkī and is of cultural

and ecological significance to tropical coastal ecosystems. This study addresses some of the missing life-history data gaps by illustrating that these highly efficient and productive juveniles readily acclimate to irradiance from full sun to about ~ 25% of ambient irradiance, providing a profound competitive advantage.

Settlement occurred in early June, which indicates the plants were fertile during the spring. This follows the fertility windows for the three *Sargassum* species that were studied by McCourt in the Gulf of California (1984). De Wreede (1976) reported that optimal embryo thallus and rhizoid growth occurred during the cooler months (Nov – March) whereas the lowest development occurred in the warmer months for *Sargassum*. Inoh (1930) explored in detail the embryo and rhizoid formation for multiple *Sargassum* species in Japan and divided them into three groups but did not explore any environmental parameters. He did notice that majority of the species released sexual cells in the spring tide, which compliments the reports for *S. muticum* in Portugal by Engelen et al. (2008). After a week, there was a slight drop in density, which is natural in the field. The decrease in density over time was also observed by Aaron-Amper et al. (2020) on all three substrates (clay, limestone, and nylon). As in the field, not all germlings survive. Those that are able to secure their rhizoids to the substrate are most likely to remain attached and continue to grow. Other substrates such as clay and nylon have been used to test seedling settlement. Aaron-Amper et al. (2020) also demonstrated that limestone tiles compared to clay and nylon rope had the lowest density of recruits in one-week but had the highest survival rate. In Korea, Redmond et al. (2014) describes successful cultivating techniques in both outdoor open water nurseries using special made string and indoor tanks consisting of “seed curtains”. Malm et al. (2003) tested settlement on different bedrocks confirming that different surface textures do affect the survival of germlings.

An integral part of survival for newly settled germlings is to secure and strengthen their holdfast to the substrate. During the first few weeks of settlement, the formation of the holdfast was evident as a dark discoidal crust surrounding the lower section of the germling that was attached to the tile. If a layer of bacteria or diatom growth is present on the surface of the substrate, this may inhibit the seedlings to anchor properly and later fall off when conditions get rough. This was evident in the clod card pilot study (Appendix 4.S7), which showed that the seeded tiles closest to the water pump were overgrown with diatoms and resulted in 100% mortality or detachment. Through observation, seedlings started to branch as early as four weeks. Growth is quick during the first few weeks and then levels off as the germlings start to focus their energy in forming branches. This may be supported by the decreasing average daily growth trend in both the full sun and shaded treatments over time. At this stage, their branches continue to expand creating more surface area for photosynthesis to occur. Instead of them growing vertically, some start to grow horizontally extending their blades farther out to maximize light capture. Aaron-Amper et al. (2020) report similar times of observed settlement and elongation of the germlings in three days and an average length of 0.57 mm after 7 days. The calculated average weekly growth for this study is 0.405 mm. A challenge for this study is that growth was measured by the vertical height and not the horizontal growth so full blade length was not captured.

ETR_m for juvenile plants showed a steady increase over time with week four having the highest maximum rate under full irradiance. This increase may represent the continued development of blades that are slowly expanding in size while simultaneously increasing in photosynthetic capacity as well. Growth may not always coincide with a plant's photosynthetic capability so the increase may demonstrate the capture and use of photons more efficiently over time for other metabolic processes. In contrast, the shaded samples decreased after two weeks and then

rebounded to initial ETR_m rates again during week four. Even with the shaded samples receiving only ~25% amount of light compared to the full irradiance samples, these germlings have the capability to bounce back to normal levels even though ETR_m rates were significantly lower than their full sun counterparts. This response may reflect how the germlings overcome the challenges of settling on a reef that are grown by towering coral or other plants. Many young germlings that grow in the tidepools are seen sprouting out from under cover of other turf algae. *Sargassum* has found ways to survive during the early weeks of development when they are most vulnerable. This result shows how long germlings take to acclimate to shaded conditions as well.

Under full sun treatment, mean E_k did not significantly alter over time as irradiance levels was not altered. Contrastingly, mean E_k under partial shade declined significantly after one week but displayed the same positive adjustment as ETR_m . This demonstrates that the germlings can acclimate to their changing environment as early as one week. In comparison to the adult plants, Kokubu et al. (2015) observed full recovery for effective quantum yield as quick as 12 hours in *S. fusiforme* after being exposed to low (~224 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) and high light levels (~959 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$). Ito et al. (2021) also revealed that *S. muticum* was able to recover photosynthetically after two hours of desiccation with the thallus losing 80% of its water content. Photosynthetic parameters increased from the bottom of the base to the tip of the adult plant reflecting the amount of irradiance available when standing up right in the water column. The tip being closest to the surface whereas the base of the plant is attached to the substrate. Gao (1990) reported similar results for *S. horneri* but there was a flip during the month of June when the plants matured. If growing in a dense meadow, shading is also highest at the bottom due to the upper canopy in addition to the blades of the plant and neighboring *Sargassum* individuals.

Surprisingly, germlings had a higher E_k rate compared to all adult plant sections. Beach (1995) also found similar photosynthetic results for two *Ulva* species being equally photosynthetic as the adult tissues. Both dominant intertidal species have evolved to adapt and be successful in these high-level irradiance exposures at an early development stage. These plants can withstand high irradiance compared to species that are categorized as shaded plants. The photosynthetic systems of shaded plants could collapse under high light exposure. Under full sun, these germlings can have access to maximum irradiance through the winter and spring months as the plants become reproductive and lose blades in the upper portions (De Wreede 1976). Canopies become bare, which opens up space for irradiance to penetrate to the bottom and allow more water flow. These high-level irradiances are also found at the collection site at Sand Island Beach Park where the *Sargassum* beds are completely exposed to high light levels. The juveniles can successfully recruit and grow because they have a strong genetic advantage that allows them to occupy such spaces. The high mean E_k rates allow these plants to be tolerant of varying irradiance levels that reflects how they are also able to grow under canopies. The stature of the adult plants in the water column allows them to sway back and forth with the water movement allowing sunlight from the surface to reach the substrate where the juveniles have settled. The cultivation of outplantable, juvenile native plants of *S. aquifolium* is a first step forward in reestablishing the local populations that historically were the dominant taxa in Waikīkī (Doty 1969; Appendix 4.S4). Current efforts to replenish other native alga on the reefs or in traditional fish ponds are carried out by community led organizations such as Kua‘āina Ulu Auamo and the Limu Hui. In the long-term, these communities will recruit native herbivores and fish to the area, which will return the reef to healthy and thriving conditions. Understanding the growth and photosynthesis of these juveniles provide better insight of how productive algae can be during

their early life stages, which is crucial to their success on the reef. Blue Carbon initiatives are becoming more of interest opening opportunities for significant studies such as this to be prioritized.

