THE EFFECT OF INCREASED TEMPERATURE AND PCO$_2$ ON RESPIRATION AND PHOTOSYNTHETIC RATES IN THE RED ALGA *Gracilaria salicornia*

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Megan Akiko Onuma

Thesis Committee:

Celia M. Smith, Chairperson
Kuʻulei S. Rodgers
Alison R. Sherwood

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Table of Contents

Acknowledgements ........................................................................................................... iii
Abstract ........................................................................................................................... iv
List of Tables ...................................................................................................................... v
List of Figures ..................................................................................................................... vi
List of Abbreviations ......................................................................................................... vii

Chapter 1: Review of the Literature .................................................................................. 1
  Global climate change and coral reefs ........................................................................... 1
  Invasive algae in Hawai‘i ............................................................................................... 3
  Gracilaria salicornia ...................................................................................................... 5
  Climate change and macroalgae ..................................................................................... 8
  Temperature and macroalgae ........................................................................................ 9
  Carbon dioxide and macroalgae ................................................................................... 11
  Proposal ......................................................................................................................... 14
  Literature Cited ............................................................................................................. 16

Chapter 2: The effects of increased temperature and pCO₂ on respiration and .......... 24
  photosynthetic rates of Gracilaria salicornia
  Introduction .................................................................................................................... 24
  Methods ......................................................................................................................... 27
    Experimental Design .................................................................................................. 27
    Statistical Analysis .................................................................................................... 31
  Results ......................................................................................................................... 33
    Respiration ................................................................................................................. 33
    Photosynthesis: ETR_{\text{max}} and E_k ...................................................................... 33
  Discussion .................................................................................................................... 34
    Comparing results with other studies ...................................................................... 34
    Temperature ................................................................................................................ 36
    Carbon Dioxide ......................................................................................................... 38
    Potential future scenarios ........................................................................................ 39
  Conclusion and future studies ..................................................................................... 42
  Literature Cited ............................................................................................................. 45

Appendix A: pH measurements ....................................................................................... 66
Appendix B: Alkalinity titrations ..................................................................................... 70
Appendix C: HOBO observations to PAR conversion ................................................... 73
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Abstract

Increasing temperatures and ocean acidification are causing widespread damage to coral reefs. Since corals and macroalgae compete for resources, this may lead to the proliferation of some macroalgal species. Understanding how macroalgae will be affected by climate change will be useful knowledge for conservation management. This study focuses on how the interactive factors of temperature and pCO$_2$ affect the respiration and photosynthesis rates of *Gracilaria salicornia*, an invasive alga in Hawai‘i originally from the eastern Pacific. Using an outdoor, continuous-flow seawater system with natural sunlight, *G. salicornia* (n=8) tissue was exposed to different temperatures and pCO$_2$ levels using a full-factorial design. At the end of a three-day exposure to treatment conditions, respiration was measured using oxygen evolution methods and photosynthesis was measured using a fluorometer to obtain rapid light curves. Each response variable was analyzed independently using multiple regression models. The models suggest temperature and temperature$^2$ had significant effects on respiration rates (P<0.03 and P<0.03, respectively). Analysis indicates pCO$_2$ had a slight but significant effect on maximum electron transfer rate (ETR$_{\text{max}}$) (P<0.04). Results indicate *G. salicornia* experiences carbon limitation, which has also been demonstrated in other species of the genus *Gracilaria*. The increase in carbon availability that accompanies ocean acidification may encourage growth in these carbon-limited species. These analyses show an increase in respiration and photosynthesis rates under select climate change scenarios, though further research will be needed to determine how these metabolic increases affect the growth and distribution of the organism.
List of Tables

Table 1.1 The effect of global climate change CO₂ levels on rhodophyte macroalgae. 15

Table 2.1: Timeline and weather conditions for the experimental periods. 51

Table 2.2: Summary of measured parameters, frequency, and instruments. 52

Table 2.3: Temperature summary statistics comparisons. 53

Table 2.4 Means of measured factors by treatment. 54

Table 2.5: Multiple regression model analysis of respiration rates. 55

Table 2.6: Summary of pooled ETRₘₐₓ, and Eₖ values. 56

Table 2.7: Multiple regression model analysis of ETRₘₐₓ values. 57

Table 2.7: Multiple regression model analysis of Eₖ values. 58

Table C.1: Summary of linear regressions for log-transformed LI-COR values and log-transformed averaged HOBO values. 75
List of Figures

Figure 2.1: Representation of tank set-up. 59
Figure 2.2: Actual picture of tank set-up at Ānuenue Fisheries Research Center. 60
Figure 2.3: Graph of the residuals by predicted values for the $ETR_{\text{max}}$ whole model. 61
Figure 2.4: Graph of $ETR_{\text{max}}$ versus pCO$_2$ values. 62
Figure 2.5: Comparison of respiration rates. 63
Figure 2.6: Comparison of $ETR_{\text{max}}$ values. 64
Figure 2.7: Comparison of $E_k$ values. 65
Figure C.1: LI-COR versus HOBO data over two trial periods. 76
Figure C.2: LI-COR versus HOBO data separated by time of observation. 77
List of Abbreviations

AFRC – Ānuenue Fisheries Research Center
CA – carbonic anhydrase
DIC – dissolved inorganic carbon
DO – dissolved oxygen concentration
E_k – minimum saturating irradiance (used with fluorometry methods)
ETR_{max} – maximum electron transport rate (used with fluorometry methods)
HIMB – Hawai‘i Institute of Marine Biology
I_k – minimum saturating irradiance (used with dissolved oxygen methods)
PAR – photosynthetically active radiation
pCO_2 – the partial pressure exerted by carbon dioxide gas in the air above a liquid
P_{max} – maximum photosynthesis rate (used with dissolved oxygen methods)
TA – total alkalinity
Chapter 1: Literature Review

Global climate change and coral reefs

Over the next century, the amount of carbon dioxide (CO₂) gas in earth’s atmosphere is projected to increase with anthropogenic input (IPCC, 2014). By 2100, atmospheric CO₂ levels are predicted to reach 940 µatm, greater than a three-fold increase from pre-industrial levels of 280 µatm (Hoegh-Guldberg et al., 2007; Pörtner et al., 2014). Carbon dioxide is a greenhouse gas, trapping and reradiating heat within the atmosphere, warming the planet (Hoegh-Guldberg et al., 2007; Pörtner et al., 2014). Much of this increase in CO₂ will be distributed by winds and diffuse into ocean waters, changing the chemistry of seawater and lowering the pH through a process known as ocean acidification. Ocean acidification will increase the absolute amount of CO₂, which in turn will cause changes in concentrations of different carbon compounds as seawater chemistry attains equilibrium (Hoegh-Guldberg et al., 2007). These changes in chemistry will affect many different life processes for diverse marine organisms, including calcification, membrane transport, photosynthesis, and reproductive success (Hoegh-Guldberg et al., 2007; Pörtner et al., 2014).

Future climate change conditions, such as decreased ocean pH and warmer ocean water temperatures, will disrupt coral reef ecosystems (Hoegh-Guldberg et al., 2007; Hughes et al., 2007; Koch et al., 2013; Kroeker et al., 2013; Pörtner et al., 2014). Corals, which provide the foundation for many tropical coastal ecosystems, are sensitive to these changes and are already beginning to face major challenges as a result (Jokiel & Brown, 2004; Hoegh-Guldberg et al., 2007; Koch et al., 2013; Kroeker, Micheli, & Gambi, 2013; Pörtner et al., 2014; Bahr, Jokiel & Rodgers, 2015; Rodgers et al., 2017). Within the past three years, widespread coral bleaching events - the loss of the algal symbiont under stressful conditions - have been reported in Hawai‘i
(Bahr, Jokiel & Rodgers, 2015; Rodgers et al., 2017), the Great Barrier Reef (Hughes et al., 2017), Taiwan (DeCarlo et al., 2017), and Indonesia (Ampou et al., 2017). Coral bleaching occurs as a stress response to high temperatures, and in severe, long-lasting cases, coral bleaching results in coral mortality (Hoegh-Guldberg, 1999; Wilkinson et al., 1999; Jokiel & Brown, 2004). In addition to rising temperatures, corals are facing a more acidic seawater environment, which inhibits their ability to calcify (Hoegh-Guldberg et al., 2007; Pörtner et al., 2014). Increased pCO$_2$ has distinct effects on the coral organism at different life stages, although there is contrasting evidence regarding the relative vulnerabilities of coral larvae, juveniles, and adult colonies to increased pCO$_2$ (Anlauf, D'Croz & O'Dea, 2011; Kroeker et al., 2013; Kroeker, Micheli & Gambi, 2013; Przeslawski, Byrne & Mellin, 2015). Coral species demonstrate varying levels of vulnerability to these conditions (Bahr, Jokiel & Rodgers, 2016; Okazaki et al., 2017), and long-term exposure to higher temperatures and ocean acidification can change community structure and reduce diversity (Hoegh-Guldberg et al., 2007; Kroeker, Gambi & Micheli, 2013; Huang & Roy, 2015).

Climate change will alter many parts of the coral ecosystem. The reaction of populations to these stressors can be summarized as follows: (1) if motile, organisms can move to new areas, (2) they can acclimate to the new conditions, (3) they can adapt genetically over multiple generations, (4) they can die, resulting in extirpation or extinction (Harley et al., 2012; Hoegh-Guldberg et al., 2017). For example, among reef fishes, the species that have small ranges and are highly dependent on coral are expected to decline (Munday et al. 2008). Like other mobile species, reef fishes will probably shift their range based on preferred temperature gradients (Munday et al. 2008). Crustose coralline algae and calcifying macroalgae show decreased photosynthetic and growth rates in ocean acidification treatments (Koch et al., 2013; Kroeker et
In general, taxa more vulnerable to climate change conditions, including coralline algae, echinoderms, and mollusks, will likely decrease in abundance while organisms with greater resilience, like crustaceans, fleshy macroalgae, and seagrasses, increase in abundance (Kroeker et al., 2013). The varying levels of vulnerability to climate change can result in changes to community structure or in more extreme cases, extirpations and extinctions (Hoegh-Guldberg et al., 2007; Kroeker et al., 2013; Kroeker, Gambi & Micheli, 2013; Kroeker, Micheli & Gambi, 2013; Pörtner et al., 2014; Huang & Roy, 2015).

Studies examining climate change in the marine environment have generally focused on corals and other calcifying invertebrates, but other important taxa have been relatively overlooked. Over 70% of marine climate change papers published between 2000-2009 examined the effect of climate change on benthic invertebrates (Wernberg, Smale & Thomas, 2012). The importance of coral research cannot be denied; however, it is also essential to examine the impact that climate change will have on other organisms, especially those that heavily influence the condition of coral reef ecosystems. Foremost among these are macroalgae, a major competitor with corals and a provider of food and oxygen to the reef, yet only 11% of publications looked at the effects of climate change on these important primary producers (Wernberg, Smale & Thomas, 2012; Koch et al., 2013). Macroalgal research is necessary to more fully understand how climate change will affect the coral reef ecosystem as a whole.

**Invasive algae in Hawai‘i**

At least 19 species of foreign marine macroalgae have been transported purposefully or accidentally to the Hawaiian Islands since the 1950s (Russell, 1992; Smith, Hunter & Smith, 2002). Many were purposefully introduced as agar or carrageenan crops for commercial purposes, while others are thought to be accidental introductions, arriving on ship hulls, in ballast
materials, as epiphytes on imported aquaculture, or as aquarium releases (Russell, 1992; Smith, Hunter & Smith, 2002; Godwin, Rodgers & Jokiel, 2006). Some of these species have become invasive-growing and spreading over large areas in a relatively short period of time.

Invasive algal blooms have financial and ecological repercussions (Russell, 1992; Cesar et al. 2002; Cesar & van Beukering, 2004; Godwin, Rodgers & Jokiel, 2006). For example, many tons of rotting algae strewn along Maui beachfront property from overgrowth of the introduced and invasive Hypnea musciformis is estimated to have cost the City and County of Maui over $20 million dollars from lowered property values, decreases in rental income, and cleanup costs (Cesar et al., 2002). Introduced and invasive algae have the potential to compete successfully for ecological niches formerly occupied by native algae (Russell, 1992). Russell demonstrated how two introduced and invasive algae in Hawai‘i (Hypnea musciformis and Acanthophora spicifera) are dominant in the competition with two native algae (Hypnea cervicornis and Laurencia nidifica) that occupy similar niches (1992). Algal mats or mounds can alter the benthic environment. For example, the invasive, mound-forming Gracilaria salicornia can create environments of hypoxia, hyperoxia, acidification, increased sedimentation, and decreased irradiance beneath the algal mound structure (Martinez, Smith & Richmond, 2012). These conditions cause stress to corals and other benthic organisms living underneath (Martinez, 2012). Invasive algae harbor different communities of epiphytes and epifauna than native algae (Fukunaga, Peyton & Thomas, 2014). Research from Hawai‘i indicates invasive algae cause changes in community composition, affect higher trophic levels, change habitats, and monopolize spatial resources (reviewed in Davidson et al., 2015).

