THE PHYSICAL AND PHYSIOLOGICAL IMPACTS OF THE INVASIVE RED
MACROALGA GRACILARIA SALICORNIA DAWSON TO CORAL REEF HABITAT
QUALITY AND CORAL HEALTH

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

DOCTOR OF PHILOSOPHY

IN

BOTANY (MARINE BIOLOGY)

MAY 2012

By

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Keywords: Gracilaria salicornia, invasive algae, recruitment, sediment, coral, coral reef management
Acknowledgements

The authors would like to thank Carey Morishige for field assistance, advice and artwork as well as: Jorg Anson, Leinson Neth, Brandi Sasaki, Sean Macduff, Chloé Brahmi, Collin Medlin, James Akana Murphy, Austin Shelton, Luc Rougee, and Jessica Rougee, Sean Knutson, Miki Sakamura-Low, Trevor Low, Cheryl Woodley, Athena Adavadanai and Lisa May, Craig Downs for field assistance; and Megan Donahue, Olga Cordero-Brana, Eric Wolanski, Zac Forsman, Sean Knutson, Luc Rougée, Dave Gulko, Kuulei Rodgers, Paul Jokiel, Andy Taylor, Steve Kolinski, and Mike Hadfield for advice.

The authors would like to acknowledge support from a Pew Fellowship in Marine Conservation to RHR, a Program of the Pew Environmental Group, the Hawaii Coral Reef Initiative to RHR, the NOAA CREST grant to RHR, the NSF URM: Environmental Biology in the Pacific Islands DBI-0829272 program to MH for JA, the University of Hawaii at Mānoa Graduate Professional Access Program to JM, and the NOAA Educational Partnership Program Graduate Science Program to JM.

All coral and larvae were collected with the permission of the Hawaii State Department of Aquatic Resources under permits number SAP 2007-30, SAP 2008-47, SAP 2009-42.
General Abstract

*Gracilaria salicornia* Dawson is a successful and prolific red invasive alga, which grows in large stands of biomass that accumulate mats as large as 5 kg m\(^{-2}\) and effectively smothers native coral reef organisms. This research aims to understand the reproductive biology and ecology of the indigenous Hawaiian coral *Porites Synaræa hawaiiensis*. *P. hawaiiensis* is a brooding coral, capable of reproducing any day of the year, producing non-swimming motile larvae that show a strong affinity for settlement near crustose coralline algae, with low variability and maximal settlement within two days.

This research also aimed to answer the central question of, how does overgrowth by invasive macroalgal species affect benthic habitat quality and coral biology on coral reefs? Data from a series of manipulative studies are presented to investigate the impacts of algal mats to physical parameters on coral reefs such as: sediment, irradiance and dissolved gas variability as well as: coral recruitment, and coral health. The algal mats attenuated irradiance by as much as 99%, effectively doubled the amount of sediment deposited to the reef area below, imposed nightly periods of hypoxia lasting from 30 minutes to 120 minutes, imposed periods of high carbon dioxide concentrations and lowered pH by as much as a tenth of a pH point as a function of algal and community respiration. When the overgrowth of the coral *Porites compressa* by algal mats was simulated, the coral bleached within two weeks and the algal symbionts increased pigment levels to enhance photosynthesis. When algal management was simulated by removing the algae, pigments in the algal symbionts returned to normal. Oxidative stress through DNA damage and elevated incidences of anaerobic respiration through the
accumulation of L-lactate was not observed, suggesting that this species of coral was resilient enough to defend and recover from this stress. These findings suggest that management control efforts to remove invasive algal mats are likely to be successful in restoring the health and recovery of this species in Hawaii. Physiological metrics of the efficacy of management action are powerful tools that are available and can give short-term information and allow for adaptive management.
# Table of Contents

**Acknowledgements** ........................................................................................................ iii

**General Abstract** ............................................................................................................. iv

**List of Tables** ................................................................................................................... x

**List of Figures** .................................................................................................................. xi

**List of Abbreviations** ................................................................................................. xiv

**Chapter 1. Interactions Among Invasive Macroalgae and Benthic Reef Species:**
**Insights into Chemically-Mediated Competition in Ecological Success of Invasive Algae** ......................................................................................................................... 1

**Abstract** .......................................................................................................................... 1

**Overview** ......................................................................................................................... 2
  - Mechanisms of Algal Species Competition: Abiotic and Biotic ................................ 3
    - Abiotic Impacts ........................................................................................................... 3
    - Hydrodynamics ......................................................................................................... 3
    - Sediment Accumulation ............................................................................................ 3
    - Attenuation of Irradiance ......................................................................................... 3
    - Abrasion ..................................................................................................................... 4
  - Space ............................................................................................................................... 4
  - Biotic Impacts ............................................................................................................... 5
    - Microbes .................................................................................................................... 5
    - Direct Physiologically Mediated Chemical Impacts .............................................. 5
  - Algal Chemical Defense and Response to Stress ..................................................... 6
  - Stress Induced Production of ROS in Plants ............................................................. 7
  - Response to Mechanical Injury ................................................................................ 8
  - Irradiance and UV Radiation Stress ......................................................................... 8
  - Thermal Induced Stress ............................................................................................. 9
  - Osmotic Stress ........................................................................................................... 9
  - Defense Response to Pathogen Attack .................................................................... 9
  - Biological and Physiological Implications of ROS Exposure ................................. 10
  - Ecological Implications of ROS Exposure .............................................................. 11
  - Volatile Halogenated Organic Compounds .............................................................. 11
  - Solubility and Transport of VHOCs in the Ocean ..................................................... 12
  - Biological and Physiological Implications of VHOC Exposure ............................ 12
  - Ecological Implications of VHOC Exposure ........................................................... 12
  - Case Studies of the Competitive Dominant Species in Hawai‘i ............................... 14
    - Eucheumoids ........................................................................................................... 14
    - Gracilaria salicornia ................................................................................................. 15
    - Other Invasive Algae in Hawai‘i ............................................................................. 16