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FIGURES

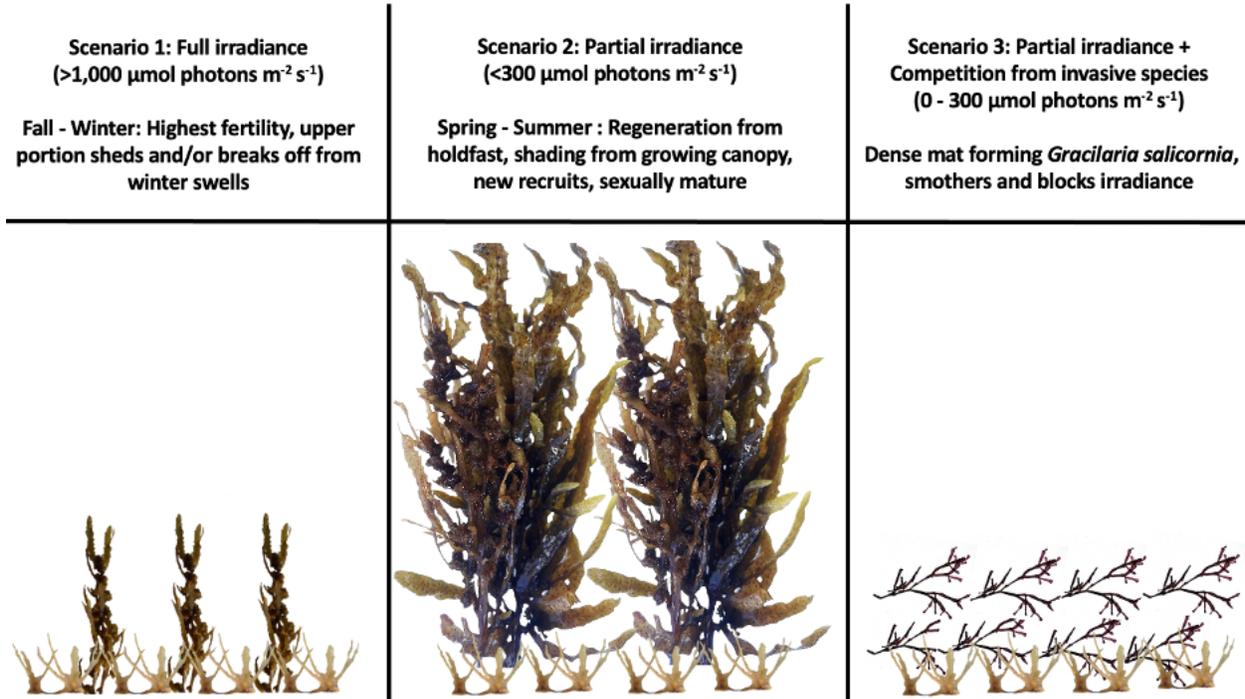


Figure 4.1. 1) Juvenile *S. aquifolium* receiving full irradiance on the reef with no competition from invasive algae. 2) Juvenile *S. aquifolium* receiving partial shading from regrowth of adult *S. aquifolium*. 3) Juvenile *S. aquifolium* being outcompeted by the invasive and mat forming red alga *Gracilaria salicornia*.



Figure 4.2. Collection site at Sand Island Beach Park and experiments conducted at the Ānuenuē Fisheries Research Center (0.65 mi away).