An important factor controlling invasive algal blooms in Hawai‘i is nutrient abundance. Increasing the nitrogen or phosphorus nutrient availability significantly increases the growth of
Hawai‘i’s marine algae, and some invasive algae are able to sequester nutrients within microenvironments created from the 3-D structure of their thalli (Stimson, Larned & McDermid, 1996; Larned, 1998). Eutrophic environments associated with wastewater injection wells and submarine groundwater discharge encourage the growth of invasive algae (Dailer, Smith & Smith, 2012). The continued discharge of sewage over several decades into Kāne‘ohe Bay has been linked to the overgrowth of the native, invasive macroalga *Dictyosphaeria cavernosa*, and the subsequent diversion of sewage outfall to deeper ocean waters caused immediate decrease in the standing crop of *D. cavernosa* within the bay (Smith *et al.*, 1981; Hunter & Evans, 1995).

The most successful invasive algae in Hawai‘i are *Avrainvillea amadelpha* (Chlorophyta) and rhodophytes *Eucheuma denticulatum* and *Kappaphycus alvarezii*, *Hypnea musciformis*, *Acanthophora spicifera*, and *Gracilaria salicornia* (Smith, Hunter & Smith, 2002; Godwin, Rodgers & Jokiel, 2006). *Gracilaria salicornia* was chosen as the focus of this research because of its ecological significance in Hawai‘i as an invasive alga.

**Gracilaria salicornia**

*Gracilaria salicornia* (C. Agardh) Dawson is a highly invasive alga in Hawai‘i that covers large swaths of shallow coastal areas around O‘ahu, Hawai‘i Island, and Moloka‘i (Rodgers & Cox, 1999; Nelson *et al.*, 2009). In its native range, *G. salicornia* is widely distributed from Africa to Indian Ocean islands, from Southwest Asia to Southeast Asia (Silva, Basson & Moe 1996) including China (Tseng, 1984), Japan (Yoshida, Nakajima & Nakata, 1990) and Taiwan (Chiang, 1985), and numerous Pacific islands including the Federated States of Micronesia, Guam (Meneses & Abbott, 1987), Mariana and Northern Mariana Islands (Meneses & Abbott, 1987; Tsuda, 1985), Palau (Ohba *et al.*, 2007) and the Solomon Islands (Womersley & Bailey, 1970). In the Hawaiian Islands, this species was first found in Hilo Bay in
1971 and is considered a non-native although the history of its introduction has not been thoroughly evaluated (Russell, 1992; C. M. Smith pers. comm., 2016; Smith, Hunter & Smith, 2002). This species and others in the genus *Gracilaria* are well studied because of their use in the agar industry (e.g. Nelson *et al.*, 1983; Raikar *et al.*, 2001).

The invasive status of *G. salicornia* in Hawai‘i derives from this plant being particularly resilient, capable of growing on hard substrates, able to acclimate to diurnal and seasonal ranges of temperatures, salinities, and light levels found in the Hawaiian Islands, as well as survive hour-long periods of desiccation (Beach *et al.*, 1997; Smith *et al.*, 2004; Phooprong, Ogawa & Hayashizaki, 2007; Martinez, Smith & Richmond, 2012). Preliminary tests have shown that *G. salicornia* is not highly preferred as a food for native reef fishes (Stimson, Larned & Conklin, 2001; Conklin, 2007; Okano, 2010). The alga can reproduce via fragments (Smith, Hunter & Smith, 2002). Mats can travel to other uncolonized areas by drifting in the water column (Conklin, 2007). These characteristics have allowed *G. salicornia* to establish in many Hawaiian coastal regions, once introduced.

The distribution around Hawai‘i and ability to spread has been documented from several sources. Established populations have been recorded around the islands of O‘ahu, Moloka‘i, and Hawai‘i Island, with more detailed distribution studies in Kāne‘ohe Bay and Waikīkī on O‘ahu, and the south shore of Moloka‘i (Rodgers & Cox, 1999; Smith *et al.*, 2004; Nelson *et al.*, 2009). Rodgers and Cox (1999) reported an ability to spread at the rate of 260-280 m yr⁻¹, and Conklin (2007) noted the ability of mats to drift in the current and establish in new areas or recolonize removal sites.

Smith *et al.* (2004) provide a broad overview of the ecology of *G. salicornia* in Waikīkī, Hawai‘i, reporting 5.2 kg m⁻² wet weight as the average biomass per area and an apical growth
rate of 0.04 g g\(^{-1}\) d\(^{-1}\) or 10.8\% per day in the field. The apical growth rate reported is much higher than found for \textit{in situ} field mats from Molokaʻi (0.03\% - 1.28\% per day, Nelson \textit{et al.}, 2009), likely associated with field conditions and self-shading. Nelson \textit{et al.} (2009) also corroborate the standing biomass per area, reporting the average biomass on reefs in Molokaʻi as approximately 5.6 kg m\(^{-2}\) wet weight. The alga’s resilience to extreme salinity and temperature variation was shown by both Smith \textit{et al.} (2004) and Phooprong, Ogawa & Hayashizaki (2007), with the ability to survive a one hour exposure to 0-50 ppt salinity and 8-37 °C. \textit{G. salicornia} can live in many different light environments, and changes pigment concentrations to photoacclimate, giving thalli diverse colors (Beach \textit{et al.} 1997). Yellow-orange tissue in the canopy of mounds, green tissue in the middle, and dark red-purple tissue in the understory signifies acclimation to high, intermediate, and low irradiance exposure, respectively (Beach \textit{et al.} 1997).

The resilience exhibited by \textit{G. salicornia} has hindered management efforts for control and removal. In a study of the efficacy of control techniques, use of temperature, salinity, desiccation, and chemical management techniques were ultimately dismissed as viable options out of concern for collateral damage to other reef species (Smith \textit{et al.}, 2004). Manual removal is a feasible but labor-intensive control option: 2000 person-hours divided among 400 volunteers to remove 20,000 kg of algae (Smith \textit{et al.}, 2004). Several studies have looked at herbivore grazing as a possible control strategy, with mixed results. Herbivorous fishes were shown to graze on the algae but when given a choice generally prefer other algal species (Smith \textit{et al.}, 2004; Conklin, 2007; Okano, 2010). Marine managers have found that one effective management technique is the rearing and out-planting of a native sea urchin \textit{Tripneustes gratilla} as a grazer for controlling algal growth (Stimson, Cunha & Philippoff, 2007; Westbrook \textit{et al.}, 2015; Neilson \textit{et al.}, in prep). This technique has shown strong success in Kāneʻohe Bay, where marine managers have
focused their efforts on removing the invasive species of *Eucheuma* and *Kappaphycus* through manual removal with aide of a boat-operated vacuum hose, yet have additionally decreased the percent cover of *G. salicornia* by out-planting urchins (Neilson *et al.*, in prep).

Recently, in March 2017, scientists observed several green sea turtles (*Chelonia mydas*) in the back lagoon of Moku o Loʻe island in Kāneʻohe Bay consuming large amounts of *G. salicornia* (Bahr *et al.*, in prep). According to visual benthic surveys, *C. mydas* consumed 41% of *G. salicornia* across 32 sites around the island, with 99% consumption in some areas (Bahr *et al.*, in prep). Although *G. salicornia* has been recorded as a significant part of the *C. mydas* diet in Kāneʻohe Bay (Russell & Balazs, 2009), *C. mydas* had not been observed in this area for 25 years, despite the establishment of *G. salicornia* beds in the back lagoon of Moku O Loʻe circa 1996 – long after the initial introduction of that alga (Rodgers & Cox, 1999; Bahr *et al.*, in prep.). An increase in nesting females during 2013 may have increased recruitment, which may in turn be the reason for the voracious foraging turtles observed here and another means of top-down control of *G. salicornia* (Bahr *et al.*, in prep).

**Climate change and macroalgae**

Because corals and macroalgae compete for space and possibly other resources, the deterioration of coral condition could result in a large-scale phase shift toward macroalgal dominated ecosystems (Hoegh-Guldberg *et al.*, 2007; Hughes *et al.*, 2007; Koch *et al.*, 2013; Kroeker, Micheli & Gambi, 2013). For example, a decrease in coral cover and recruitment, and an increase in coral mortality were linked to a macroalgal phase shift after an intense bleaching event on the Great Barrier Reef (Hughes *et al.*, 2007). Sites examined close to a naturally occurring CO₂ vent showed increased cover by fleshy macroalgae, compared to coral-dominated control sites (Enochs *et al.*, 2015). Compared to the wealth of studies conducted on corals under
climate change scenarios, there are relatively few studies documenting macroalgal responses to any of the global change parameters (Gao, Helbling & Häder, 2012; Harley et al., 2012; Koch et al., 2013; Ji et al., 2016). Future marine resource managers may be responsible for more algal-dominated ecosystems. Thus, it becomes increasingly important to know how these ecosystems will operate under conditions that reflect the changes that are underway.

At a local level, understanding the potential impacts of climate change on *G. salicornia* and other invasive algae could help us to predict some of the changes that are quickly manifesting in our ecosystem. Hawai‘i’s coral reef ecosystems protect the shorelines, support ecosystem diversity, help feed Hawai‘i’s people, and provide economic benefits by promoting tourism (Moberg & Folke, 1999; Cesar & van Beukering, 2004). Confronted with the loss of this significant resource, it is important to know how a major competitor - an invasive macroalga - will fare under these changing conditions. The following sections summarize the effects of increased temperature and increased pCO₂ on macroalgae, with emphasis on species in the genus *Gracilaria*.

**Temperature and macroalgae**

Many studies concerning the effects of temperature on algae focus on the immediate reaction of algae to different temperatures rather than on long term effects. For example, many studies explore Q₁₀ values, or the rate of a process (such as photosynthesis, respiration, or growth) at T₁ °C divided by the rate of the same process at T₁ - 10°C (Davison, 1991). These studies generally grow the algae at an ambient temperature, and then expose the algae to various temperature regimes for a short period of time to see how the algae react to sudden and extreme changes. Q₁₀ studies across terrestrial and marine botany generate physiological performance curves, with lowered performance at temperature extremes and a bell-shaped curve with maxima
at the species-specific optimal temperature (Laing, 2012; Harley et al., 2012). Though $Q_{10}$
studies are clearly informative, they should be approached with caution when estimating the
effects of gradual global warming of sea surface temperatures. Short-term (minutes to hours)
response of algae to temperature change is often contradictory to long-term (days to weeks)
response (Li, 1980; Davison, 1991). Thus, experiments looking to mimic the effects of climate
change should allow time for the organism to acclimate to treatment conditions.

Determining the long-term (days to weeks) effects of temperature change is more
challenging as it consumes more time and resources. However, studies that allow an acclimation
period are more representative of how organisms will react to such changes over a longer period
(Koch et al., 2013). Unfortunately, there is very little information on the length of time needed
for algae to acclimate to temperature treatment conditions (Davison, 1991). Many algal studies
looking at long-term effects of temperature will acclimate samples to treatments for several
weeks, but there is evidence that acclimation begins within hours of exposure to a temperature
change (Davison, 1991). Multi-week acclimation periods in manipulative experiments can be
problematic in climate change research for experimental design reasons. Climate change
researchers often apply treatments using large header chambers that flow into individual tanks.
To avoid pseudo replication, these experiments should be replicated several times to determine if
any observed effects are a result of treatment conditions or the processes taking place in a mixing
chamber (Wernberg, Smale & Thomsen, 2012). Including a multi-week acclimation period into
each experimental trial limits the number of trials that can be performed in a specific amount of
time, and therefore experimental runs need to be planned accordingly.