**Dissertation Questions and Hypotheses** ..................................................................... 17

**Chapter 2. Invasive Algal Mats Degrade Coral Reef Physical Habitat Quality** .... 20

**Abstract** ........................................................................................................................ 20
Chapter 4. The Impacts of *Gracilaria salicornia* on Coral Recruitment ............................................. 57
Abstract ......................................................................................................................... 57

Introduction .................................................................................................................. 57
Materials and Methods ................................................................................................. 60
Lab Survivorship and Settlement Assay ....................................................................... 60
Field Survivorship and Settlement Assay ....................................................................... 61
Statistical Analyses ......................................................................................................... 63
Lab Survivorship and Settlement Assay ....................................................................... 64
Field Survivorship and Settlement Assay ....................................................................... 64
Results ............................................................................................................................ 65
Lab Tests for Survivorship and Settlement ................................................................... 65
*In-situ* Survivorship and Settlement ........................................................................... 68
Discussion ...................................................................................................................... 72
Lab Tests for Survivorship and Settlement ................................................................... 72
*In-situ* Field Survivorship and Settlement .................................................................. 73
Conclusions ..................................................................................................................... 75

Chapter 5. Physiological Impacts of Overgrowth by *Gracilaria salicornia* on *Porites compressa* ............................................................................................................. 76
Abstract .......................................................................................................................... 76

Introduction ..................................................................................................................... 77
Materials and Methods ................................................................................................. 79
Site Description and Experimental Design ................................................................... 79
Field Transplant Experiment .......................................................................................... 81
Biochemical Analyses ..................................................................................................... 81
Zooxanthellae Density ...................................................................................................... 82
Algal Pigment Extraction ............................................................................................... 82
Oxidative Stress .............................................................................................................. 83
Metabolic Condition ....................................................................................................... 83
Statistical Analyses ......................................................................................................... 84
Zooxanthellae Density ...................................................................................................... 84
Algal Pigment Extraction ............................................................................................... 84
Oxidative Stress .............................................................................................................. 85
Metabolic Condition ....................................................................................................... 85
Results ............................................................................................................................... 85
Zooxanthellae Density ...................................................................................................... 91
Algal Pigments ................................................................................................................. 93
Chlorophyll *a* (µg cm⁻²) ................................................................................................. 93
Chlorophyll *c₂* (µg cm⁻²) ............................................................................................... 93
Total Carotenoid Content (µg cm⁻²) ................................................................................ 94
Chlorophyll *a* Content (µg cell⁻¹) ............................................................................... 94
Chlorophyll *c₂* Content (µg cell⁻¹) ............................................................................... 94
Total Carotenoid Content (µg cell⁻¹) ................................................................................ 95
Chlorophyll *a:c* Ratio .................................................................................................... 95
Oxidative Stress .............................................................................................................. 96
Metabolic Condition ........................................................................................................ 96
Discussion ......................................................................................................................... 96
Zooxanthellae Density ........................................................................................................ 97
Algal Pigments .................................................................................................................. 99
  Pigment by Surface Area ................................................................................................. 99
  Pigment by Zooxanthella Cell ......................................................................................... 99
Oxidative Stress ................................................................................................................ 101
Metabolic Condition ....................................................................................................... 102
Conclusions ...................................................................................................................... 103

Chapter 6: Gracilaria salicornia as a Competitive Dominant Invader and the Potential for Management Success ................................................................. 105
Summary ............................................................................................................................ 105
G. salicornia as a Successful Invader ................................................................................ 107
Organisms Living with G. salicornia .................................................................................. 108
Lessons Learned and Future Research ........................................................................... 109
Strategies and Challenges of Management in Hawai‘i ...................................................... 109
  Short-Term Control Measures: Manual Removal ......................................................... 110
  Long-Term Control Measures: Enhancing Grazing ...................................................... 111
The Future of Research for Management ..................................................................... 111
Final Conclusions .............................................................................................................. 113

Literature Cited .................................................................................................................. 115
List of Tables

Table 2.1 Dissolved oxygen and pH extreme maxima and minima, mean and standard deviation data from six algal mats and six reference pairs over a 24 h diurnal cycle .................................................................................................................................................. 31
Table 3.1 Observations of planulae occurring throughout the months of January–May and July-August for *Porites hawaiiensis* .............................................................................................................................................. 47
Table 4.1 Lab Survivorship, Settlement Assay .......................................................................................................................................................................................... 66
Table 4.2 Field Survivorship, Settlement Assay .................................................................................................................................................................................. 70
Table 5.1 Descriptive statistics for health variables. ............................................................................................................................................................................. 86
Table 5.2 Results of tests of significance for health parameter analyses ................................................. 88
List of Figures