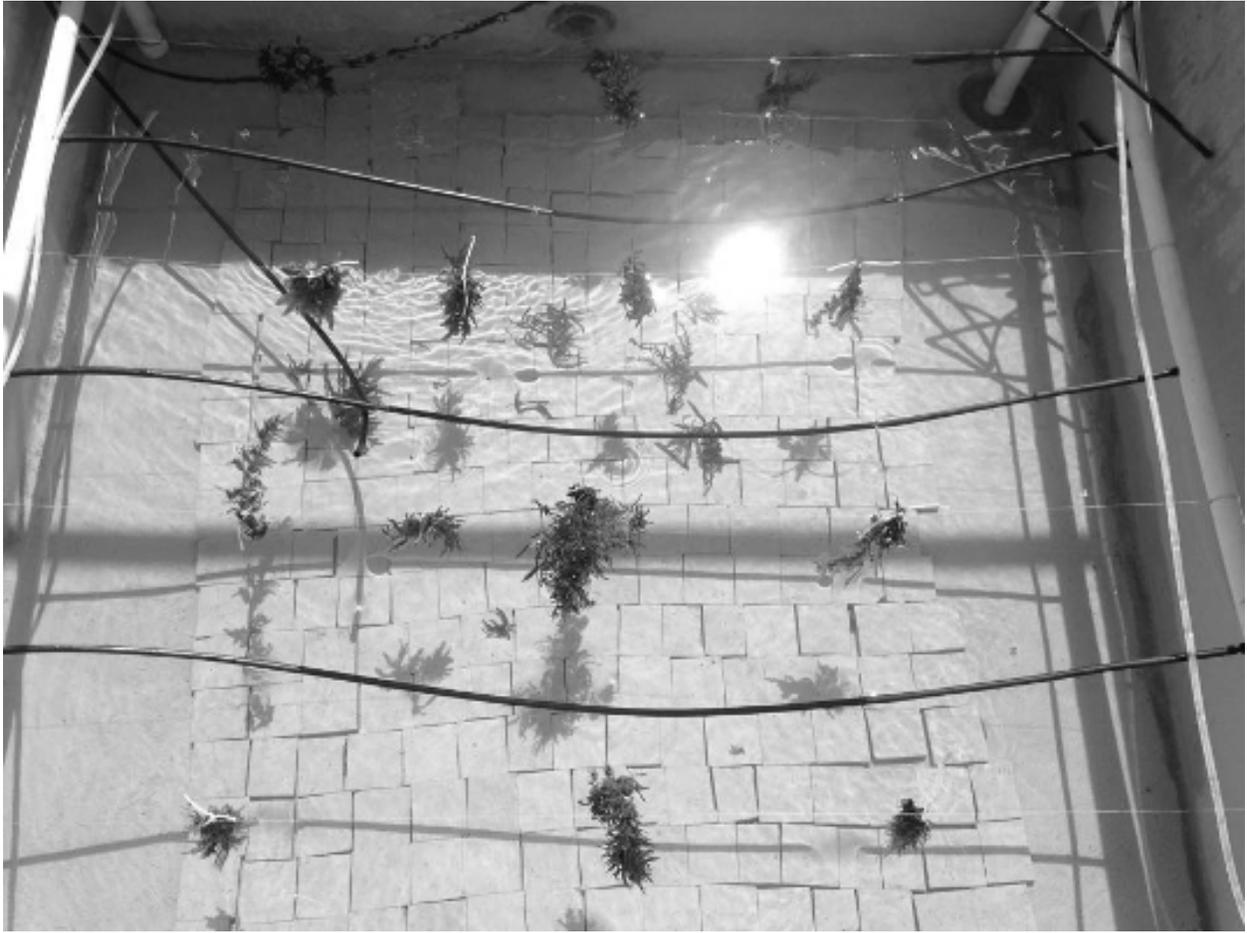


Figure 4.3. Reproductive *Sargassum* plants hanging on the surface of the water above settlement tiles in settling tank. Black tubes = air tubes.

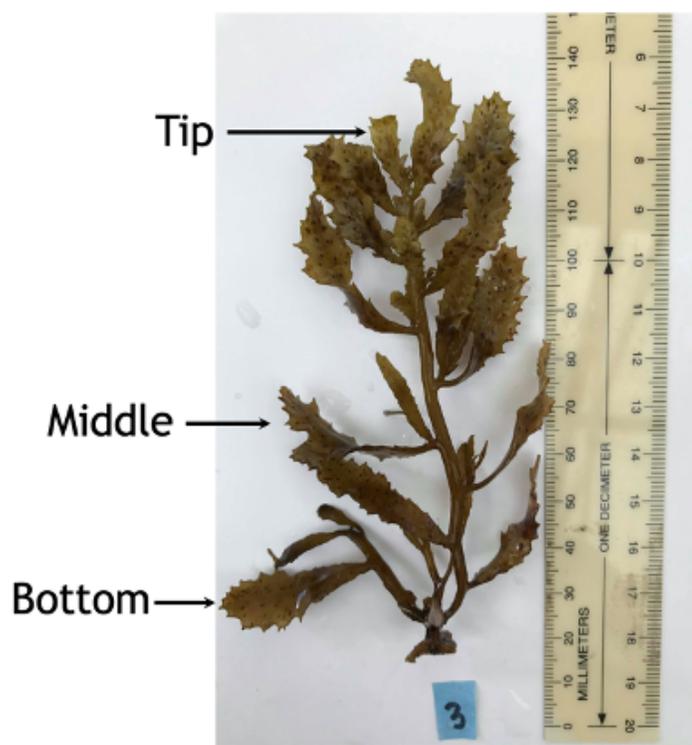


Figure 4.4. Labeled tip, middle, and bottom section of *Sargassum* where photosynthetic readings were measured.

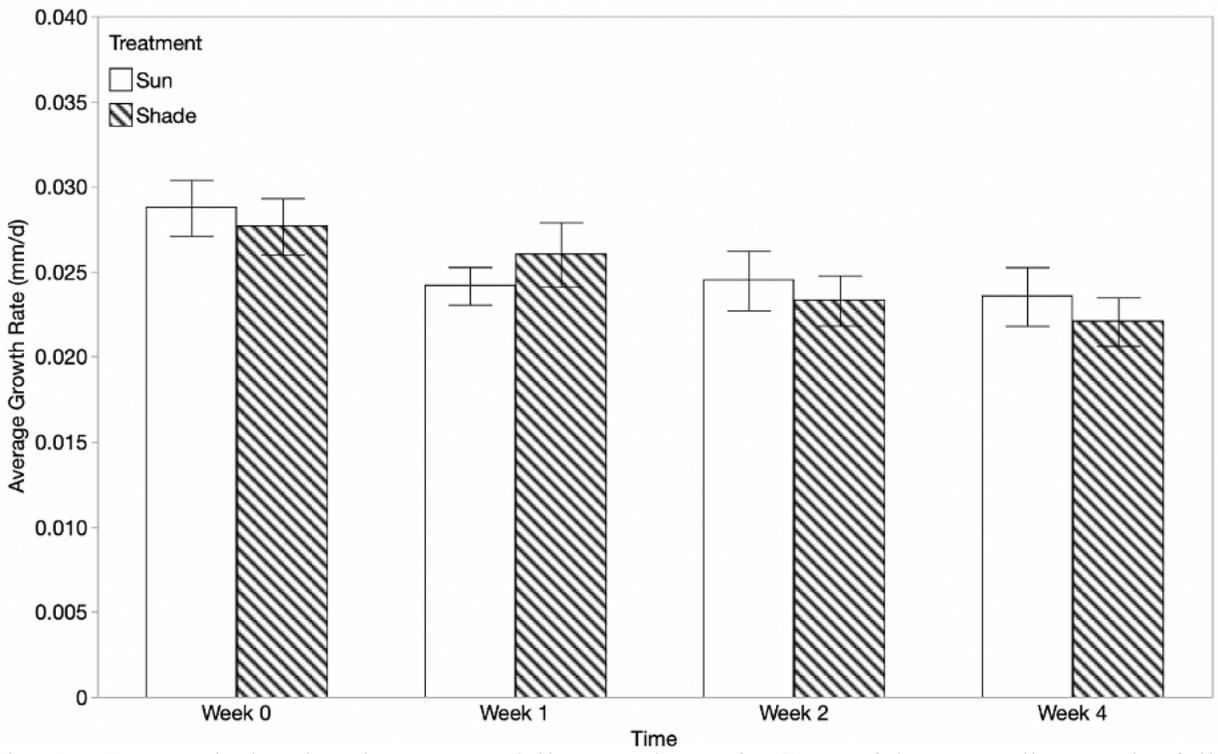


Fig. 4.5. Bar graph showing the average daily growth rate for *S. aquifolium* germlings under full sun (white bar) and shaded (striped bar) conditions. Error bars represent 1 SE.