A few patterns emerge from long-term studies that have either included a temperature
acclimation period or grown the plant samples at the treatment temperature. When comparing
algal samples of the same species grown at a range of different temperatures, those grown at lower temperatures had higher maximum photosynthetic rates (Davison, 1991). Regardless of the temperature at which the algae were grown, the optimal temperature for photosynthesis was usually higher than the optimal temperature for growth, which highlights the fact that one process cannot be used as a direct proxy for the other in temperature experiments (Li, 1980; Lapointe, Tenore & Dawes, 1984; Davison, 1991; Koch et al., 2013). Li (1980) describes growth in phytoplankton as “the integrated expression of cellular processes,” meaning growth rates depend not only on photosynthetic rates, but also on other factors such as respiration and decay. Therefore, discrepancies in optimal temperature values for growth and photosynthesis may reflect an increased cost in respiration and excretion at higher temperatures (Li, 1980; Lapointe, Tenore & Dawes, 1984; Robarts & Zohary, 1987). In manipulative experiments with phytoplankton grown for several generations under different temperatures, the temperature for maximum photosynthetic rate was usually several degrees higher than the temperature at which phytoplankton were grown (Li, 1980). The relationship between respiration and temperature is not well studied in macroalgae, although research indicates that increases in temperature correlate with increases in dark respiration and photorespiration (Lapointe, Tenore & Dawes, 1984; Koch et al., 2013). Several studies on cyanobacteria suggest an exponential relationship between water temperature and respiration rates (reviewed in Robarts & Zohary, 1987).

**Carbon dioxide and macroalgae**

Inorganic carbon in aquatic habitats exists in three principle forms: carbon dioxide (CO₂), bicarbonate (HCO₃⁻), and carbonate (CO₃²⁻). These carbon compounds exist in equilibrium with each other according to Equations 1.1 and 1.2 (Dickson, Sabine, & Christian, 2007). In seawater at normal pH (8.0-8.2), the concentration of CO₂ is approximately 10 µM, which is low
compared to the 2000 µM concentration of HCO$_3^−$. Adding CO$_2$ to seawater acidifies the water by increasing the concentration of dissolved hydrogen ions (H$^+$), lowering the pH. Equation 1.1 indicates that seawater with higher pH values will have a higher ratio of [HCO$_3^−$] to [CO$_2$], whereas seawater with lower pH values will have a lower ratio, favoring CO$_2$. Understanding the ways these carbon compounds interact is necessary for interpreting how chemistry changes can affect life processes.

Equation 1.1: $\text{CO}_2(\text{aq}) + \text{H}_2\text{O} \leftrightarrow \text{HCO}_3^−(\text{aq}) + \text{H}^+(\text{aq})$

Equation 1.2: $\text{HCO}_3^−(\text{aq}) \leftrightarrow \text{CO}_3^{2−}(\text{aq}) + \text{H}^+(\text{aq})$

Carbon dioxide availability is integral to all plant photosynthesis. In the balanced equation of photosynthesis, carbon dioxide combines with water and solar energy within plant cells to produce glucose. For some algae, the amount of dissolved inorganic carbon (DIC) available in seawater is not enough to maximize growth; these plants are termed “carbon limited” (Koch et al., 2013). Although CO$_2$ is the carbon compounds necessary for photosynthesis, some algae have evolved ways to use the more abundant compound, HCO$_3^−$ (Koch et al., 2013; Raven & Beardall, 2014). For example, many macroalgae use the enzyme carbonic anhydrase (CA) to convert HCO$_3^−$ into CO$_2$, which then passively diffuses into algal cells for photosynthetic use (Koch et al., 2013; Raven & Beardall, 2014). Changes in the concentrations of seawater carbon molecules may have widespread, species-specific effects depending on how different algae take in carbon for photosynthesis and whether they are carbon limited at current DIC levels.

There is a wealth of research on how increased carbon concentrations affect macroalgae but it is questionable how much of this research is applicable to global climate change scenarios.
Most of the research on this subject conducted before the year 2000 focused on inorganic carbon as a nutrient. Manipulative experiments on macroalgae used treatments of enhanced CO$_2$ or HCO$_3^-$ to determine the effects of increased DIC as a fertilizer for algal growth, but most of these treatments exponentially exceeded the predicted pCO$_2$ under climate change scenarios (e.g. DeBusk & Ryther, 1984; García-Sanchez, Fernández & Niell, 1994). Other experiments analyzed the short-term effects (minutes to hours) of exposure to high pCO$_2$ conditions (e.g. Haglund et al., 1992; Rivers & Peckol, 1995). Because of their short time scales and extreme carbon concentrations, both of these methodologies should be reviewed with caution when attempting to assess how long-term and gradual increases of pCO$_2$ associated with global climate change will affect macroalgae.

Several studies have examined the effect of pH and inorganic carbon concentration on members of red algal genus *Gracilaria*. For at least three species (*G. tikvahiae*, *G. secundata*, and *G. lemaneiformis*), increasing the amount of available carbon, through CO$_2$ bubbling or NaHCO$_3$ supplements, and maintaining a pH between 7.85-8.00 led to increases in growth rates (DeBusk & Ryther, 1984; Lignell & Pedersén, 1989; Xu, Zou & Gao, 2010). Growth rates sharply declined at pH values above 8.6 and gradually declined at pH values below 7.8, regardless of carbon abundance (DeBusk & Ryther, 1984; Lignell & Pedersén, 1989). Multiple species of *Gracilaria*, including *G. lemaneiformis*, *G. secundata*, *G. tenuistipitata*, and *G. tikvahiae*, have shown the capacity to use HCO$_3^-$ as a carbon source for photosynthesis through the use of CA. These species however do show a higher affinity for CO$_2$ (Lignell & Pedersén, 1989; Haglund et al., 1992; García-Sanchéz, Fernández, & Niell, 1994; Xu, Zou & Gao, 2010). One study found lowered growth rates, photosynthetic rates, pigment content, and Rubisco content in *G. tenuistipitata* grown at high DIC concentrations; however, the treatments used were
50x the highest predicted levels of CO₂ under global climate change scenarios (Garcia-Sanchéz, Fernández, & Niell, 1994).

Research oriented specifically toward climate change scenarios with macroalgae have shown a positive, neutral, or negative relationship between pCO₂ and growth/photosynthesis for an alga, based on species differences (Table 1.1; Gao, Helbling & Häder, 2012; Harley et al., 2012; Koch et al., 2013; Johnson, Price & Smith, 2014; Ji et al. 2016). No studies have been conducted on G. salicornia specifically, and few studies have looked at the interaction between both pCO₂ and temperature on the photosynthesis and respiration of a fleshy macroalgae.

Proposal

This research will examine the effects of increased temperature and increased pCO₂ on the respiration and photosynthetic rates of G. salicornia. The amount of increase in temperature and pCO₂ will be set by climate change projection models for the year 2100. Response variables will be analyzed for interactive effects between temperature and pCO₂. The experiment will be carried out in outdoor, flow-through aquaria to best replicate natural light conditions, day/night period, salinity, ambient temperature, ambient pH and water chemistry, and environmental variation.
Table 1.1 Literature summary: effect of global climate change CO₂ levels on fleshy rhodophytes

<table>
<thead>
<tr>
<th>Reference</th>
<th>Species</th>
<th>Irradiance source</th>
<th>Experiment type</th>
<th>Nutrients added</th>
<th>Variables measured</th>
<th>Effects of elevated CO₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Israel &amp; Hophy (2002)</td>
<td><em>Hypnea cornuta</em></td>
<td>Natural sunlight</td>
<td>Natural field setting</td>
<td>No</td>
<td>Growth</td>
<td>negative</td>
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<tr>
<td></td>
<td><em>Hypnea musciformis</em></td>
<td>Unspecified</td>
<td>Tanks with aeration</td>
<td>Yes</td>
<td>Growth</td>
<td>neutral</td>
</tr>
<tr>
<td></td>
<td><em>Porphyra sp.</em></td>
<td>Unspecified</td>
<td>Tanks with aeration</td>
<td>Yes</td>
<td>Growth</td>
<td>neutral</td>
</tr>
<tr>
<td></td>
<td><em>Pterocladia capillacea</em></td>
<td>Unspecified</td>
<td>Tanks with aeration</td>
<td>Yes</td>
<td>Growth</td>
<td>neutral</td>
</tr>
<tr>
<td></td>
<td><em>Pterocladia capillacea</em></td>
<td>Natural sunlight</td>
<td>Natural field setting</td>
<td>No</td>
<td>Growth</td>
<td>neutral</td>
</tr>
<tr>
<td></td>
<td><em>Solieria sp.</em></td>
<td>Unspecified</td>
<td>Tanks with aeration</td>
<td>Yes</td>
<td>Growth</td>
<td>neutral</td>
</tr>
<tr>
<td>Johnson, Price &amp; Smith (2014)</td>
<td><em>Acanthophora spicifera</em></td>
<td>Natural sunlight with shade cloth: to simulate 5 m ocean depth</td>
<td>Tanks with aeration</td>
<td>No</td>
<td>Growth</td>
<td>neutral</td>
</tr>
<tr>
<td></td>
<td><em>Hypnea pannosa</em></td>
<td>Natural sunlight with shade cloth: to simulate 5 m ocean depth</td>
<td>Tanks with aeration</td>
<td>No</td>
<td>Growth</td>
<td>positive</td>
</tr>
<tr>
<td>Kram <em>et al.</em>, (2016)</td>
<td><em>Plocamium cartilagineum</em></td>
<td>Full-spectrum fluorescent bulbs</td>
<td>Flow-through tanks with aeration</td>
<td>No</td>
<td>Growth</td>
<td>positive</td>
</tr>
<tr>
<td>Ho &amp; Carpenter (2017)</td>
<td><em>Amansia rhodantha</em></td>
<td>Natural sunlight with fiberglass covers: attenuation to simulate back reef (&lt;3 m depth)</td>
<td>Flow-through flumes</td>
<td>No</td>
<td>Growth</td>
<td>neutral</td>
</tr>
</tbody>
</table>
Literature Cited


Bahr KD, Coffey DM, Rodgers K, Balazs GH. In prep. Green sea turtles on the move for food: *Honu* in Hawai‘i grazing their way into new habitats.


Chapter 2: The effects of increased temperature and pCO$_2$ on respiration and photosynthetic rates of *Gracilaria salicornia*

Introduction

With the warming and acidification of the oceans, tropical coral reef ecosystems are expected to change dramatically over the next decades (Hoegh-Guldberg *et al.*, 2007; Pörtner *et al.*, 2014). Warmer sea surface temperatures are directly linked to large-scale coral bleaching events and increased pCO$_2$ in the ocean is disrupting the ability to build strong calcified skeletons (Jokiel & Brown, 2004; Hoegh-Guldberg, 1999; Hoegh-Guldberg *et al.*, 2007; Doney *et al.*, 2009; Bahr, Jokiel & Rodgers, 2015; Rodgers *et al.*, 2017). Within the past three years, a worldwide series of bleaching events have caused damage to corals in many areas including Australia (Hughes *et al.*, 2017), Asia (Ampou *et al.*, 2017; DeCarlo *et al.*, 2017), and in the Hawaiian Islands (Bahr, Jokiel & Rodgers, 2015; Rodgers *et al.*, 2017). Beyond corals, other calcifying organisms, such as mollusks, echinoderms, crustaceans, coralline algae, and coccolithophores, will be affected by the chemical changes inherent in ocean acidification to varying degrees (Kroeker *et al.*, 2013; Pörtner *et al.*, 2014). These major environmental changes are predicted to devastate much of the world’s remaining coral reefs (Hoegh-Guldberg *et al.*, 2007).

Corals and fleshy macroalgae live in a balance dictated in part by competition. They compete for shared resources including space and sunlight (McCook, Jompa & Diaz-Pulido, 2001). In the aftermath of a bleaching event, Australian researchers found that macroalgae overgrew corals and inhibited coral recovery when herbivorous fish were excluded, demonstrating the potential for macroalgal phase shifts in overfished areas after a disturbance event (Hughes *et al.*, 2007). Surveys of sites along a naturally occurring CO$_2$ gradient near a
volcanic vent demonstrated a shift from high coral cover in ambient seawater to algal dominance (macroalgae and turf algae) in more acidified zones (Enarchs et al., 2015). If the next century brings major competitive disadvantages for corals, macroalgae may outcompete corals and become dominant on many reefs (Hughes et al., 2007; Hoegh-Guldberg et al., 2007; Porzio, Buia & Hall-Spencer, 2011; Koch et al., 2013; Enoch et al., 2015).