Figure 2.1 Map of the study area at Moku o Lo‘e, Kāne‘ohe Bay, O‘ahu, Hawai‘i. Replicate paired study sites were along a 100 m transect in the lagoonal portion of southern portion of the windward side of the island.......................................................... 24

Figure 2.2 a) Sediment traps were placed under algal mats and 1 m away for a reference site. b) Probes from data logging sondes were placed into mats of Gracilaria salicornia and 1 m away for a reference site. .......................................................... 26

Figure 2.3 The relationship between dissolved oxygen and pH in a diurnal cycle for (a) six algal mats and (b) six reference pairs. Horizontal bars indicate the time from sunrise that these values occurred.......................................................... 32

Figure 2.4 Dissolved oxygen (solid line) and mean pH (dashed line) over a 24 hr diurnal cycle in time from sunrise at site 372 under an algal mat (a) and the reference pair (b). Mean dissolved oxygen (solid line) and mean pH (dashed line) over a 24 hr cycle in time from sunrise for (c) six algal mats and (d) six reference pairs............ 33

Figure 3.1 Porites hawaiiensis colonies growing on an assemblage of crustose coralline algae and turf in the intertidal zone on O‘ahu, Hawai‘i (scale bar 10 mm)............. 43

Figure 3.2 Porites hawaiiensis releasing, zooxanthellate planula larvae. Due to lack of buoyancy and weak swimming characteristics, once the larvae are released they scoot from the parent colony or are carried away by ciliary or water motion........... 48

Figure 3.3 Mean number of planulae released per day (cumulative) by Porites hawaiiensis colonies during the study periods one (June 2007) and two (Sept 2007 to Feb 2008). ............................................................................................................. 48

Figure 3.4 Planula release normalized to maximum daily planula production versus lunar day, for 4 complete lunar cycles for 9 reproductive colonies (October 2007 to January 2008)............................................................................................................. 49

Figure 3.5 Average number of planulae released per day (cumulative) per coral regressed against initial size of the coral colony (n=26). Open circles and the solid line for the June 2007 study and open squares with the dashed line for the Sept 2007 to Feb 2008 study............................................................................................................. 50

Figure 3.6 Mean percent of larvae settled at 48 hrs ± 2 SE in response to Hydrolithon sp. (CCA) and 48 hour microbial biofilm conditioned glass............................................. 52
Figure 4.1 a) Map of the study area at Moku o Lo‘e, Kāne‘ohe Bay, O‘ahu, Hawai‘i, b) The experimental design consisted of nine paired sites along a 1 m transect parallel to the shore, algal mat and reference sites were at least 1 m apart.

Figure 4.2 The mean percentages of a) larval survivorship, b) larval settlement, over time in the laboratory assay. Reference in white bars and algal mat group in shaded bars. Error bars are SD.

Figure 4.3 The mean percentages of a) larval survivorship for reference and algal groups at two and six days, b) overall larval settlement for reference and algal groups at two and four days of initial larvae, c) larvae settled on the top or bottom of the settlement chamber or substrate of initial larvae at two days, d) larvae settled on the top or bottom of the settlement chamber or substrate by proportion of initial larvae at six days, e) larvae settled on the top or bottom of the settlement chamber or substrate by proportion of survival at two days, f) larvae settled on the top or bottom of the settlement chamber or substrate by proportion of survival at six days, g) larvae settled on the top or bottom of the settlement chamber or substrate by proportion of total settled at two days, and h) larvae settled on the top or bottom of the settlement chamber or substrate by proportion total settled larvae at six days of deployment in the field assay. Reference in white bars and algal mat group in shaded bars. Error bars are SD.

Figure 4.4 a) Settlement chambers at two days. Left side is the reference group and the right side is the algal mat group, which had accumulated fine sediment. b) Close up of settled larvae on top of the substrate and the bottom of the settlement dish for the reference group. c) Close up of settled larvae on top of the substrate and adjacent to Gracilaria salicornia recruits.

Figure 5.1 a) Map of the study area at Moku o Lo‘e, Kāne‘ohe Bay, O‘ahu, Hawai‘i, b) The experimental design consisted of nine paired sites along a 1 m transect parallel to the shore, algal mat and reference sites were at least 1 m apart.

Figure 5.2 P. compressa at different timepoints of the experiment: a) one week under the algal mats, left to right reference and algal mat groups; b) two weeks under the algal mats, left to right reference and algal mat groups; c) three weeks into recovery, left to right, reference, algal exposure recovery transplant, algal mat group; d) five weeks into recovery, left to right, reference, algal exposure recovery transplant and algal mat groups; e) Coral under algal mats for seven weeks, the dark rings inside are coral tissue.
Figure 5.3 a) Mean chlorophyll a cm$^{-2}$, b) Mean chlorophyll c$_2$ cm$^{-2}$, c) Mean carotenoids cm$^{-2}$, d) Mean chlorophyll a cell$^{-1}$, e) Mean chlorophyll c$_2$ cell$^{-1}$, f) Mean carotenoids cell$^{-1}$, g) Mean chl a:c ratio, h) Mean zooxanthellae density cm$^2$, i) Mean DNA AP sites per 10$^5$ base pairs over time, j) concentration of L-lactate in samples on weeks one, two and five. Shaded bars represent the algal group and white bars represent the reference group; Shaded bars represent the algal group and white bars represent the reference group.
### List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tr>
<td>°C</td>
<td>Degrees Celsius</td>
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<tr>
<td>CCA</td>
<td>Crustose Coralline Algae</td>
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<td>cm</td>
<td>Centimeters</td>
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<td>CM</td>
<td>Covariance matrix</td>
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Chapter 1. Interactions Among Invasive Macroalgae and Benthic Reef Species: Insights into Chemically-Mediated Competition in Ecological Success of Invasive Algae