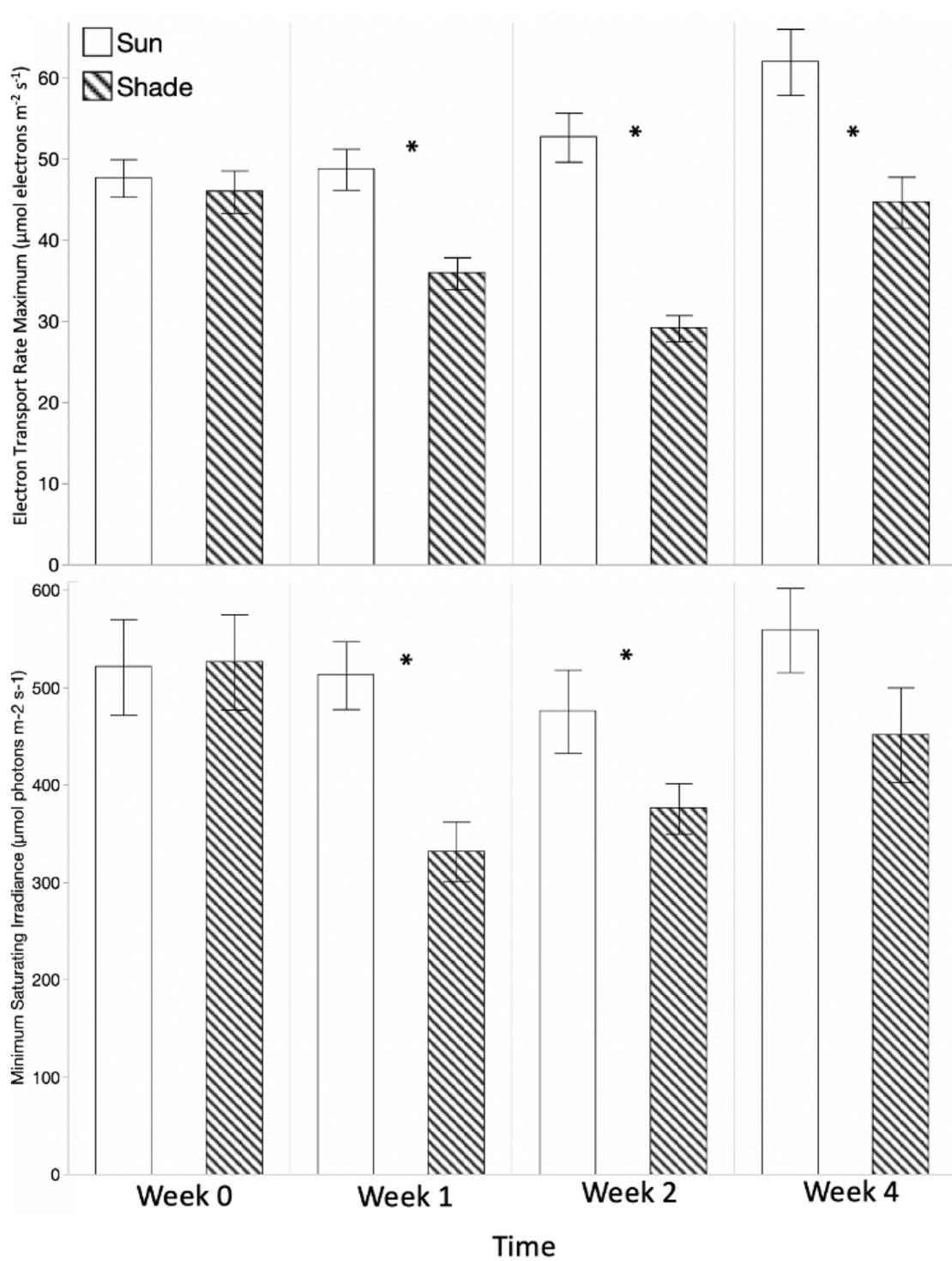


Figure 4.6. Mean ETR_m and E_k starting from the beginning (Week 0), after one week, two weeks and four weeks under full sun (white bar) and shaded (striped bar) conditions. n = 20. Error bars are standard error. * = significant difference (P < 0.05) between treatments.