Compared to corals, there is relatively little known about how fleshy macroalgae will respond to climate change (Wernberg, Smale & Thomsen, 2012). Of marine climate change research conducted between years 2000-2009, only 11% focused on macroalgae, compared to over 70% that focused on benthic invertebrates including coral (Wernberg, Smale & Thomsen, 2012). Within the category of macroalgae, focus has been given to calcifying algae including coralline algae, and results have generally shown reduced growth and reduced abundance in high pCO$_2$ environments (Koch et al., 2013; Kroeker et al., 2013; Kroeker, Micheli & Gambi, 2013; Johnson, Price & Smith, 2014; Kram et al., 2016) There are fewer studies and less clear results regarding climate change and fleshy macroalgae. From short-term manipulative pCO$_2$ experiments, researchers have found that a small percentage of fleshy macroalgae show an increase in growth rates under high pCO$_2$ conditions, a few show decreased growth rates, but the majority show no significant response (e.g. Israel & Hophy, 2002; Diaz-Pulido et al., 2011; Johnson, Price & Smith, 2014; Kram et al., 2016; Ho & Carpenter, 2017). The long-term effect of temperature on algal growth is defined by individual optimal growth temperatures and temperature threshold limits, although how these will change with acclimation and adaptation is not well studied (Harley et al., 2012). Our lack of knowledge makes it difficult to predict how macroalgal communities will react to climate change, which is concerning because macroalgae may become the dominant on reefs.
In the Hawaiian Islands, overgrowth by invasive macroalgae has been a problem since the late 1970s, if not earlier (Russell, 1992). At least 19 species of foreign marine macroalgae have been transported purposefully or accidentally to the Hawaiian Islands since the 1950s and some of these algae have shown the capacity for rapid growth and spread (Russell, 1992; Smith, Hunter & Smith, 2002). There are financial, ecological, and social consequences of invasive algal blooms including loss of property value, clean-up and management costs (Cesar et al. 2002), competition for native algal niches (Russell, 1992), impacts to native gathering practices, modification of the benthic environment, increased stress to corals (Martinez, Smith & Richmond, 2012), and changes in invertebrate abundances (Fukunaga, Peyton & Thomas, 2014).

One of the most invasive species in Hawai‘i is the rhodophyte *Gracilaria salicornia* (C. Ag.) Dawson (Smith, Hunter & Smith, 2002; Godwin, Rodgers & Jokiel, 2006). The success of *G. salicornia* in Hawai‘i stems in part from the alga’s resilience; it is capable of growing on hard substrates, able to acclimate to diurnal and seasonal ranges of temperatures, salinities, and light levels found in the Hawaiian Islands, as well as survive hour-long periods of desiccation (Beach et al., 1997; Smith et al., 2004; Phooprong, Ogawa & Hayashizaki, 2007; Martinez, Smith & Richmond, 2012). *G. salicornia* is able to spread quickly at the rate of 260-280 m y⁻¹ and has established populations around the islands of O‘ahu, Moloka‘i, and Hawai‘i Island, with more detailed distribution studies in Kāne‘ohe Bay and Waikīkī on O‘ahu, and the south shore of Moloka‘i (Rodgers & Cox, 1999; Smith et al., 2004; Nelson et al., 2009). Once established, it grows quickly at the rate of 10.8% per day, covering the substrate in large mounds amounting to over 5.2 kg m⁻² wet weight in densely covered areas (Smith et al. 2004). To this date, no studies have been conducted to see how *G. salicornia* will fare under climate change conditions.
Within the limited research on macroalgae, there is a need for experiments that (1) mimic daily cycles and the large environmental variance found in the tropical coral reef ecosystem, and (2) examine pCO₂ and temperature for interactive effects (Gao, Helbling & Häder, 2012; Koch et al., 2013). This experiment addresses these knowledge gaps by examining the individual and interactive effects of temperature and pCO₂ on algal metabolism in *G. salicornia*. The response variables measured were dark respiration rate via oxygen evolution methods, and the rate of photosynthesis via fluorometric analysis. Outdoor, continuous-flow seawater systems were used in this experiment to capture the environmental variability inherent in natural ecosystems (Jokiel, Bahr & Rodgers, 2014).

**Methods**

**Experimental design**

Tissue samples of *Gracilaria salicornia* (C. Agardh) E. Y. Dawson (~4 g wet weight) were collected from a ~60 m² collection location near the Coral Reef Ecology Lab on Moku o Lo‘e, O‘ahu. To encompass genetic variability, all samples were collected at least 1 m apart. All samples were collected between 0.5-1.5 m depth from healthy, ungrazed tissue of uniform green-gray color. Tissues were hand-cleaned of all macroscopic sediment, epiphytes, and epifauna using soft brushes and metal fine point picks.

Tissues were transported to Ānuenue Fisheries Research Center (AFRC) in seawater-filled, sealed plastic bags within a cooler with ice packs. They were placed in aquaria filled with 5 µm filtered seawater with manipulated pH and temperature, detailed below. On the day of collection, maximum electron transfer rates (ETR<sub>max</sub>) were measured for each collected tissue using a Pulse Amplitude Moderated Fluorometer (Junior PAM, Walz, Germany). The eight most-similar ETR<sub>max</sub> readings were used to choose the tissue samples experimental trial, to
lessen the variance from sun/shade acclimation states. Excess tissue samples were discarded. A total of thirty-two tissue samples were used during the four experimental trials.

In the aquarium system, there were two levels of pH: ambient and acidified (~ -0.2 pH units from normal seawater) and two levels of temperature: ambient and heated (~ +1°C from normal seawater) in a full factorial design (Figures 2.1 and 2.2). The increased temperature and acidification simulate potential climate conditions in the year 2100, following the International Panel on Climate Change 2014 report (Pörtner et al., 2014). pH was adjusted by bubbling CO₂ gas into header tanks and a temperature difference was maintained using titanium water heaters submerged in the header tanks, following Jokiel, Bahr & Rodgers (2014), to maintain consistency in all aquaria. Tissues were given approximately 52 h to acclimate to treatment conditions prior to initial measurements of response variables (Lignell & Pedersén, 1989).

Each experimental trial ran for four days from initial collection and exposure to treatment conditions to the final measurements. Subsequently, all tissues were dried until stable dry-weight was achieved (five days). Four experimental trials were conducted between October and December of 2016 (Table 2.1).

Respiration measurements were taken 52 h after placement into treatment condition. These measurements were conducted at night between 21:00-00:00 h. To measure respiration, each sample was placed in a double-walled, lidded, metal thermos container with 400 mL of filtered seawater from its corresponding aquarium. Initial dissolved oxygen (DO) readings were conducted, containers were sealed, and a laboratory rotator (Laboratory Rotator Model G2, New Brunswick Scientific Co. Inc., New Brunswick, NJ, USA) was used to gently agitate the samples over a 1 h period. The laboratory rotator was set at its lowest setting (40 rpm) to disrupt the boundary layer around the tissue sample for realistic gas exchange rates while minimizing
mixing at the air-water interface. 1 h after initial DO readings, final DO readings were taken. All DO measurements were recorded using a YSI 556 Multiprobe System (YSI Inc., Yellow Springs, OH, USA) with randomized sampling order. Four control containers containing only seawater were included, to account for the interchange of oxygen between the air and water during the hour-long interval. Following respiration measurements all tissue samples were returned to their original tanks.

The third morning after sample collection, rapid light curves were generated fluorometrically (Junior PAM, Walz Germany) between 10:00-11:00 h to measure photosynthetic rates. Samples were removed from aquaria and placed in plastic containers in a uniform partial-light environment for approximately 10 min before initiating PAM measurements, individually, in random order. Once completed, all samples were dried in a drying oven at 60°C for five days and weighed. Dry weight was used to normalize respiration rates.

Through the duration of the experiment, the temperature, salinity, total alkalinity (TA), light, and pH in the aquaria were regularly monitored (Table 2.2 and 2.3) to assure consistency between trials and calculate pCO₂ using the CO₂sys software program (Pierrot, Lewis & Wallace, 2006). Temperature and light measurements were recorded every 30 min in each aquarium for the duration of the trial (HOBO Pendant Data Loggers, Onset, MA, USA). Several summary statistics of temperature (Table 2.4) were used to generate models in JMP software and results were compared to determine which would be most informative. The most useful metric for temperature was the mean temperature value from 2300-0200 h (“mean mid-night temp”), likely from low variance (Table 2.4).
To measure photosynthetically active radiation (PAR), a LI-COR Spherical Underwater Quantum Sensor (Lincoln, NE, USA) was deployed in the water bath holding the aquaria, at the same depth as the algal tissue. The sensor was attached to a data logger (LI-1400, LI-COR, Lincoln, NE, USA) that measured PAR every 30 min from 0630-1900 h for the duration of the trial. Due to researcher error, only two of the four trials were measured for PAR.

Salinity was measured in each aquarium twice per trial (YSI 556 Multiprobe System, calibrated with YSI Conductivity Calibrator Solution #3169).

pH was determined for each aquarium with a spectrophotometer (Molecular Devices SpectraMax M2, CA, USA) at the Hawai‘i Institute of Marine Biology (HIMB) twice per trial within three hours of seawater sample collection, using meta-cresol purple dye (Acrōs Organics, Thermo Fisher Scientific, Belgium) as an indicator (Clayton & Byrne, 1993, in Dickson, Sabine & Christian, 2007). For a detailed description of the spectrophotometric analysis methodology, see Appendix A.

For each aquarium, TA titrations were conducted using an automatic titrator (Titrino Plus 887, Metrohm AG, Switzerland, calibrated with YSI pH 4.00 and Oaklon pH 7.00 buffer solutions) at HIMB within 48 hours of seawater sample collection. For a detailed description of the alkalinity titration procedures, see Appendix B.

Respiration was calculated as oxygen consumed and converted to µmol O₂ g dw⁻¹ h⁻¹. PAR exposure for each individual tank was calculated by experimentally deriving a site-specific equation to convert lux (HOBO logger unit of measurement) to PAR µmol photons m⁻² s⁻¹ (Appendix C). All logger light measurements were subsequently converted to PAR. Two calculations were employed to calculate PAR: (1) an average value of light measured between 0700-1730, and (2) the last PAR measurement taken before tissues were analyzed with Junior
PAM. pH values obtained by spectrophotometric analysis were converted to pCO$_2$ values using the CO$_2$sys software for Microsoft Excel (Pierrot, Lewis & Wallace, 2006).

**Statistical Analysis**

This study used a full-factorial, blocked design with randomly assigned treatments. Initially, the experimental unit was the header, the location of treatment assignment. Because each header fed two tanks, each containing individual thalli, these individuals could not be considered independent observations. However, during the analysis, headers were not shown to have a significant effect on any of the response variables (Tables 2.6-2.8), allowing individual tanks to be treated as experimental units. Thus, 32 samples were spread equally among four treatments, yielding a sample size of eight (n=8). Two samples died during the experiment and were excluded from analysis.

The experiment was blocked by week-long experimental trials. The eight tanks had fixed locations, but treatments were randomly assigned among the tanks at the start of each trial. Algae were haphazardly distributed into tanks after collection and cleaning. The order of respiration and PAM measurements was randomized for each trial. The order of salinity, temperature, pH, and total alkalinity measurements was not randomized.

Although an ANOVA had been planned for data analysis, seasonal changes in the ambient temperature of the seawater over the course of the experimental period led to an inability to maintain statistically significant temperature categories in the aquaria. As a result, generalized linear models were used to analyze data. All measured factors were treated as continuous variables and “Header” and “Tank” were evaluated as categorical variables. The three response variables measured and analyzed were respiration, maximum electron transport rate (ETR$_{max}$), and the minimum saturating irradiance (E$_k$). Each response variable was checked
for normality, homoscedasticity, and multivariate normality using graphical analysis of histograms and residual plots. For each response variable, a whole model was created which contained “Header” and “Tank” (nested in “Treatment”) to ensure that neither header nor tank had a significant effect.

During the course of the experiment, mortality occurred in two samples. The first sample death may be linked to water stagnation from equipment failure within one aquarium. The second tissue died from unknown circumstances, despite the fact that all the parameters it was exposed to (temperature, pH, light) were duplicated in an adjacent tank and received water from the same header tank. It was therefore assumed that the mortality was not associated with factors being tested, but instead a factor inherent to the tissue itself (e.g. history prior to collection, recent injury). Both samples were excluded from all statistical analyses.

This study aimed to constrain pCO$_2$ levels at or below 940 µatm, the upper limit projected for year 2100 (Pörtner et al., 2014). pCO$_2$ is difficult to measure continuously, thus pH was measured as a proxy for pCO$_2$, aiming to keep the pH values in the CO$_2$ treatments within 0.40 pH units from the pH of ambient seawater. However, three tissue samples experienced conditions outside of the established range (pCO$_2$ values of 1035, 1062, and 1232 µatm; pH values of 7.67, 7.66, and 7.61). Though these pCO$_2$ values were higher than desired, these values were retained in the analysis. *G. salicornia* is a dense, mound-forming alga capable of weaving thalli together so tightly that less than 1% of ambient irradiance penetrates to the bottom of the mound (Beach et al., 1997; Martinez, Smith & Richmond, 2012). Within these mounds, thalli experience conditions different from ambient seawater, including significantly lower pH (Martinez, Smith & Richmond, 2012). Martinez and colleagues report a minimum pH value of 7.47 within a *G. salicornia* mound (Martinez, Smith & Richmond, 2012). If pH values within
mounds of *G. salicornia* are significantly lower than ambient seawater values, data that fall within these pH ranges are still considered ecologically significant.