Abstract
Coastal areas in Hawai‘i have been invaded by several competitively dominant alien macroalgal species that alter the physical environment and compete with native organisms. Competitively dominant invasive plants can persist in extreme and stressful habitats. More subtly, these competitive dominant algae also alter abiotic factors such as hydrodynamic flow, light penetration, sediment accumulation, and water chemistry (via physiologically mediated processes) that involve the production and release of toxic chemicals such as reactive oxygen species (ROS) and volatile halogenated organic compounds (VHOC) as a consequent response of the algae to stress. Several macroalgae including invasive genera and species in Hawai‘i produce ROS in response to mechanical injury, high irradiance stress, thermal induced stress, osmotic stress, and as a response to pathogen attack. ROS is damaging to the algae so it is reduced to water with haloperoxidase enzymes, which simultaneously produce VHOC. The major VHOC produced are bromoform, chloroform, dibromomethane, and diiodomethane. VHOCs can be produced at rates that have been found to have antiepiphitic properties, inhibit bacterial growth, and deter grazing of herbivores. Of the multitude of mechanisms of competition, any one or a combination of mechanisms have the potential to alter ecological and biological processes of herbivores, algae, and corals. Eucheuma denticulatum, Kappaphycus alvarezii, Kappaphycus striatum, and Gracilaria sp. are known to produce ROS and VHOC in response to stress and it is likely that these metabolites functionally affect coral reef organisms in Hawai‘i, possibly aiding the successful invasion and persistence of these species.
**Overview**

As human impacts on our oceans increase it is important to understand stressors affecting coral reefs worldwide. Disturbances such as the intrusion of alien invasive species are increasing with documented effects on reef structure and function. The coral reef ecosystems of the Hawaiian Archipelago have evolved as unique sets of biological communities, largely affected by the islands’ relative isolation. Six introductions of prolific macroalgae have occurred in the last 60 years subjecting native Hawaiian reef organisms to new competitive interactions with unfamiliar species (Smith et al. 2002). Strategies for the management and conservation of reefs afflicted with competitive pressures from alien invasive species are currently being designed, tested, and implemented. Some research has been conducted to understand the effects of invasive marine plants on reef community structure, although the interactions that these competitive dominant species have with native species are still poorly understood. In order to understand and then manipulate the ecological role that these invasive species have, we must understand the range of physical and biological conditions that are altered as invasive species grow to dominance. Worldwide, comprehensive reviews describe specific research that aimed to understand these specific mechanisms (Chadwick and Morrow 2011, Fong and Paul 2011, Ritson-Williams et al. 2009, Birrell et al. 2008, McCook et al. 2001), but as each competitive dominant species has specific interactions, we almost need a series of case studies before an integrated paradigm can be gained.

Algae can physically abrade other organisms, alter hydrodynamic flow, increase rates of sediment accumulation and re-suspension, reduce light penetration, alter bioavailability of nutrients, and produce allelopathic toxins. Few studies have addressed the role of photosynthesis and respiratory influence on dissolved gases or metabolite production and the resulting capacity for causing oxidative stress in nearby organisms (Potin 2008, Dring 2006). Effects can also be indirect whereby an algal species is a vector for other species that induces negative impacts such as bacteria, epiflora, or epifauna. A synopsis of the existing literature reveals that the potential mechanisms of competition between coral and algae for each species combination may be different and that data gaps remain.
Mechanisms of Algal Species Competition: Abiotic and Biotic

**Abiotic Impacts**

**Hydrodynamics**

Water flow is an important property for marine organisms. In addition to supporting larval transport, removing sediment, and bringing zooplankton prey and nutrients, it is especially important to regulate the boundary layer surrounding the coral, mediating the exchange rate and flux of molecules with the open ocean, such as nutrients, waste, O$_2$, and CO$_2$. The reduction of flow can decrease the exchange rate and flux of these molecules and have negative impacts to affected organisms, for example, the buildup of respiratory gases can induce photorespiration and cause photoinhibition in photosynthetic organisms (Nakamura 2010). Several studies have shown that flow (Escartin and Aubrey 1995, Carpenter and Williams 1993) as well as advection (Worcester 1995) can be reduced by macrophytes. Large mats of macroalgae certainly have the ability to reduce water flow.

**Sediment Accumulation**

It is generally accepted that macroalgae can cause accelerated accumulation of sediments (Fabricius 2005); however few studies have actually tested this. Purcell (2000) reports correlations between the presence of algae and sediment from field studies. Stamski and Field (2006) detect and measure sediment attached to algae in the field, but do not present data on accumulation of sediment under algae. The presence of sediment has been found to prevent algal (Umar et al. 1998) and invertebrate larvae (Birrell et al. 2005) from recruiting, induce coral disease (Garrison et al. 2005) as well as increase nutrients and smother and directly kill benthic organisms (Fabricius 2005).