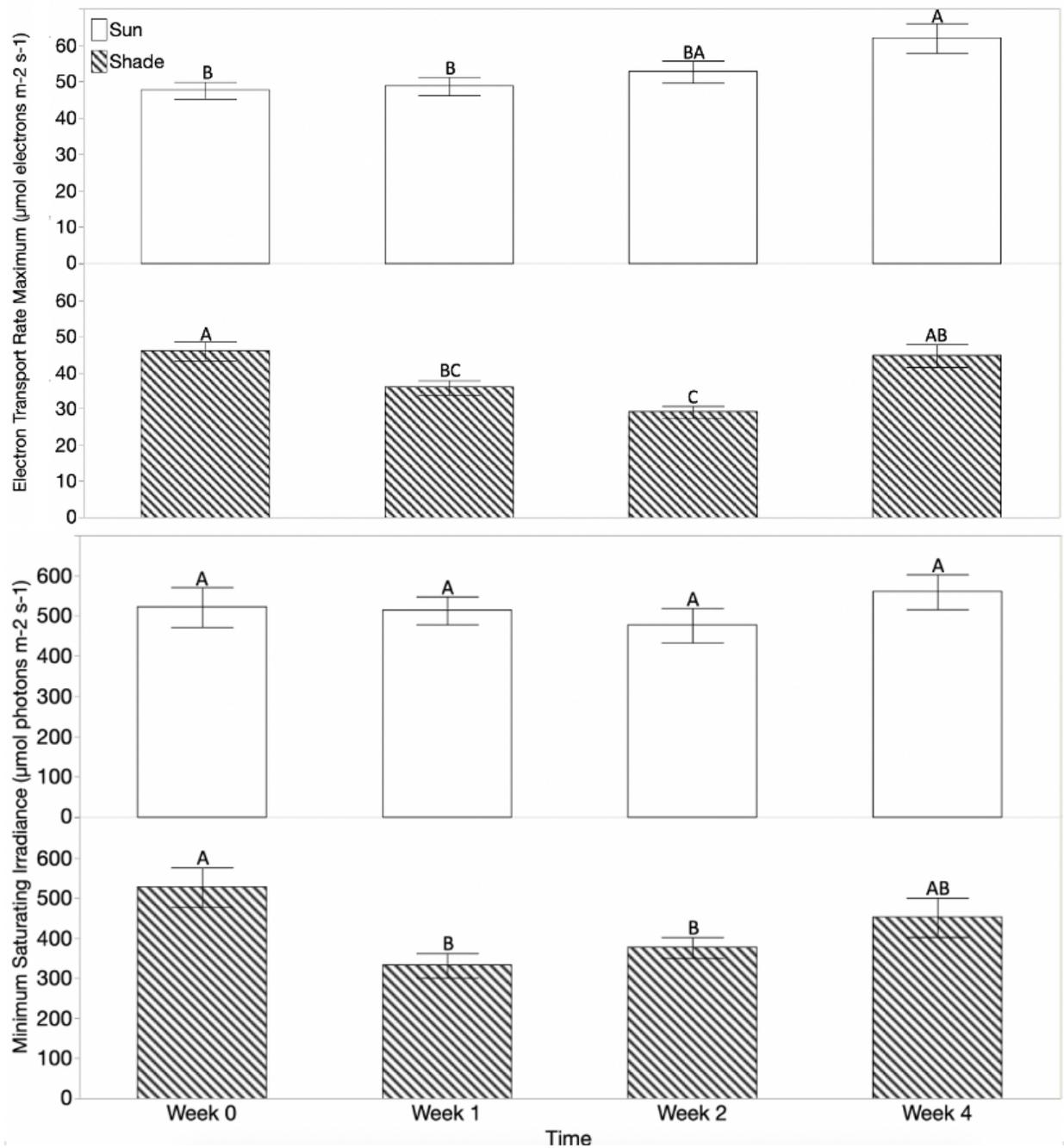


Figure 4.7. Mean ETR_m and E_k across time under full sun (white bar) and shaded (striped bar) conditions. $n = 20$. Error bars are standard error. Weeks not connected by the same letter are significantly different.

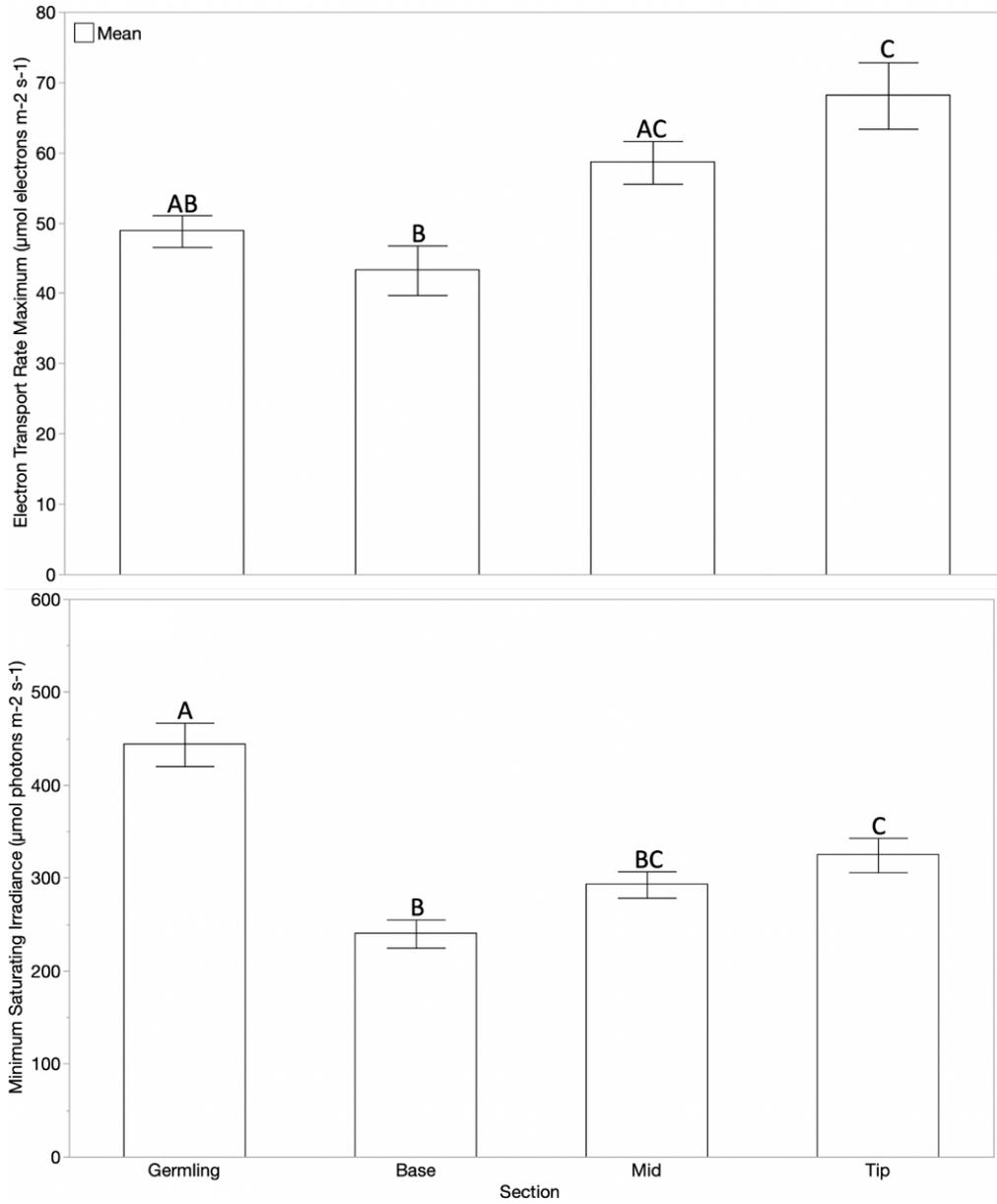
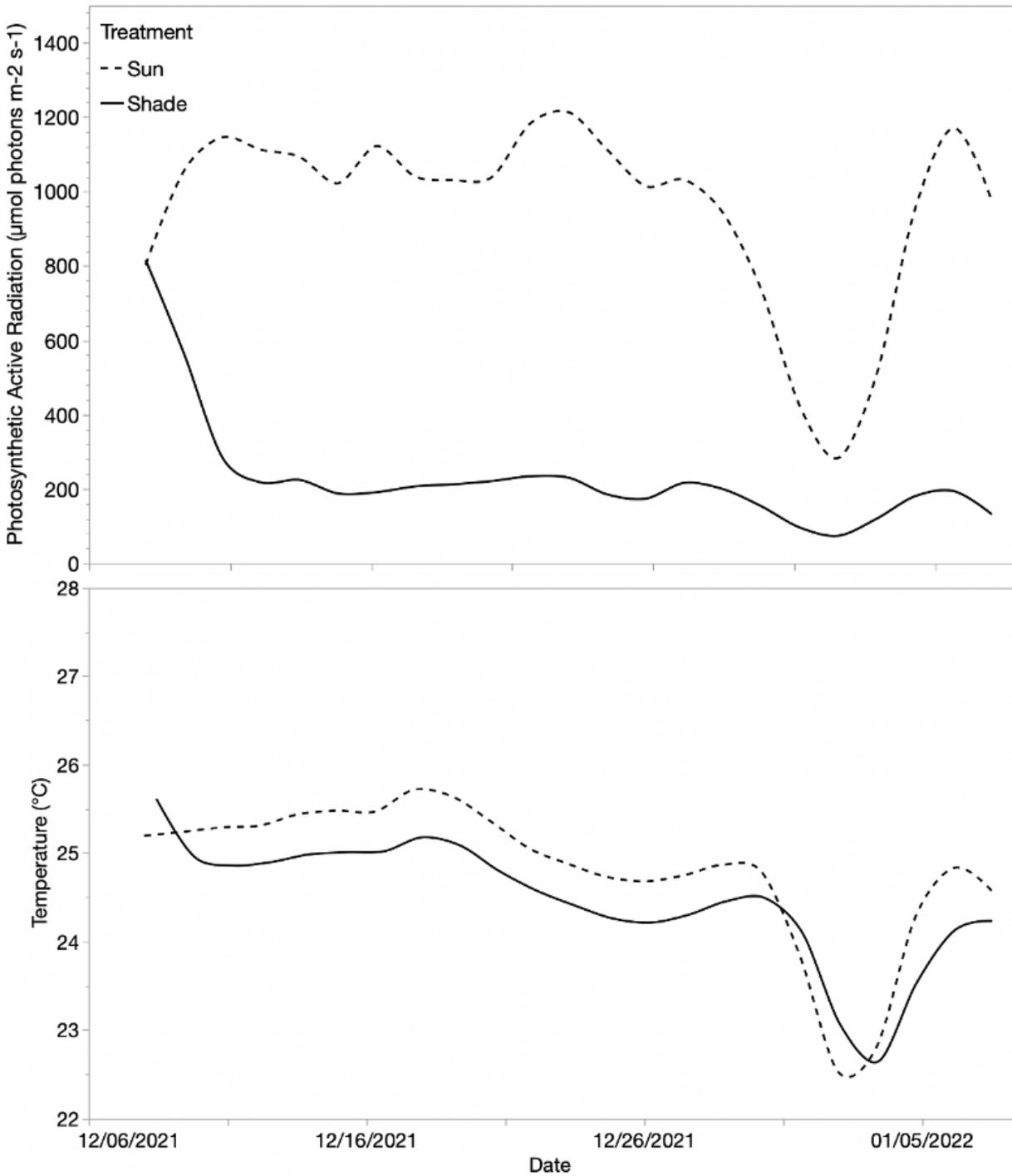