**Results**

**Respiration**

Pooled respiration rates ranged from -5.03 to 15.89 μmol O₂ h⁻¹ g⁻¹ DW, with an average value of 6.64 and standard deviation 5.79 μmol O₂ h⁻¹ g⁻¹ DW. Respiration rates appeared to be influenced most strongly by the average mid-night temperatures and the square of the average mid-night temperatures. The complete model (Table 2.5) indicated that pCO₂ values did not have a significant effect on respiration values.

**Photosynthesis: ETR₁₀₀₀ and Eₖ**

Pooled ETR₁₀₀₀ values ranged from 24.72 to 85.40 μmol e⁻ m⁻² s⁻¹ (Table 2.6). The effects of mean mid-night temperature, pCO₂, and PAR (average between 0700-1730 h) were examined. The only factor in this model that had a significant effect was the pCO₂ treatment (Table 2.7). However, upon examining the residuals, three outlier data points were determined to have strong influence over the model (Figures 2.3 and 2.4). These outliers were extreme ETR₁₀₀₀ values at extreme pCO₂ values and were included in the analysis, but it should be noted that when excluded, the pCO₂ factor ceased to be significant.

Pooled Eₖ values ranged from 164.1 to 715.1 μ mol photons m⁻² s⁻¹ (Table 2.6). The statistical model showed that the categorical variable “tank” (nested under “treatment”) had a significant effect on Eₖ (Table 2.8). Therefore, whether the experimental treatments conditions or the confounding factor of “tank” was the dominant factor explaining Eₖ cannot be differentiated. Effect tests indicated that mean midnight temperature and the PAR measurement taken directly before Junior PAM measurements were significant factors. Data tables are included for reference.
purposes, however, effects of treatment conditions cannot be determined as significant because of the significance of the tank as a confounding factor.

**Discussion**

Respiration and photosynthesis are two metabolic processes that contribute to the overall growth rate of a plant. From a mass balance perspective, Equation 2.1 (Carr, Duthie & Taylor, 1997) gives a simple model of plant growth:

Equation 2.1: \[ S = P - R - L \]

In this equation, \( S \) represents the change in standing stock of the plant (plant growth) as a function of the accumulation of biomass through gross photosynthesis (\( P \)), the loss of biomass through respiration (\( R \)) and other losses (\( L \)) (Carr, Duthie & Taylor, 1997). From this straightforward model, we can see that increasing photosynthesis contributes to positive growth and increasing respiration contributes to reductions in growth. Understanding how different climate change factors will affect these processes in *G. salicornia* can inform predictions of how successful this invasive alga will be in the next century.

**Comparing results with other studies**

Rates of respiration measured here were pooled and compared with those reported in Phooprong, Ogawa & Hayashizaki (2007). Respiration rates were measured for *G. salicornia* samples from three different locations (Okinawa, Japan; Rayong, Thailand; Phuket, Thailand) across a gradient of temperatures (Phooprong, Ogawa & Hayashizaki, 2007). Respiration rates from treatments at 25 °C and 30 °C, the temperatures closest to those in this study, are included in Figure 2.5 for comparison. Similar to the study presented here, temperature had a significant, positive effect on respiration rates (Phooprong, Ogawa & Hayashizaki, 2007). The respiration
rates found in this study were lower than the respiration rates found by Phooprong et al., which may be tied to methodological differences. In this study, respiration rates were measured at night, several hours after any light exposure. In contrast, Phooprong et al. exposed plants to 15 min of darkness (zero irradiance for respiration measurements) before initiating DO measurements (Phooprong, Ogawa & Hayashizaki, 2007). A minimum of 30 min is considered an appropriate darkness interval to avoid increased respiration rates due to the phenomenon of light enhanced dark respiration - a period of elevated respiration rates directly following exposure to light (Florez-Sarasa et al., 2012). Thus, higher respiration rates measured by Phooprong et al. may be attributable to light enhanced dark respiration resulting from an abbreviated dark period.

ETR_{\text{max}} values and E_k values from this study were pooled and compared with those from two previous experiments: Spafford, 2011 and Kawachi et al., in prep. (Figures 2.6 and 2.7). Spafford’s research examined photosynthetic parameters for G. salicornia at different photoacclimation states (high irradiance and low irradiance). The tissues collected by Spafford were orange (high irradiance acclimation) or red (low irradiance acclimation) expressing different pigment concentrations based on photoacclimation state (Beach et al., 1997; Spafford, 2011). The tissue collected in this study and by Kawachi et al. was photoacclimated to intermediate irradiance and green in color (Kawachi M, pers. comm., 2017). These distinct photoacclimation states influence ETR_{\text{max}} and E_k values, with green tissue demonstrating the highest values and red tissue at the lowest values for both variables.

This result disagrees with earlier findings by Beach et al. (1997), comparing oxygen evolution light curves with in-vivo absorbance spectra in G. salicornia tissue acclimated to low irradiance (red color), intermediate irradiance (green color), and high irradiance (orange color).
For reference, $P_{\text{max}}$ and $I_k$ are conceptually similar to $\text{ETR}_{\text{max}}$ and $E_k$, respectively, but the first pair is used to describe data obtained through oxygen evolution and the latter pair is used with data obtained through fluorometry. Maximum photosynthesis rate ($P_{\text{max}}$) values were negatively correlated with in-vivo absorbance peaks at wavelength 568 nm (associated with R-phycoerythrin) (Beach et al. 1997). Minimum saturating irradiance ($I_k$) values were positively correlated with peaks at wavelength 495 nm (associated with R-phycoerythrin and carotenoids). Red tissue was associated with a high peak at 568 nm and low $P_{\text{max}}$, and a low peak at 495 nm and low $I_k$. Orange tissue showed the opposite tendencies: a low peak at 568 and high $P_{\text{max}}$, and a high peak at 495 and high $I_k$. Green tissue peaks were comparatively intermediate, indicating that $P_{\text{max}}$ and $I_k$ should be intermediate values relative to values from red and orange tissue. In this study, however, green tissue showed distinctly higher $\text{ETR}_{\text{max}}$ and $E_k$ values compared to red and orange tissue.

Temperature

$Q_{10}$ studies posit that the rate of a process, such as photosynthesis, will increase with increasing temperature until an optimal thermal temperature is reached, after which the rate of the process will decrease rapidly (Davison, 1991; Laing, 2012). However, our research found no significant effects of temperature on $\text{ETR}_{\text{max}}$. The discrepancy may stem from the fact that changes associated with short-term exposure to temperatures seen in the $Q_{10}$ studies do not necessarily reflect the physiology of an acclimated plant (Li, 1980; Davison, 1991). Other experiments which did allow algae to acclimate to temperature conditions found a correlation between lower growth temperature plants and higher maximum photosynthesis rates (Davison, 1991). Although an acclimation period of two days is not unprecedented (Lignell & Pedersén, 1989), it is possible that the samples did not fully acclimate to temperature conditions prior to
measurements. Another possibility is that our temperature range was not broad enough to produce a significant effect. Q$_{10}$ studies often expose plants to a broad range of temperatures, with differences exceeding 20 °C between minimum and maximum temperatures (e.g. Phooprong, Ogawa & Hayashizaki, 2007). In contrast, the temperature range in this study was smaller (2 °C) to approximate climate change conditions within the next century.

The increase in respiration with increasing temperature that we observed aligns with the results of previous studies. Several studies that allow an acclimation period or grow the algae at specific temperature regimes have shown a link between increasing temperature and increasing dark respiration (Lapointe, Tenore & Dawes, 1984; Robarts & Zohary, 1987; Phooprong, Ogawa & Hayashizaki, 2007; Koch et al., 2013). This increased respiration at higher temperatures is hypothesized to be the reason for the disconnect between optimal growth temperature and optimal photosynthesis temperatures (Li, 1980; Lapointe, Tenore & Dawes, 1984; Robarts & Zohary, 1987).

If 52 h was insufficient acclimation time, then the increased respiration rate observed in this study may reflect the increased cost of energy for acclimation to treatment conditions. The amount of time needed for an alga to acclimate to a different temperature regime is not well studied (Davison, 1991). There is evidence that some algae may not need acclimation periods at all so long as the new temperature treatment falls within a range of temperatures that do not cause temperature stress to the alga (Li, 1980; Lapointe, Tenore & Dawes, 1984). In one experiment, *Gracilaria tikvahiae* samples grown in an outdoor facility at 25-30 °C were transplanted into chambers and exposed to constant temperatures of 15, 20, 25, and 30 °C (Lapointe, Tenore & Dawes, 1984). Each sample’s growth rate was monitored prior to and following the temperature change. Results indicated that the algal samples did not need time to
adjust to the temperature change; instead they continued to have exponential growth rates immediately after the transplantation. The sole exception was the algae transplanted into the 15 °C treatment, the thermal extreme for this clone of *Gracilaria tikvahiae*, which produced a lag of 5-9 days before the algae stabilized into steady-state growth (Lapointe, Tenore & Dawes, 1984). The temperatures experienced by algae in this experiment did not have a large range (22.24 °C minimum temperature and 29.65 °C maximum temperature observed over all trials) and were far from the thermal extremes of this alga (Smith *et al.*, 2004; Phooprong, Ogawa & Hayashizaki, 2007). Therefore, it seems probable that the increased respiration values observed are not caused by the increased energy cost of acclimation.

**Carbon Dioxide**

Prior to this study, at least three species in the genus *Gracilaria* showed increased growth with increased carbon dioxide availability (DeBusk & Ryther, 1984; Lignell & Pedersén, 1989; Xu, Zou & Gao, 2010). Although growth was not measured in this experiment, an increase in ETR$_{\text{max}}$ was observed at higher pCO$_2$. Our results imply carbon limitation in *G. salicornia*, which would be consistent with the carbon limitation found in *G. tikvahiae* (DeBusk & Ryther, 1984), *G. segundata* (Lignell & Pedersén, 1989), and *G. lemaneiformis* (Xu, Zou & Gao, 2010). If *G. salicornia* is a carbon limited species, then climate change may increase the growth rate of this already invasive species (Harley *et al.*, 2012; Koch *et al.*, 2013). However, further testing is necessary to determine whether these potential increases in growth rate at higher pCO$_2$ values will be outweighed by accelerated respiration rates at higher temperatures, which could inhibit growth.

Most of the species studied in this genus and a majority of rhodophytes in general are able to use HCO$_3^-$ via carbonic anhydrase (Lignell & Pedersén, 1989; Haglund *et al.*, 1992;
Andria et al., 2001; Moulin et al., 2011; Koch et al., 2013). It would be imprudent to assume that *G. salicornia* can also use HCO$_3^-$ solely based on the characteristics of other species; however, our results are reasonable if this assumption were true, given preferred use of CO$_2$ over HCO$_3^-$ and the increased availability of CO$_2$ at lower pH values (Lignell & Pedersén, 1989; Haglund et al., 1992). The different ways that algae acquire and use carbon are one cause of the contrasting, species-specific reactions that can occur when exposed to increased pCO$_2$ (Gao, Helbling & Häder, 2012; Koch et al., 2013; Johnson, Price & Smith, 2014).

**Potential future scenarios**

There is evidence that *G. salicornia* in Hawai‘i will maintain or increase growth rates into the next century. The increase in ETR$_{\text{max}}$ photosynthetic rates with increasing pCO$_2$ implies carbon limitation in *G. salicornia*. Carbon limitation in marine flora is the premise that the photosynthesis and growth rates of a specific species are not maximized at present-day dissolved inorganic carbon (DIC) concentrations (Koch et al., 2013). Although we did not measure growth in *G. salicornia* directly, it has been shown for this alga that photosynthesis and growth rates are highly correlated (Smith et al. 2004). If *G. salicornia* is carbon limited, then future levels of increased pCO$_2$ will likely result in elevated growth rates (Harley et al., 2012; Koch et al., 2013) if the increase in respiration rates with warmer temperatures is low.