**Attenuation of Irradiance**

Irradiance is a fundamentally important resource for zooxanthellate corals. Photosynthetically active radition (PAR) provides energy for the photosynthetic production of carbohydrates and subsequent production of additional metabolites
transferred from zooxanthellae to coral host. In addition, irradiance has been regarded as a cue for settlement of corals (Mundy and Babcock 1998), is involved in the regulation of heterotrophic feeding behavior (Gorbunov and Falkowski 2002), and can regulate reproductive timing (Levy et al. 2007). A shading effect on the irradiance that coral receive has the potential to influence all of these important functions. Irradiance in optically clear waters at shallow depths on reefs commonly reaches 2,000 µmol photons m\(^{-2}\) s\(^{-1}\) photosynthetically active radiation (PAR), and in many cases this light is in excess of what zooxanthellae can process. Shading effects can prevent coral from achieving maximal photosynthesis (Langdon and Atkinson 2005, Porter et al. 1984) or reaching the compensation point for respiration (Langdon and Atkinson 2005, Levy et al. 2004, Porter et al. 1984, Falkowski and Dubinski 1981, Falkowski and Dubinski 1981) when light levels fall below 600 µmol photons m\(^{-2}\) s\(^{-1}\) and 350 µmol photons m\(^{-2}\) s\(^{-1}\) respectively. Additionally, as irradiance decreases the rate of coral calcification also decreases (Marubini et al. 2001). In two cases, the attenuation of light has been noted to have positive effects. Coral shaded by a canopy of Sargassum sp. avoided bleaching during a widespread bleaching event on the Great Barrier Reef (Jompa et al. 2008). Additionally, Muller and van Woesik (2009), report that light intensity influences the rate of coral-disease progression and when coral diseased with “white plague” were shaded, the progression of the disease was slower than for coral that were not shaded.

**Abrasion**

In some cases, evidence of algal abrasion has been detected with adult coral (Box and Mumby 2007) and coral recruits (Gleason 1996). This phenomenon is influenced by both algal characteristics and water motion, and is more likely a concern when algae and coral are living in high energy habitats and less likely in low energy habitats or underneath a dense algal mat with dampened water flow.

**Space**

Space is often a limiting factor on coral reefs due to competition with other organisms. Photosynthetic organisms like coral can only make use of space that falls within areas
with sufficient light. Space preemption by macroalgae has been identified as an initial competitive interaction with coral, either preventing the settlement of larvae or outward growth of juveniles or adults (Box and Mumby 2007, Mumby 2006). In addition, algal borders may regulate the linear expansion of coral growth.

**Biotic Impacts**

**Microbes**

One of the few detected positive associations that macroalgae have with coral is the influence on recruitment. Metabolites (Morse et al. 1996, Morse et al. 1994, Morse and Morse 1991, Morse et al. 1988) of crustose coralline algae (CCA) and associated bacterial biofilms (Tran and Hadfield 2011, Webster et al. 2004, Negri et al. 2001, Heyward and Negri 1999) possess cues for larval settlement. Conversely, algae can have negative impacts to coral that are mediated by bacterial communities. The presence of macroalgae promoted an increase in harmful bacteria and the subsequent death of coral tissue in laboratory studies (Smith et al. 2006). Additionally, proximity to macroalgae reduced survivorship of coral larvae under laboratory conditions with antibiotics (Vermeij et al. 2009).

**Direct Physiologically Mediated Chemical Impacts**

Turf (Wangpraseurt et al. 2012) and canopy forming macroalgae (Hauri et al. 2010) have been found to create microenvironments in which dissolved oxygen can become depleted, presumably from high oxygen consumption and through the reduction of mass exchange processes. These kinds of interactions are likely at the interface between algae and coral or under large canopies or mats of algae. Water flow likely plays an important role in reducing the boundary layer and the resultant regulation of mass transfer processes on the benthos.
**Algal Chemical Defense and Response to Stress**

Many macroalgae produce secondary metabolic compounds and toxins, which may serve an array of ecological functions. Secondary metabolic compounds are scattered amongst a diversity of classes including: terpenes, polyketides, amino-acid derived compounds, shikimates, nucleosides, prenylated quinines, and hydroquinones (Potin 2008). Physiologically-mediated metabolic processes and stress-induced metabolic products are not typically considered allelopathic as they can also serve direct functions within the algae; however the capacity of these products, or the ancillary metabolites of these products to be transported out of the alga and their ability to affect other organisms, is becoming apparent. Such examples might include products of respiration (CO₂) and reactive oxygen species (ROS) produced through a variety of mechanisms. ROS are a class of reactive oxygen compounds that can act as both oxidants and reductants. Singlet oxygen, superoxide radicals, hydrogen peroxide, and hydroxyl radicals are all types of ROS.