Figure 4.8. Photosynthetic responses of ETR_m and E_k for 11-week-old germlings and the base, mid, and tip sections of reproductive adult plants (n = 10). Sections not connected by the same letter are significantly different. Standard error bars.

APPENDIX



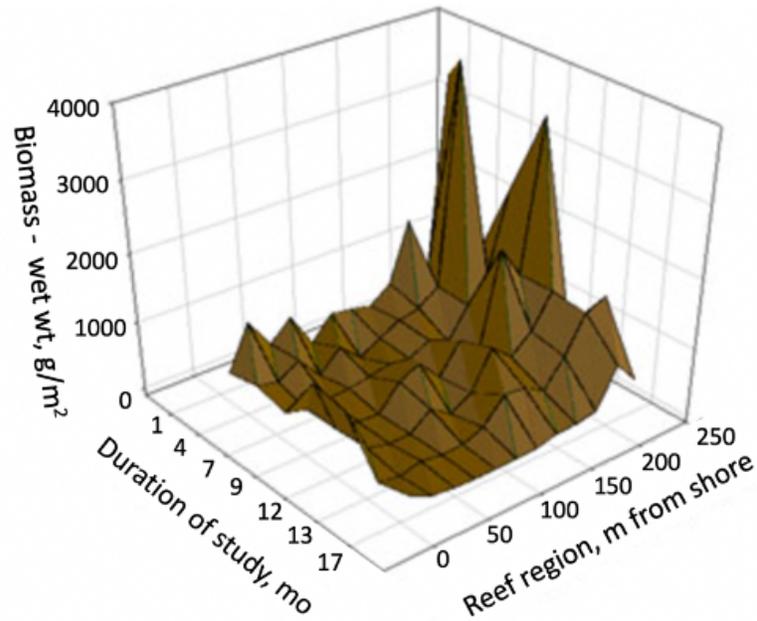
Appendix 4.S1 Environmental parameters immersed in mesocosms, for photosynthetic active radiation and temperature, for the full sun (dotted line) versus partial irradiance (solid line) mesocosms. Temperature recorded by HOBO data logger and LICOR 4-pi sensor.

Number of plants horizontally placed on LICOR sensor	Irradiance ($\mu\text{mol photons m}^{-2} \text{ s}^{-1}$)
0 plants (full sun)	1400
1 plant	600
2 plants	300
3 plants	150
4 plants	15
4 plants (standing up)	150

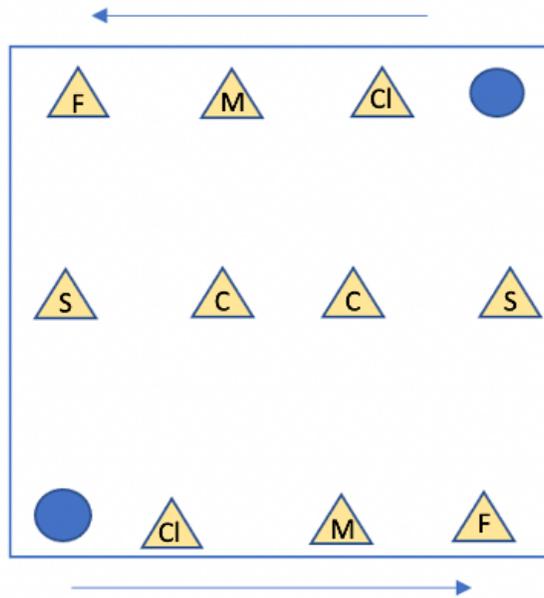
Appendix 4.S2 PAR measurements for simulated *Sargassum aquifolium* canopy from Arlynn Nedlic's study (unpublished 2019).

Appendix 4.S3 Tukey's ordered differences report for comparing ETR_m and E_k between germlings and the base, mid, and tip sections of adult plants.