*G. salicornia* is known to grow in climates warmer than Hawai‘i (22 °C-30 °C), such as Okinawa, Japan (21 °C–32 °C) and along the coast of Thailand (25 °C–35 °C), which demonstrates that the species has the potential to thrive in warmer environments (Phooprong, Ogawa & Hayashizaki, 2007). If Hawai‘i sea surface maximum temperatures increase by 1-3 °C over the next century, *G. salicornia* will likely still be within its thermal limits, presuming that the genes contributing to thermal tolerance in the western Pacific population are present in the
Hawai‘i population as well. Genetic sequencing has indicated that the Hawai‘i population of *G. salicornia* is closely related to the Thailand population, with populations from Malaysia and the Philippines as out-groups, implying that the genetic capacity for thermal tolerance may be conserved (Yang, Geraldino & Kim, 2013).

High temperature events in Hawai‘i during 2014 and 2015 were associated with widespread coral bleaching across the state including Kāne‘ohe Bay and Hanauma Bay (Bahr, Jokiel & Rodgers, 2015; Rodgers et al., 2017). However, these warming events may have affected invasive macroalgae as well. Invasive algae, primarily *Eucheuma denticulatum* and *Kappaphycus* alvarezii but also *Acanthophora spicifera* and *G. salicornia*, have established high abundance in Kāne‘ohe Bay since the 1990s and have been the target of focused removal efforts (Rodgers & Cox, 1999; Neilson et al., in prep). Following the 2014/2015 warming events, researchers and managers noticed a drastic decrease in the abundance of *Eucheuma*/ *Kappaphycus* spp., but no noticeable decrease in *G. salicornia* abundance (Neilson BJ, pers. comm., 2017; Wall CB, pers. comm., 2017); however, further research is needed to address confounding factors, such as decreased trade wind velocity and decreased water flow, before temperature can be confirmed as the trigger for the algal decrease (Wall CB, pers. comm., 2017). Growth experiments indicate that *Eucheuma*/ *Kappaphycus* spp. have a lower thermal maximum than *G. salicornia* (Stimson J, unpublished data, 2017). Temperature-triggered changes in algal abundance and community structure have been demonstrated elsewhere for this specific warming event. Cox, Spalding & Foster reported a decline in native *Padina* and *Dictyota* spp. and a rise in invasive *Avrainvillea amadelpha* on the south shore of O‘ahu following the 2014 warming event (2017). Similar changes in algal abundance linked to temperature have been recorded in temperate climate zones, with high mortality in kelp after extreme temperature events (Martínez,
Cárdenas & Pinto, 2003; Wernberg et al., 2016). The change of the Pacific Decadal Oscillation, a long-term and large-scale climate pattern affecting the North Pacific, from cool phase to warm phase may be one factor driving these higher temperatures now and into the next decades (Rodgers et al., 2015). Together, these observations imply that in the next decades G. salicornia may have a competitive advantage over other invasive and native algae in warming waters, although more research is necessary.

Lastly, seawater changes within the next decades are expected to drastically decrease the abundance of coral on reefs (Hoegh-Guldberg et al., 2007; Hughes et al. 2007; Kroeker, Micheli & Gambi, 2013; Pörtner et al., 2014). On reefs, coral and algae compete for space and sunlight, and the increasing coral mortality rates may create space and rubble substrate in sunlit areas, offering the opportunity for algal expansion (McCook, Jompa & Diaz-Pulido, 2001; Hoegh-Guldberg, 2007; Hughes et al. 2007; Koch et al. 2013). The combination of an increased amount of habitat space, the thermal tolerance to survive in warming Hawaiian coastal areas through the next century, and the increase in carbon dioxide availability for this apparently carbon limited plant are all indications that G. salicornia will continue to be an invasive presence into the future.

The consequences of the sustained or increased abundance of G. salicornia on Hawai‘i’s reefs will include effects on several ecological levels. G. salicornia has shown the ability to compete successfully against native algal species that may occupy similar ecological niches (Russell, 1992). An increase in the abundance of G. salicornia may therefore increase the competition with similar native species such as Laurencia nidifica and Gracilaria coronopifolia (Russell, 1992). Increasing G. salicornia would lead to increased abundance in associated epiphytes and epifauna, which are distinct from the communities of epibionts on native algae.
Overgrowth by *G. salicornia* creates harmful conditions for corals as the algal mounds significantly change the physical (sediment buildup and decreased irradiance) and chemical (lowered pH, periods of hypoxia and hyperoxia) conditions beneath them (Martinez, 2012; Martinez, Smith & Richmond, 2012). Additionally, presence of *G. salicornia* led to decreased survivorship in coral larvae in lab experiments, an effect that would decrease coral ability to recolonize algal dominated reefs, though these results were not replicated in field studies (Martinez, 2012). Decreased abundance of native algal species, changes in invertebrate abundances, and further deterioration of corals are some of the effects that can be expected with *G. salicornia*’s abundance increase.

### Conclusion and future studies

In Hawai‘i, *G. salicornia* is one of several macroalgae that may invade and overgrow coral reefs within the next century. Understanding how pCO$_2$ and temperature each affect photosynthesis and respiration can help us to understand how the alga reacts to these factors at a physiological level. In this experiment, it was found that respiration increases with increasing temperature and photosynthesis increases with increasing pCO$_2$. The increase in photosynthesis rates may be indicative of carbon limitation at current DIC concentrations, which would mean increased growth with increased pCO$_2$. Meta-analyses from multiple studies may show patterns in how different factors and combinations of factors affect macroalgae at the physiological and community level (Johnson, Price & Smith, 2014).

There are a number of gaps to fill for future studies. A direct step after this research would be a similar study that incorporates a growth measurement of *G. salicornia*, with a longer acclimation and trial period. Because interactive effects of pCO$_2$ and temperature were not significant for this alga, the full-factorial experimental design could be exchanged for two single-
factor concurrent experiments. This change would allow for a greater number of replicates per experimental trial.

Recent occurrences including the 2014/2015 warming events and subsequent decrease in *Eucheuma/Kappaphycus* spp. abundance (Neilson BJ, pers. comm., 2017; Wall CB, pers. comm., 2017) and the increased green sea turtle foraging behavior (Bahr et al., in prep) merit further investigation. Currently, manual alien algae removal efforts in Kāne‘ohe Bay by the State of Hawai‘i Department of Aquatic Resources focus specifically on species of *Eucheuma and Kappaphycus*. Further, abundance of *G. salicornia* decreases as an effect of sea urchin outplanting (Neilson et al., in prep). If growth by species of *Eucheuma or Kappaphycus* is more temperature sensitive than growth of *G. salicornia*, as has been observed in the field, this could have important repercussions for management (Neilson BJ, pers. comm., 2017; Wall CB, pers. comm., 2017). If the next decades in Hawai‘i bring high temperatures as predicted from both global climate change and the Pacific Decadal Oscillation warm phase (Rodgers et al. 2015), then a shift in priority from species of *Eucheuma and Kappaphycus* to *G. salicornia* and other invasive algae may be important.

Climate change will occur gradually over decades, and long-term (months to years) studies on how this will affect macroalgae are lacking. Immediate changes in algal physiology when exposed to a treatment are not identical to long-term changes, when the algae have had a chance to acclimate (Li, 1980; Davison, 1991) and week-long experiments may not capture the full range of effects that treatments will have over time. Long-term studies might even include periods of reproduction, to examine the effect of treatment on multiple life stages. Replicating a community in a mesocosm, with corals, macroalgae, crustose coralline algae, and invertebrates
in long-term manipulative experiments is ideal in ecological climate change research, but even without those resources, individual linkages can be examined.
**Literature Cited**


Bahr KD, Coffey DM, Rodgers K, Balazs GH. In prep. Green sea turtles on the move for food: *Honu* in Hawai‘i grazing their way into new habitats.


Kawachi MS, Van Houtan KS, Smith CM. In prep. Elevated nutrients promote growth of coral reef macroalgae in Hawai‘i.


Table 2.1: Timeline and weather conditions for the experimental periods from the National Weather Service Forecast Office, Honolulu International Airport weather station (located ~5 km from experiment location).

<table>
<thead>
<tr>
<th>Trial number</th>
<th>Trial Dates (year 2016)</th>
<th>Max Temp (°C)</th>
<th>Min Temp (°C)</th>
<th>Average day length (hr:min)</th>
<th>Total rainfall during trial (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Oct. 17-20</td>
<td>30.6</td>
<td>23.9</td>
<td>11:37</td>
<td>0.0762</td>
</tr>
<tr>
<td>2</td>
<td>Oct. 31- Nov. 3</td>
<td>29.4</td>
<td>22.2</td>
<td>11:21</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>Nov. 7-10</td>
<td>29.4</td>
<td>18.9</td>
<td>11:14</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>Nov. 28- Dec. 1</td>
<td>27.8</td>
<td>21.1</td>
<td>10:57</td>
<td>0.4572</td>
</tr>
</tbody>
</table>
Table 2.2: Summary of measured parameters, frequency, and instruments

<table>
<thead>
<tr>
<th>Measured parameter</th>
<th>Frequency of measurement during trial period</th>
<th>Measurement Instrument</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dissolved oxygen</td>
<td>Once, for respiration rate determination</td>
<td>YSI 556 Multiprobe System</td>
</tr>
<tr>
<td>Temperature</td>
<td>Every 30 min</td>
<td>HOBO data logger</td>
</tr>
<tr>
<td>Salinity</td>
<td>Once per trial</td>
<td>YSI 556 Multiprobe System</td>
</tr>
<tr>
<td>Total Alkalinity</td>
<td>Twice per trial</td>
<td>Titrino Plus 877, Metrohm</td>
</tr>
<tr>
<td>Electron Transfer Rate</td>
<td>Once, for photosynthetic rate determination</td>
<td>Junior PAM, Walz Germany</td>
</tr>
<tr>
<td>pH</td>
<td>Twice per trial</td>
<td>Spectrophotometer</td>
</tr>
</tbody>
</table>
### Table 2.3: Temperature summary statistics comparisons

<table>
<thead>
<tr>
<th>Summary statistic</th>
<th>Average (°C)</th>
<th>Standard deviation (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average of all temperature values for each sample</td>
<td>26.78</td>
<td>1.08</td>
</tr>
<tr>
<td>Average of temperature values from 1100-1400 h for each sample</td>
<td>27.83</td>
<td>1.08</td>
</tr>
<tr>
<td>Average of temperature values from 2300-0200 h for each sample</td>
<td>26.43</td>
<td>1.07</td>
</tr>
</tbody>
</table>
Table 2.4: Means of measured factors by treatment

<table>
<thead>
<tr>
<th>Treatment category</th>
<th>Mean midnight temperature (°C)</th>
<th>pH</th>
<th>Salinity (ppt)</th>
<th>Total Alkalinity (equivalents)</th>
<th>PAR average 0700-1730 (µmol photons m⁻² s⁻¹)</th>
<th>pCO₂ values (µatm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Std Dev</td>
<td>Mean</td>
<td>Std Dev</td>
<td>Mean</td>
<td>Std Dev</td>
</tr>
<tr>
<td>ambient temp, +CO₂</td>
<td>26.14</td>
<td>1.09</td>
<td>7.81</td>
<td>0.10</td>
<td>34.44</td>
<td>0.17</td>
</tr>
<tr>
<td>ambient temp, normal pH</td>
<td>26.31</td>
<td>1.32</td>
<td>8.04</td>
<td>0.01</td>
<td>34.52</td>
<td>0.05</td>
</tr>
<tr>
<td>increased temp, +CO₂</td>
<td>26.54</td>
<td>1.05</td>
<td>7.81</td>
<td>0.11</td>
<td>34.53</td>
<td>0.08</td>
</tr>
<tr>
<td>increased temp, normal pH</td>
<td>26.72</td>
<td>0.94</td>
<td>8.03</td>
<td>0.01</td>
<td>34.52</td>
<td>0.07</td>
</tr>
</tbody>
</table>
Table 2.5: Multiple regression model analysis (effect tests and parameter estimates) of respiration rates ($R^2 = 0.74$). Values significant at the level of $\alpha = 0.05$ are marked with an asterisk (*).