**Reactive Oxygen Species:**

\(^1\)O₂ (Singlet Oxygen)

Singlet oxygen (\(^1\)O₂) is dioxygen whereby two 2p electrons have a parallel spin, as opposed to anti-parallel in O₂, making it more reactive than O₂. Singlet oxygen can be produced in many ways, including photosensitation, and is highly reactive and can modify proteins, nucleic acids, and lipids (Ledford and Niyogi 2005).

O₂⁻ (Superoxide Radical)

The superoxide radical (O₂⁻) is an anionic form of O₂, which has an extra electron and is thus more reactive. It can act as an oxidant or reductant and it can diffuse across membranes at a slow rate with a lifetime of 50 µs (Lesser 2006). It can be directly converted to hydroperoxide radicals, peroxynitrate, or to hydrogen peroxide (H₂O₂) by reaction with other molecules, enzymes, or spontaneously (Fridovich 1998).
**$H_2O_2$ (Hydrogen Peroxide)**

Hydrogen peroxide ($H_2O_2$) is an uncharged, readily diffuseable ROS with water-like properties that is less reactive than others, but able to diffuse readily throughout the cell and across biological membranes, aquaporins, and aquaporin homologues (Bienert et al. 2006, Lesser 2006). $H_2O_2$ is also known to be involved in a variety of signaling pathways inducing programmed cell death (PCD) and Mitogen-Activated Protein Kinase (MAPK) (Lesser 2006, Apel and Hirt 2004) as well as biochemical processes such as inhibition of protein phosphatases, activation of transcription factors, and as a signal for gene expression (Apel and Hirt 2004). In photosynthetic organisms, $H_2O_2$ can damage DNA and enzymes involved in carbon fixation (Lesser 2006). In the presence of semiquinones, or transition metals via the Fenton reaction, $H_2O_2$ can be converted to $OH^-$. $H_2O_2$ has a dark half-life of 5.5 days in seawater, which makes it one of the more stable and long-lived of the ROS (Yuan and Shiller 2001).

**$OH^-$ (Hydroxyl Radical)**

The most reactive oxygen radical is the hydroxyl radical ($OH^-$). It can initiate free radical chain reactions, oxidize membrane lipids, and cause proteins and nucleic acids to denature (Lesser 2006, Fridovich 1998).

**Stress Induced Production of ROS in Plants**

ROS can be produced in the chloroplast, mitochondria, and endoplasmic reticulum of cells as byproducts of natural biochemical reactions as well as via stress initiated processes. Abiotic stresses, such as increased light irradiance, exposure to UV, increased temperatures, changes in salinity, mechanical stress, and exposure to xenobiotics and pollutants, can all induce ROS production. Additionally, biotic stresses including infection by pathogens, such as bacteria and other algae, can induce defense responses involving oxidative bursts of ROS. In algae, these responses can be genera- or species-specific with only some species exhibiting this behavior. One of the conditions that marine algae have been known to experience as a response of degradation due to ROS is called “ice-ice disease,” (Mtolera et al. 1996, Largo et al. 1995a, Largo et al. 1995b, Mtolera et al. 1995, Collen and Pedersen 1994). Specifically, ice-ice disease refers to the
condition of thalli and branch tips appearing white (looking like ice) and becoming necrotic, the presence of a distinct smell of chlorine, and the eventual colonization by epiphytes (Pedersen et al. 1996).

**Response to Mechanical Injury**
Research has shown that when macroalgae are mechanically injured they may respond with an oxidative burst. Collen and Pedersen (1994) were among the first to detect an \( \text{H}_2\text{O}_2 \) burst in response to mechanical stress imposed on an alga. They found that mechanical stress imposed to 10 g of the rhodophyte, *Eucheuma platycladum* Schmitz, induces an oxidative burst of 7 µm \( \text{H}_2\text{O}_2 \) after 10 minutes. Additionally, Collen et al. (1995) detected \( \text{H}_2\text{O}_2 \) production from *E. denticulatum* of up to 12 µm after just 10 minutes from imposing mechanical stress. Even with mechanical stress imposed and the potential presence of \( \text{H}_2\text{O}_2 \), these competitive dominant species may be able to recover from mechanical stress and wounding without developing ice-ice disease as the rhodophyte, *Kappaphycus alvarezii*, does following experimental wounding (Largo et al. 1995b). Multiple studies by Ross et al. (2006, 2005a, 2005b) suggest that an oxidative burst of ROS, including \( \text{H}_2\text{O}_2 \), is involved in wound repair of the chlorophyte, *Dasycladus vermicularis*, through oxidative cross-linking and activation of upstream signaling events.

**Irradiance and UV Radiation Stress**
If photosynthetic light harvesting complexes absorb more photons than they can process, a number of protective processes take place, including ROS production (Dring 2006). More specifically, plants can be photoinhibited when they are exposed to irradiances that exceed the capacity of CO\(_2\) assimilation and the over-reduction of the electron transport chain can inactivate photosystem II. An excess of electrons in the photosynthetic apparatus can be dissipated either thermally (i.e., non-photochemical quenching) or via electron acceptors in the chloroplast (i.e., photochemical quenching) and if there is a shortage of electron acceptors loose electrons can react with O\(_2\) to form ROS.
**Thermal Induced Stress**

The effects of freezing temperatures on temperate algae in intertidal zones have been studied and found to stimulate ROS production in some cases (Dring 2006). Elevated temperatures can change thylakoid membrane fluidity and render them energetically uncoupled but still capable of splitting water, thereby accumulating ROS via the Mehler reaction (Tchernov et al. 2004). It is also important to note that elevated temperatures can lower the light induced photoinhibition maxima (Lesser 2006). Largo et al. (2005b) found that *K. alvarezii* experienced decreased growth at 30 ºC and developed ice-ice disease with tissue loss between 33–35 ºC in laboratory experiments.