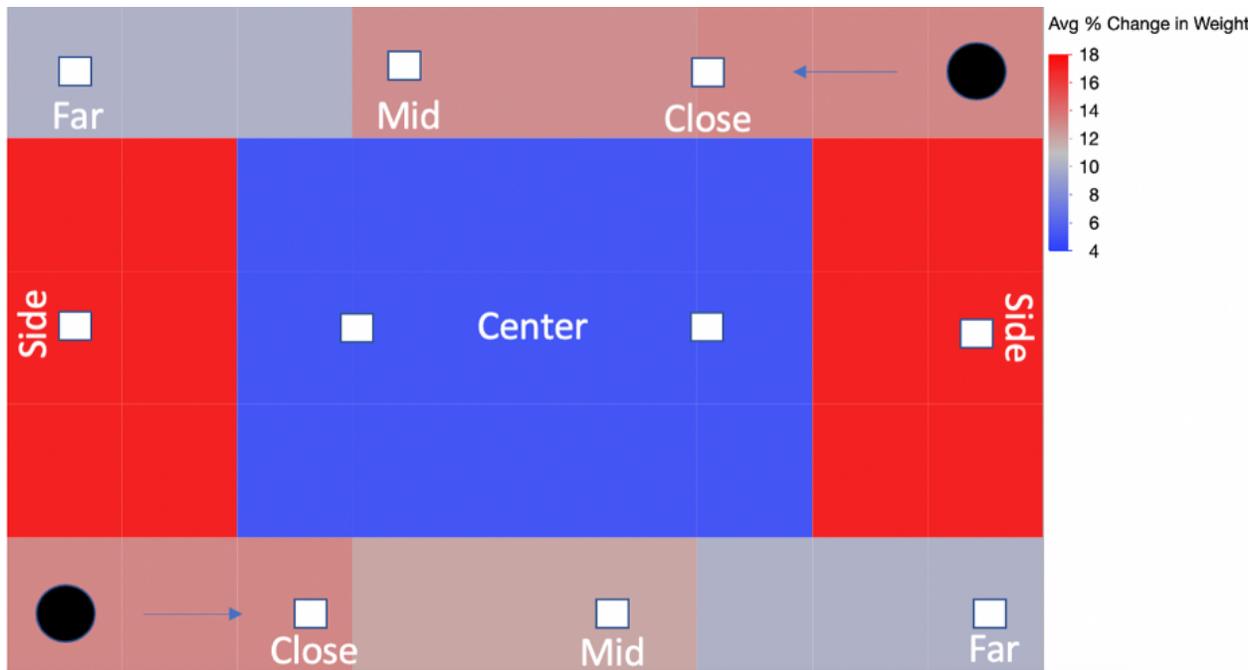
	Level	-Level	Diff	Std Err Dif	Lower CL	Upper CL	p-Value
ETR_m	Tip	Base	24.85080	4.830266	12.2192	37.48241	<0.0001
	Tip	Germling	19.17321	3.654644	9.6160	28.73045	<0.0001
	Mid	Base	15.35150	4.830266	2.7199	27.98311	0.0106
	Mid	Germling	9.67391	3.654644	0.1167	19.23115	0.0461
	Tip	Mid	9.49930	4.830266	-3.1323	22.13091	0.2080
	Germling	Base	5.67759	3.654644	-3.8797	15.23483	0.4101
E_k	Germling	Base	255.1809	49.14221	126.669	383.6925	<0.001
	Germling	Mid	202.3056	49.14221	73.794	330.8172	0.0005
	Germling	Tip	170.5290	49.14221	42.017	299.0406	0.0043
	Tip	Base	84.6519	64.95023	-85.199	254.5030	0.5631
	Mid	Base	52.8753	64.95023	-116.976	222.7264	0.8477
	Tip	Mid	31.7766	64.95023	-138.074	201.6277	0.9613



Appendix 4.S4 Biomass wet weight of *Sargassum aquifolium* (= *S. echinocarpum*) that was collected between 1966 – 1968 on the reef flat in front of the Waikīkī Natatorium ocean-facing seawall. Data from Doty, M. S. (1969).



Appendix 4.S5 Illustration of clod card positions in experimental water bath containing water pumps. F = Far, M = Mid, Cl = Close, C = Center, S = Side, ● = Water pump, ▲ = Clod card, → = Water pressure direction.



Appendix 4.S6. Average change in weight (%) in water bath. The water pumps (black circles) are positioned in opposite corners diagonal to each other, and clod cards (white boxes) are placed in different positions throughout the water bath. The water direction (blue arrows) expelled from the water pumps create a circular movement in the water bath.

Appendix 4.S7. *Pilot Study: Measuring flow rate differences with clod cards using Round 3 Germlings*

Testing effects of water flow in mesocosms. Two out of six water baths were used in this pilot study to test for water pressure differences using Plaster of Paris clod cards (Jokiel and Morrissey 1993, Hart et al. 2002). Both water baths received the same amount of water flow and aeration that were delivered via tubing. The experimental mesocosm had two 500 mpg Supreme Aqua-Mag Water Pumps (Danner Manufacturing, Inc.) located on opposite ends, vertical of each other to create a circular water motion in the tank. The control mesocosm did not have any water pumps. A total of 40 clod cards were constructed using Plaster of Paris Dry Mix (DAP Products) as described by Doty (1971). Clod cards were attached to 5 cm² plastic squares (number 2 plastic, Acme Plastics, Inc.) using marine epoxy (J-B Weld 8277 Underwater Adhesive Epoxy). Once the marine epoxy dried, the plastic squares were attached to settling tiles using rubber bands. The settling tiles provided the same height as the germlings and served as a weight against the water pressure. Ten clod cards were placed in each mesocosm with two replicates per position. There were five positions in the water bath: 1) farthest away from water pump = far, 2) midway between the water pump and wall = mid, 3) closest to the water pump = close, 4) on the side of the water pump where the water bounced off the wall = side, and 5) at the center of water bath where little to no water pressure occurred = center (Appendix 4.S5). Two experimental runs were conducted with the clod cards remaining in the water bath for 18.5 h or 5.5 h.

Effect of water pressure on germlings. Round 3 germlings were used in this experiment. Once water pressure difference in the water bath was confirmed, 5-week-old seeded limestone tiles were placed in the same location of the clod cards in each water bath for one week. Each water bath contained a LI-COR Spherical Underwater Quantum Sensor to record PAR and HOBO data loggers that measured both irradiance and temperature every 15 minutes.

Clod card pilot study and photosynthesis between blade sections. A one-way ANOVA was used to test whether percent change in weight differed across tile positions in each mesocosm, and if photosynthesis was different between the adult plant sections and germlings.