<table>
<thead>
<tr>
<th>Source</th>
<th>Nparm</th>
<th>DF</th>
<th>Sum of Squares</th>
<th>F Ratio</th>
<th>Prob &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>pCO2 values</td>
<td>1</td>
<td>1</td>
<td>54.76966</td>
<td>2.3823</td>
<td>0.1510</td>
</tr>
<tr>
<td>Treatment_Cat[Tank]</td>
<td>12</td>
<td>12</td>
<td>392.64153</td>
<td>1.4232</td>
<td>0.2832</td>
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<tr>
<td>Header</td>
<td>3</td>
<td>3</td>
<td>81.77512</td>
<td>1.1857</td>
<td>0.3598</td>
</tr>
<tr>
<td>mean mid night temp</td>
<td>1</td>
<td>1</td>
<td>159.46045</td>
<td>6.9361</td>
<td>0.0233*</td>
</tr>
<tr>
<td>mean mid night temp*mean mid night temp</td>
<td>1</td>
<td>1</td>
<td>143.98345</td>
<td>6.2629</td>
<td>0.0294*</td>
</tr>
</tbody>
</table>

| Term                                | Estimate   | Std Error | t Ratio | Prob>|t| |
|-------------------------------------|-------------|-----------|---------|-----|
| Intercept                           | -100.6568   | 36.45083  | -2.76  | 0.0185* |
| pCO2 values                         | 0.0164813   | 0.010678  | 1.54  | 0.1510 |
| Tank[1]:Treatment_Cat[cool, CO2]    | -4.391973   | 4.276425  | -1.03  | 0.3265 |
| Tank[2]:Treatment_Cat[cool, CO2]    | 12.366235   | 5.369305  | 2.30  | 0.0418* |
| Tank[2]:Treatment_Cat[cool, norm]   | -6.162523   | 6.853204  | -0.90  | 0.3878 |
| Tank[2]:Treatment_Cat[hot, CO2]     | -2.259006   | 4.677362  | -0.48  | 0.6386 |
| Tank[3]:Treatment_Cat[cool, norm]   | -8.170384   | 4.48763   | -1.82  | 0.0959 |
| Tank[4]:Treatment_Cat[cool, CO2]    | 4.4410629   | 3.297811  | 1.35  | 0.2052 |
| Tank[5]:Treatment_Cat[cool, CO2]    | -3.224216   | 5.031248  | -0.64  | 0.5348 |
| Tank[5]:Treatment_Cat[hot, CO2]     | 0.2386172   | 5.174219  | 0.05  | 0.9640 |
| Tank[6]:Treatment_Cat[cool, CO2]    | 2.5294539   | 4.118306  | 0.61  | 0.5516 |
| Tank[7]:Treatment_Cat[cool, norm]   | -10.08822   | 4.813103  | -2.10  | 0.0600 |
| Tank[8]:Treatment_Cat[cool, CO2]    | -0.662496   | 5.473208  | -0.12  | 0.9058 |
| Tank[8]:Treatment_Cat[cool, norm]   | 0.3322521   | 5.539338  | 0.06  | 0.9532 |
| Header[1]                           | -4.962094   | 3.082751  | -1.61  | 0.1358 |
| Header[2]                           | 0.5013599   | 3.355918  | 0.15  | 0.8839 |
| Header[3]                           | -1.786201   | 4.031497  | -0.44  | 0.6663 |
| mean mid night temp                 | 3.5788475   | 1.358893  | 2.63  | 0.0233* |
| (mean mid night temp-26.4265)*(mean mid night temp-26.4265) | 3.9358068   | 1.5727   | 2.50  | 0.0294* |
Table 2.6: Summary of pooled $\text{ETR}_{\text{max}}$, and $E_k$ values.

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>St. Dev.</th>
<th>Min.</th>
<th>Max.</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{ETR}_{\text{max}}$ ($\mu\text{mol e} \cdot \text{m}^2 \cdot \text{s}^{-1}$)</td>
<td>47.31</td>
<td>14.15</td>
<td>24.72</td>
<td>85.40</td>
</tr>
<tr>
<td>$E_k$ ($\mu\text{mol photons m}^2 \cdot \text{s}^{-1}$)</td>
<td>367.3</td>
<td>144.3</td>
<td>164.1</td>
<td>715.1</td>
</tr>
</tbody>
</table>
Table 2.7: Multiple regression model analysis (effect tests and parameter estimates) of ETR$_{\text{max}}$ values ($R^2 = 0.77$). Values significant at the level of $\alpha = 0.05$ are marked with an asterisk (*).

<table>
<thead>
<tr>
<th>Source</th>
<th>Nparm</th>
<th>DF</th>
<th>Sum of Squares</th>
<th>F Ratio</th>
<th>Prob &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>mean mid night temp</td>
<td>1</td>
<td>1</td>
<td>272.71249</td>
<td>2.2943</td>
<td>0.1580</td>
</tr>
<tr>
<td>pCO2 values</td>
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<td>1</td>
<td>674.08391</td>
<td>5.6711</td>
<td>0.0364*</td>
</tr>
<tr>
<td>PAR avg 700-1730</td>
<td>1</td>
<td>1</td>
<td>42.73079</td>
<td>0.3595</td>
<td>0.5609</td>
</tr>
<tr>
<td>Treatment_Cat[Tank]</td>
<td>12</td>
<td>12</td>
<td>979.35794</td>
<td>0.6866</td>
<td>0.7362</td>
</tr>
<tr>
<td>Header</td>
<td>3</td>
<td>3</td>
<td>381.54817</td>
<td>1.0700</td>
<td>0.4014</td>
</tr>
</tbody>
</table>

| Term                      | Estimate | Std Error | t Ratio | Prob>|t| |
|--------------------------|----------|-----------|---------|-------|
| Intercept                | -93.36209| 72.48884  | -1.29   | 0.2242 |
| mean mid night temp      | 4.3517836| 2.873026  | 1.51    | 0.1580 |
| pCO2 values              | 0.0526896| 0.022125  | 2.38    | 0.0364*|
| PAR avg 700-1730         | -0.041859| 0.069815  | -0.60   | 0.5609 |
| Tank[1]:Treatment_Cat[cool, CO2] | 15.04281 | 9.732353 | 1.55    | 0.1505 |
| Tank[2]:Treatment_Cat[cool, CO2] | -11.11109| 12.77084 | -0.87   | 0.4029 |
| Tank[2]:Treatment_Cat[cool, norm] | 15.659065| 15.00714 | 1.04    | 0.3191 |
| Tank[3]:Treatment_Cat[hot, CO2] | -7.318108| 10.53774 | -0.69   | 0.5018 |
| Tank[4]:Treatment_Cat[cool, CO2] | 2.0966482| 10.43099 | 0.20    | 0.8444 |
| Tank[5]:Treatment_Cat[cool, norm] | -2.197423| 7.185643 | -0.31   | 0.7655 |
| Tank[5]:Treatment_Cat[cool, CO2] | 17.120559| 11.35435 | 1.51    | 0.1598 |
| Tank[6]:Treatment_Cat[hot, CO2] | 1.0390469| 11.85469 | 0.09    | 0.9317 |
| Tank[7]:Treatment_Cat[cool, norm] | 7.6644968| 9.558157 | 0.80    | 0.4396 |
| Tank[8]:Treatment_Cat[cool, CO2] | -0.445841| 10.21297 | -0.04   | 0.9660 |
| Tank[8]:Treatment_Cat[cool, norm] | -5.365603| 10.35979 | -0.52   | 0.6148 |
| Header[1]                 | 5.4047044| 11.46578 | 0.47    | 0.6466 |
| Header[2]                 | -6.153538| 6.922712 | -0.89   | 0.3931 |
| Header[3]                 | -13.84851| 7.769408 | -1.78   | 0.1023 |
Table 2.8: Multiple regression model analysis (effect tests and parameter estimates) of $E_k$ values ($R^2 = 0.93$). Values significant at the level of $\alpha = 0.05$ are marked with an asterisk (*).

<table>
<thead>
<tr>
<th>Source</th>
<th>Nparm</th>
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<th>Sum of Squares</th>
<th>F Ratio</th>
<th>Prob &gt; F</th>
</tr>
</thead>
<tbody>
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<td>mean mid night temp</td>
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<td>1</td>
<td>19298.08</td>
<td>4.8601</td>
<td>0.0497*</td>
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<td>1</td>
<td>7651.21</td>
<td>1.9269</td>
<td>0.1926</td>
</tr>
<tr>
<td>Treatment_Cat[Tank]</td>
<td>12</td>
<td>12</td>
<td>211361.41</td>
<td>4.4358</td>
<td>0.0097*</td>
</tr>
<tr>
<td>PAR before PAM</td>
<td>1</td>
<td>1</td>
<td>27812.03</td>
<td>7.0043</td>
<td>0.0227*</td>
</tr>
<tr>
<td>Header</td>
<td>3</td>
<td>3</td>
<td>14425.02</td>
<td>1.2110</td>
<td>0.3514</td>
</tr>
</tbody>
</table>

| Term                          | Estimate | Std Error | t Ratio | Prob>|t| |
|-------------------------------|----------|-----------|---------|--------|
| Intercept                     | 1489.7906| 511.3846  | 2.91    | 0.0141*|
| mean mid night temp           | -39.72275| 18.01841  | -2.20   | 0.0497*|
| pCO2 values                   | 0.1969359| 0.141871  | 1.39    | 0.1926 |
| Tank[1]:Treatment_Cat[cool, CO2] | 116.20585| 54.94273  | 2.12    | 0.0581 |
| Tank[2]:Treatment_Cat[cool, CO2] | -136.3667| 73.73894  | -1.85   | 0.0914 |
| Tank[2]:Treatment_Cat[cool, norm] | 55.494449| 83.9632   | 0.66    | 0.5223 |
| Tank[2]:Treatment_Cat[hot, CO2] | -169.7469| 62.08165  | -2.73   | 0.0194*|
| Tank[3]:Treatment_Cat[cool, norm] | 5.8186821| 57.69482  | 0.10    | 0.9215 |
| Tank[4]:Treatment_Cat[cool, CO2] | -123.0186| 40.63728  | -3.03   | 0.0115*|
| Tank[5]:Treatment_Cat[cool, CO2] | 41.366339| 65.39677  | 0.63    | 0.5400 |
| Tank[5]:Treatment_Cat[hot, CO2] | 18.160414| 69.66733  | 0.26    | 0.7992 |
| Tank[6]:Treatment_Cat[cool, CO2] | -64.93524| 55.07522  | -1.18   | 0.2633 |
| Tank[7]:Treatment_Cat[cool, norm] | 12.516165| 58.32975  | 0.21    | 0.8340 |
| Tank[8]:Treatment_Cat[cool, CO2] | 292.52723| 62.46509  | 4.68    | 0.0007*|
| Tank[8]:Treatment_Cat[cool, norm] | -164.3833| 68.27046  | -2.41   | 0.0347*|
| PAR before PAM                | -6.526965| 2.466204  | -2.65   | 0.0227*|
| Header[1]                     | 2.0116702| 42.75776  | 0.05    | 0.9633 |
| Header[2]                     | -42.73803| 44.4315   | -0.96   | 0.3568 |
| Header[3]                     | -26.80649| 52.92359  | -0.51   | 0.6225 |
Figure 2.1: Representation of tank set-up. Seawater from Honolulu harbor is pumped into the header tanks where treatments (heat/no heat; CO₂/no CO₂) were applied. Water flowed from the elevated headers into eight aquaria (two per header) each containing an algal sample. Actual aquarium positions were randomized in each trial.
Figure 2.2: Photograph of tank set-up at Ānuenue Fisheries Research Center. Seawater flows continuously from elevated header tanks through PVC pipes and plastic tubing into individual aquaria. Wooden scaffolds keep the plastic tubes submerged so seawater flows in from the bottom, ensuring mixing. Excess water overflows from the top of the aquaria into larger water baths.
Figure 2.3: Graph of the residuals by predicted values for the ETR\textsubscript{max} whole model. Blue line indicates the linear regression of the model. Visual outliers are indicated within dotted circle.
Figure 2.4: ETR$_{\text{max}}$ plotted against pCO$_2$ values (µmol electron m$^{-2}$s$^{-1}$ and µatm, respectively). The same three outliers as in Figure 2.3 are indicated by the dotted circle.
Figure 2.5: Comparison of respiration rates. Means (x) and standard deviations (bars) of respiration rates from this study and Phooprong, Ogawa & Hayashida (2007).
Comparison with past studies: $\text{ETR}_{\text{max}}$

Mean $\text{ETR}_{\text{max}}$ ($\mu$mol e$^{-}$ m$^{-2}$ s$^{-1}$)

1. This study, (30 observations)
2. Kawachi et al., in prep., (n=9)
3. Spafford, 2011 high irradiance (n=7)
4. Spafford, 2011 low irradiance (n=9)

Figure 2.6: Comparison of $\text{ETR}_{\text{max}}$. Means and standard errors shown are from this study, Kawachi et al., in prep and Spafford (2011).
Figure 2.7: Comparison of $E_k$ values. Means and standard errors shown are from this study, Kawachi et al., in prep and Spafford (2011).
Appendix A: pH Measurements

pH measurements were run spectrophotometrically using metacresol purple (mCP) indicator dye according to Standard Operating Procedure (SOP) 6b (Dickson, Sabine & Christian, 2007). Spectrophotometry measurements from the first trial were conducted using a computer assisted HP Diode Array, Botany, University of Hawai‘i at Mānoa. Spectrophotometry measurements for subsequent trials were conducted using a Molecular Devices SpectraMax M2 (CA, USA) spectrophotometer and Softmax software located at the Hawai‘i Institute of Marine Biology (HIMB).