**Osmotic Stress**

Several studies have been done to try to understand the effects of osmotic stress responses in algae, mostly from hypo-osmotic stress (reduction of salinity). Often a tolerance response is measured in terms of optimal photosynthetic function, growth rates, or lethality (Largo et al. 2005a, Largo et al. 2005b, Ask and Azanza 2002). For example, exposure to salinity, below 20 ppt for *E. denticulatum* and 15 ppt for *K. alvarezii* for a week, have been found to be lethal via slow algal deterioration and symptoms of ice-ice disease (Largo et al. 2005b). Additionally, algal deterioration in *K. alvarezii* was found to occur under reduced salinity conditions (20 ppt) by the addition of selected strains of bacteria (Largo et al. 2005a). Few studies have examined the role of ROS and osmotic stress; however in a study by Collen et al. (1995), after mechanical stress injury was imposed on *E. denticulatum*, an enhanced oxidative burst measured as $\text{H}_2\text{O}_2$ was detected with decreased salinities of 24 and 12 ppt. Coelho et al. (2002) ran experiments to probe the role of ROS in an osmotic stress response and report that hyperosmotic stress is involved with the induction of ROS production and stimulation of non-selective ion channels that increase $\text{Ca}^{2+}$ ions intracellularly in the embryonic cells of *Fucus serratus*.

**Defense Response to Pathogen Attack**

Some macroalgae produce ROS in response to the recognition of pathogen associated molecular patterns (PAMPS) or microbial associated molecular patterns (MAMPS) by
algal cell wall components (Potin 2008, Dring 2006, Lesser 2006, Apel and Hirt 2004). MAMPs include: agars, carrageenans, and alginates (cell wall components of algae), the amino acid L-asparagine (from an alga), and lipopolysaccharides (from the outer membranes of gram-negative bacteria) as well as numerous unidentified substances that stimulate signaling cascades that result in an oxidative burst response (Potin 2008, Dring 2006, Lesser 2006, Apel and Hirt 2004).

**Biological and Physiological Implications of ROS Exposure**

Exposure to ROS can result in a variety of physiological and cellular damage. Oxidative damage refers to damage induced by the reaction of ROS with biological molecules. Specifically, ROS are known to cause damage to cellular components such as lipids, proteins, and DNA (Potin 2008, Lesser 2006). ROS can affect membranes by lipid peroxidation to create such compounds as aldehydes, malondialdehyde, and hydrocarbons (Lesser 2006). In many cases cell death may occur, especially when mitochondrial membranes are affected. ROS can react with amino acids by modifying them, fragmenting the peptide chain, aggregating cross-linked reaction products, altering electrical charge, and increasing the susceptibility to removal and degradation. Of course, any change to a protein structure, such as an enzyme, can deactivate its function. ROS can react with DNA sugar and base moieties, which can be degraded, broken, or cross-linked to proteins thereby making lesions, causing deletions, and mutations in DNA.

Organisms have evolved strategies for mitigating ROS, including a series of antioxidant enzymes, and molecules. Important enzymatic defenses are superoxide dismutase (catalyzes the conversion of superoxide to hydrogen peroxide), catalase and peroxidase (catalyzes the conversion of hydrogen peroxide to water), and glutathione peroxidase (catalyzes the conversion of hydrogen peroxide to water using glutathione as a reductant). Important non-enzymatic defenses include: ascorbic acid (acts as a reductant to scavenge superoxide, hydrogen peroxide, and hydroxyl radicals), glutathione (acts as a reductant to scavenge superoxide, hydrogen peroxide, and hydroxyl radicals), tocopherol (acts as a reductant to quench ROS, excited triplet state chlorophyll, and singlet oxygen as well as
dissipate excess excitation energy via the xanthophyll cycle, and protect the photosynthetic apparatus from overexcitation), uric acid (acts as a reductant to scavenge singlet oxygen, and hydroxyl radicals), mannitol (acts as a reductant to scavenge hydroxyl radicals), dimethylsulfide and dimethylsulfoniopropionate (acts as a reductant to scavenge hydroxyl radicals), and mycosporine-like amino acids (absorb UV radiation, scavenge singlet oxygen and superoxide).

**Ecological Implications of ROS Exposure**
While direct evidence is lacking, ROS release has been implicated in mechanical stress as a defense against herbivory (Potin 2008, Collen and Pedersen 1994). Results from mechanical injury studies do show that oxidative bursts can occur within a short timeframe (e.g., 10 minutes), certainly giving grazers a chance to be exposed to ROS before complete digestion of foods. It does seem feasible that any organism that readily and consistently grazes on a ROS-rich food source, must have its own physiological defenses to inactivate ROS effects or toxicity exposure.

By increasing pH as a result of ROS production by non-calcareous algae, calcification may be accelerated and the bioavailability of bicarbonate may become limited for tissue growth because ROS react with available protons that also react with bicarbonate to produce CO$_2$ for photosynthesis, which is important for algae that do not use bicarbonate as a nutrient source) (McConnaughey 2000).