During the first run, the clod cards were left in the water baths for 18.5 h. This resulted in the clod cards closest to the water pumps to dissolve more than 30% of initial weight leading to inaccurate measurements (Jokiel and Morrissey 1993). Hence, the next set of clod cards were placed in the tank for a shorter period, 5.5 h. Clod cards that were placed on the side, mid, and close positions in the experimental water bath decreased significantly in weight compared to those that were in the center of the mesocosm (All $P < 0.05$). The clod cards that were placed farthest away from the water pump still decreased in weight and was only significantly lower compared to those placed on the sides ($P = 0.0276$) (Supp. Fig. 3S). There were no significant differences in weight between all positions in the control water bath (ANOVA $P = 0.4599$).

After a few days, significant diatom growth on the tiles in the high-water motion tank coincided with 100% mortality of germling for the tiles closest to the water pumps. Further mesocosm experiments were run with no water motion; this allowed germling growth to remain consistent and keep diatom growth at bay.

CHAPTER 5: OVERALL CONCLUSION

This dissertation provides 1) an assessment of the benthic community in a 2-year climate change study providing a holistic view of what the marine plant community in Hawai‘i may look like within the next century under predicted future climate change conditions, 2) the first reported growth and photosynthetic rates of collected fresh samples for the dominant native green alga *Microdictyon setchellianum* under different irradiance regimes while exploring historical data from Doty’s (1979) Waikīkī study, and finally 3) perspective on a new tool to restore the native brown alga *Sargassum aquifolium* or limu kala populations in part by characterizing early life stages of growth and photosynthesis.

Mesocosm experimental setups have played an integral role in providing researchers the opportunity to explore complex questions in a more focused and controlled environment. Even though the layout is on a smaller scale compared to the open ocean, mesocosms provide the luxury to manipulate environmental factors such as salinity, water temperature, irradiance, nutrients, carbon dioxide, and more. This allows scientists to hone in on specific questions to assess the effects of these variables that have been changing so rapidly in the world today. Bahr et al. (2019), Timmers et al. (2020), and McLachlan et al. (2022) have reported that overall communities within this climate change mesocosm study have not been negatively impacted in terms of species diversity but instead have shown signs of shifting. Similarly, the macroalgal communities that were assessed in the same mesocosms also appear to shift, and not dramatically change. Species diversity and photosynthetic rates were not significantly different across all treatments. Species composition slightly differed but was also not significant. This demonstrates

that the native marine plants are resilient and will be able to persist under the predicted future conditions, which can help recruit and increase herbivory on a reef.

Microdictyon setchellianum is found in many Central Pacific reefs making this alga a significant player in those marine ecosystems but is not easily studied elsewhere in the Pacific basin. If this alga disappears as our oceans change, coral reef communities of fish will lose a principal primary producer that contributes about 75% of reef cover in certain regions. This species' ability to photoacclimate under low to high light levels allow it to have wide depth distribution from the intertidal to deep waters as one of the most common green algae. More focused studies on *Microdictyon setchellianum*, such as nutrient and herbivory studies in Hawai'i, need to be carried out as it is a keystone species in one of the world's last pristine waters in the Papahānaumokuākea Marine National Monument and across the Main Hawaiian Islands.

The restoration tools were developed to successfully cultivate *Sargassum aquifolium* germlings from reproductive adult plants. Seeded limestone tiles served as a successful platform for germlings to settle, anchor and grow for at least 5 months in mesocosms at the Ānuenuē Fisheries Research Center. One of the main challenges with the mesocosm study is that wave exposure and water motion that is naturally found on the rocky shoreline were not simulated in the tanks. This led to diatom growth that required extensive and consistent cleansing of the tiles. Wave exposure is a crucial part in the growth process as it keeps the germlings from biofouling and provides that natural pressure the germlings need to build a strong and robust holdfast to anchor them to the substrate.

Overall, my research sets the stage for new questions to be answered for our limu community and future work. With climate change as an ongoing challenge, the 2-year mesocosm study provided valuable insight on the future health of the reefs in Kāne'ōhe Bay. This opens up

questions as to what reefs may look like in other parts of the Pacific region such as the FSM. Would the algal community in the FSM show the same resiliency? Specific to the experiment, would the established communities on the mesocosm walls be the same if the material of the mesocosms (polypropylene) was different? Because the algal data was collected at the end of the experiment, did the algal community change from the beginning to the end or did it stay consistent throughout the duration of the experiment?

The first reported measurements of growth and photosynthesis for *Microdictyon* provides understanding as to how productive the communities are in shallow waters. Because *Microdictyon* has a wide depth distribution, the next question to ask is how photosynthetic are the meadows in PMNM where irradiance is limited? How are these lush meadows sustaining themselves at 64 m depths? Do they have a slower growth rate compared to the communities under higher irradiance levels? Questions regarding herbivory also need to be explored as these dense meadows support healthy herbivore populations in these pristine waters.

The cultivation process of *Sargassum aquifolium* germlings is a major step in restoring the local populations on the reefs and preserving the culture and traditions of Hawai'i. The germlings were cultivated for only 5 months due to time constraints, which leads to the need for further experimentation. The next step for this project is to determine at what age are the germlings robust enough to be outplanted on the reef? Would seeded tiles that are placed on the reef have faster growth rates compared to seeded tiles that remain in the mesocosm? Will the limestone tiles serve as a long-term, successful platform for continued growth compared to an actual reef? Answering critical questions such as these will help refine this cultivation process that aims to re-establish the native limu kala populations for both ecological and cultural significance.

My research relates to the broader Pacific community because of the commonality of species that are found across the region. Although my research took place in Hawai‘i, the selection of the two native species, *Microdictyon setchellianum* and *Sargassum aquifolium*, were intentional as both species are found in the FSM as well. As like many of our native algal species, very little is known about their basic biology despite their essential roles in the marine ecosystem. Few experimental studies are underway for the Western Pacific’s inhabited islands and resource managers have no current information. The checklists of benthic algae for the Micronesia region need to be updated as algal species may have disappeared and/or have been introduced over time. These checklists provide the identification names and location of where each alga was observed but lack information such as percent cover or abundance that is helpful in knowing how much is present on the reef. Algal surveys have been conducted during reef assessments, but proper identification is lacking. There is a dire need to 1) fill in the missing basic biology of our native plants, 2) understand their ecophysiological responses to our changing oceans in terms of growth, physiology, and competition, 3) and improve documentation and identification efforts. To properly manage and protect our marine biodiversity and resources, we need to ensure we have a strong foundation that is built on the best science to inform policy-making decisions and secure the livelihoods of the local communities.