Mixing of the dye: Metacresol purple tablets (Acrōs Organics, Thermo Fisher Scientific, Belgium) 76.49 g of metacresol purple (mCP) tablets were mixed with 100 mL deionized water to produce a 2 mM solution. The dye was then mixed with dilute NaOH\(_{(aq)}\) to bring the pH of the dye close to 7.9 (Dr. Meter pH-100)

Calibration of the dye: The pH of dye differs slightly from the pH of each of sample, therefore addition of the indicator dye changes the pH of each sample slightly. To account for this, each batch of mCP dye must be calibrated. Beakers containing 50 mL of filtered seawater were prepared across a spectrum of pH values between 8.5 and 7.7, using CO\(_2\) and dilute NaOH\(_{(aq)}\) to alter the pH values. The approximate pH values of each prepared seawater solution were 8.3, 8.1, 8.0, 7.9, and 7.8.

For each seawater solution sample, the following steps were taken.

1. Using a water bath, samples were maintained at 25°C during the spectrophotometry procedure.

2. The spectrophotometric analysis software (Softmax Pro) was set to analyze absorbance values at wavelengths 578 and 434, with a reference wavelength at 730.
3. A cuvette and pipette were rinsed with sample seawater twice. 3 mL of sample seawater were pipetted into the cuvette.

4. The cuvette exterior was wiped thoroughly with a tissue (Kimwipe) to remove excess oil and dust.

5. The cuvette was placed into the spectrophotometer (Molecular Devices SpectraMax M2, CA, USA) and analyzed as a blank sample.

6. The cuvette was removed from the spectrophotometric machine and 50 µL of mCP indicator dye was added to the cuvette. The cuvette was covered and slowly inverted to ensure proper mixing.

7. The cuvette was wiped thoroughly with a tissue.

8. The cuvette was placed into the spectrophotometer for analysis of absorbance values.

9. For dye calibration purposes, steps 6-9 were repeated once with the same sample of water in the same cuvette.

10. All absorbance values and blank values were recorded.*

11. All absorbance values (first addition of dye and second addition of dye) were corrected using Eqs. 1 & 2.

* Some spectrophotometer software automatically corrects absorbances using the blank samples and reference wavelengths. In this case, only absorbance values of wavelengths 578 and 434 must be recorded, and Eqs. 1 and 2 below may be ignored.

Using this protocol, a regression line was created from the ratio of the absorbance values after the first addition of dye, and the difference between the ratio of the absorbance values after the second addition of dye (Eq. 3). This regression allowed an approximation of the change in the pH resulting from the addition of dye so that compensation can be made (Clayton & Bryne, 1993 in Dickson, Sabine & Christian, 2007).
Equations 1 & 2: Calculating corrected absorbance values

corr$_{578}$ = (raw$_{578}$ − blank$_{578}$) − (raw$_{730}$ − blank$_{730}$)
corr$_{434}$ = (raw$_{434}$ − blank$_{434}$) − (raw$_{730}$ − blank$_{730}$)

blank$_{578}$, blank$_{434}$, and blank$_{730}$ are the absorbance values obtained from spectrophotometric analysis of a blank/undyed sample.

raw$_{578}$, raw$_{434}$, raw$_{730}$ are the raw absorbance values obtained from spectrophotometric analysis of a dyed sample.

corr$_{578}$, corr$_{434}$ are the absorbance values corrected using blank and reference absorbances.

Equation 3: Equation for the calibration linear regression

\[ \Delta (A_{578} / A_{434}) = a + b (A_{578} / A_{434}) \]

$A_{578}$ and $A_{434}$ are the corrected absorbance values at wavelengths 578 and 434, respectively.

\( \Delta (A_{578} / A_{434}) \) is the change in the absorbance ratio between the first addition of dye and the second addition of dye.

\( a \) and \( b \) are the intercept and slope specific to the calibrated dye batch and are used to correct for the pH changes resulting from adding dye to the seawater sample.

Measuring the pH of a sample: Measuring the pH of a sample is similar to calibrating the dye batch. The same procedure, excluding step 10, was used to measure the absorbances of each sample. The ratio of $A_{578}/A_{434}$ was then calculated from these values and corrected for the dye measurement using the slope and intercept values from the calibration procedure (Eq. 4). A pH value was then calculated from the corrected absorbance ratios (Eq. 5). Temperature and salinity measurements of the sample were also necessary for these calculations.

Equation 4: Calculating the corrected absorbance values

\[ (A_{578} / A_{434})_{corr} = (A_{578} / A_{434}) - [a + b(A_{578} / A_{434})] \]

$A_{578}$ and $A_{434}$ are the sample’s measured absorbance values at wavelengths 578 and 434, respectively.
\((A_{578} / A_{434})_{corr}\) is the corrected ratio, which takes into account the pH change caused by the addition of indicator dye. This is the ratio used for the seawater sample pH determination calculations.

Equation 5: Calculating the pH of the seawater sample

\[
\text{pH} = pK_2 + \log_{10} \left[ \frac{(A_{578} / A_{434})_{corr} - 0.00691}{2.222 - 0.133(A_{578} / A_{434})_{corr}} \right]
\]

\[
pK_2 = \frac{1245.69}{T/K} + 3.8275 + 0.00211(35 - S)
\]

\(T\) is temperature at the time of spectrophotometry measurement (estimated here at 298.15K). \(K\) is the unit Kelvin, making the final value unitless. \(S\) is salinity of the sample in parts per thousand (estimated here at 34.5 ppt)

**Literature Cited**


Appendix B: Alkalinity Titrations

Alkalinity titrations were done using an automatic titrator (Titrino Plus 877, Metrohm) with a pH glass electrode (9101 Herisau, Metrohm) and 0.1 N HCl. The procedure for alkalinity titrations without an automatic titrator is outlined in Dickson, Sabine & Christian (2007) (SOP 3b: Determination of total alkalinity in seawater using an open cell titration).

Calibrating the pH electrode: Prior to each use, the titrator’s pH electrode was calibrated. The pH electrode was rinsed with deionized water and gently dried with a tissue (Kimwipe) before and between calibrations. NBS buffer solutions of pH 4.00 ± 0.01 (YSI 3821) and 7.00 ± 0.01 (Oaklon) were used during calibration. After the calibration, a slope percentage is given which indicates the level of functionality of the pH electrode. For this experiment, all slope values were above 98%.

Preparing the automatic titrator: Prior to each use, a preparation sequence was run on the titrator. This preparation sequence pumps acid through the tubing system to remove any bubbles that may have become trapped in the tubes. The software runs the preparation function automatically with user prompting. For this experiment, the burette tip was placed into a waste beaker of seawater and during the prep function, tubes were gently tapped to facilitate the removal of air bubbles.

Titrating a seawater sample: The automatic titrator system incorporates a pH electrode, a thermometer, and a burette for dispensing acid. The pH electrode reads the pH of the solution indicating when an inflection point is reached. The thermometer is used to correct for variance caused by temperature. The burette tip automatically dispenses dilute HCl acid at intervals to detect the volume of acid needed to neutralize the alkalinity in the sample, and the titrator software calculates TA from this value. Included in the set-up is an air pump attached to a length
of thin tubing. During the titration, carbon dioxide evolves in solution and the air pump helps to purge this gas from the sample after the first inflection point. All of these components were rinsed with filtered seawater and dried gently with a tissue before and between sample measurements.

1. All bottles containing sample seawater were kept in a water bath at 25°C (293.15 K) prior to titration to stabilize temperatures.

2. Approximately 100 mL of seawater was placed into a clean beaker using an automatic pipettor.

3. Seawater was added or removed from the beaker using a plastic dropper pipette until the seawater weighed between 100.000-100.100 g using a balance with ±0.001 g precision (Mettler Toledo XS403S). Weights were inputted into the automatic titrator software to assist in calculations.

4. The pH electrode, thermometer, and burette were all submerged in the sample beaker and a holder was used to maintain their position.

5. A magnetic stir bar was inserted into the beaker and the user prompted the automatic program to begin a titration.

6. The start time of each titration, sample weight, and sample temperature were manually recorded.

7. After the first pH inflection point was reached (displayed on the titrator monitor), air was added to the sample.

8. At the end of the titration, the pH and total alkalinity were manually recorded.
Maintaining accuracy assurance using certified reference materials (CRM): Every two weeks, a CRM was run to check the accuracy of the titrator using the same procedure required for typical seawater samples as detailed above.

Literature Cited

Appendix C: HOBO observations (lux) to PAR (µmol photons m\(^{-2}\) s\(^{-1}\)) conversion

HOBO Pendant Data Loggers (Onset, MA, USA) referred to as “HOBOs” were placed close to the algal tissue in each aquarium to take light and temperature measurements every 30 min. A single LI-COR Sperical Underwater Quantum Sensor (Lincoln, NE, USA) was also deployed in the water bath, adjacent to two aquaria, at the same depth as the algal samples. The sensor was attached to a data logger (LI-1400, LI-COR, Lincoln, NE, USA) which was programmed to measure light every 30 min between 0630-1900. The HOBO measures light in lux across a wide spectrum (~150-1200 nm wavelength) and measures only light received at 90° (cosine sensor). The LI-COR sensor is calibrated to measure Photosynthetically Active Radiation (PAR), which is light in the 400-700 nm wavelength range, coming in from all angles (360° or 4π sensor). In biology, PAR is often measured in µmol photons m\(^{-2}\) s\(^{-1}\) and is sometimes referred to as Photosynthetic Photon Flux Density (PPFD) (Björn, 1994). This is a quantum light unit, meaning that it measures the quanta of photons received per unit area per unit time, and is more applicable to photosynthetic studies due to the quantized nature of photosynthetic chemistry (Thimijan & Heins, 1982; Bolton & Hall, 1991). Many references give a simple linear conversion equation for converting sunlight from lux to PAR (Thimijan & Heins, 1982).

However, the ratio of total radiation to PAR is affected by many factors, such as location, season, and sky conditions (Jacovides et al., 2004). Additionally, in this case we are not only trying to convert between units, but also trying to convert a planar measurement to a 3D measurement. For this reason, we decided to look at the correlation between the HOBO measurements and the LI-COR measurements to create our own conversion equation.

To create a place-specific conversion of lux to PPFD, we looked at all light measurements between the hours of 0630-1900. We averaged the measurements taken by the
HOBOs in the two tanks closest to the LI-COR sensor. The LI-COR sensor only recorded data from two trials, due to researcher error. We paired the averaged HOBO measurements from those two trials with the LI-COR data and matched the time-stamps of each observation. A graph of LI-COR versus averaged HOBO data was created (Figure C.1). After visual inspection, it appeared that light values from the morning (0630-1130) and light values from the afternoon (1400-1900) followed two distinct regression curves, while light values from midday (1200-1330) showed no pattern at all (Figure C.2). After log-transforming both variables to create a more linear relationship, a linear regression was performed on morning, noon, and afternoon data sets. The linear regression fitted the morning and afternoon data very well (Table C.1), but did not fit the noon data. Since no clear pattern could be found for the noon data, HOBO values before 1230 were clumped into morning and values at or above 1230 were clumped into afternoon. Using the two equations, all individual HOBO data was converted from lux values measured with a cosine meter to the analogous µmol photons m$^{-2}$ s$^{-1}$ measured with a 4π sensor.

**Literature Cited**


Table C.1: Summary of linear regressions for log-transformed LI-COR values and log-transformed averaged HOBO values.

<table>
<thead>
<tr>
<th>Time of observations</th>
<th>R² value</th>
<th>P value</th>
<th>Equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morning</td>
<td>0.91</td>
<td>&lt;0.0001</td>
<td>Log (LI-COR) = -1.231 + 0.723 Log(HOBO)</td>
</tr>
<tr>
<td>Noon</td>
<td>0.00</td>
<td>.927</td>
<td>Log (LI-COR) = 4.219 + 0.0131 Log(HOBO)</td>
</tr>
<tr>
<td>Afternoon</td>
<td>0.84</td>
<td>&lt;0.0001</td>
<td>Log(LI-COR) = -0.819 + 0.564 Log(HOBO)</td>
</tr>
</tbody>
</table>
Figure C.1: LI-COR versus HOBO data over two trial periods.
Figure C.2: LI-COR versus HOBO data separated by time of observation (morning 0630-1130, midday 1200-1330, or afternoon 1400-1900).