**Volatile Halogenated Organic Compounds**
Some species of algae are known to produce volatile halogenated organic compounds (VHOC), which can remain dissolved in water, but eventually diffuse into the atmosphere. VHOC are largely produced in concert with or following an oxidative burst, primarily from H$_2$O$_2$. The most commonly produced VHOCs found in the largest quantities are bromoform (CHBr$_3$), chloroform (CHCl$_3$), dibromomethane (CH$_2$Br$_2$), and diiodomethane (CH$_2$I$_2$); however there are numerous other compounds, which are also
formed in trace amounts. Additionally, haloamines can also be formed and are regarded to be particularly toxic (Pedersen et al. 1996a, 1996b).

VHOCs are generated when haloperoxidases catalyze the reaction of H\textsubscript{2}O\textsubscript{2} with halide ions (Br\textsuperscript{−}, Cl\textsuperscript{−}, I\textsuperscript{−}) and organic substrates. The function of this reaction had initially been believed to be only a detoxification mechanism of H\textsubscript{2}O\textsubscript{2} (Manley and Barbero 2001, Pedersen et al. 1996a, Pedersen et al. 1996b, Collen et al. 1994). In addition to haloperoxidases, methyltransferase enzymes can also form halomethanes (Manley 2002). Several haloperoxidases have been identified across the Chlorophyta, Ochrophyta, and Rhodophyta. Mainly, types of bromoperoxidases and iodoperoxidases have been identified and many of these enzymes have transient metal porphyrin centers with vanadium (Dring 2006). Haloperoxidases are in chloroplasts and outer cellular surfaces (Manley 2002).

**Solubility and Transport of VHOCs in the Ocean**

Bromoform exists in flux between the ocean and the atmosphere and it is present mostly at shallow depths, it is estimated at concentrations up to 20 pmol L\textsuperscript{−1} in Hawai‘i (Quack and Wallace 2003).

**Biological and Physiological Implications of VHOC Exposure**

VHOCs have been suggested to be toxic (Collen et al. 1995), but few studies have actually tested this hypothesis. Gibson et al. (1979) found that bromoform is lethally toxic to menhaden fish and shrimp ranging from 7–40 ppm concentrations. These concentrations are larger than have been measured in the ocean from algal production.

**Ecological Implications of VHOC Exposure**

Limited research has been done to study the role that the emission of VHOC may have as an ecological defense strategy; however it does appear that VHOCs may serve functions against bacteria, algal epiphytes, grazers, and other macroalgal competitors with other macroalgae. Paul et al. (2006a) found that the alga, Asparagopsis armata, produces and
releases bromoform \((1,110 \pm 393 \text{ SE ng gDW}^{-1} \text{ h}^{-1})\) and dibromoacetic acid \((539 \pm 166 \text{ SE ng gDW}^{-1} \text{ h}^{-1})\) and could reduce bacterial density and inhibit bacterial colonization of strains of *Vibrio sp.*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus sp.*, but failed to do so when Br was unavailable as a co-factor. A study by Denboh et al. (1997) found that bromoform concentrations of 3 ppm could inhibit the development of sporelings of the brown alga, *Laminaria angustata*. Ohsawa et al. 2001 found that the production of bromoform from the alga *Corallina pilulifera* has anti-epiphytic properties with microalgae at release rates as small as 0.09 ng min\(^{-1}\) cm\(^{-2}\). In a separate study by Paul et al. (2006b), *A. armata*, with brominated products, showed deterred grazing activity by an amphipod. Gibson et al. (1979) also found that bromoform release rates as low as 19 mg l\(^{-1}\) induced an avoidance behavior (from the source) of the shrimp, *Penaeus aztecus*, and release rates induced a narcotic like effect at 31 mg l\(^{-1}\) and above. It seems that the majority of observed effects of VHOC exposure to marine organisms are negative; however, interestingly, dibromomethane was found to be a larval settlement inducer of sea urchin larvae of *Strongylocentrotus nudus* (Oshiro et al. 1999, Taniguchi et al. 1994).

In addition to VHOCs, other haloperoxidase-mediated halogenated compounds have been found to affect bacteria. Wever et al. (1991) proposed that algae could produce hypobromous acid (HOBr) as a host defense. Borchardt et al. (2001) found that haloperoxidases in the alga, *Laminaria digitata*, could catalyze reactions between H\(_2\)O\(_2\) and Br and Cl ions to produce hypobromous (HOBr) and hypochlorous (HOCl) acid, which are microbicidal compounds. Additionally, they found that these acids react with acylated homoserine lactones, which are integral components involved with bacterial cell to cell communication and thus potentially affect biofilm formations. Bromamine was found to be algicidal to the freshwater alga, *Chlorella pyrenoidosa*, while chloramines were found to be algistatic (inhibits further growth of algae, but doesn’t kill existing algae) (Kott et al. 1966).
Case Studies of the Competitive Dominant Species in Hawai‘i

Eucheumoids

Eucheuma denticulatum, Kappaphycus alvarezii, and Kappaphycus striatum are closely related species. Kappaphycus alvarezii was introduced to Hawai‘i in 1974, while K. striatum was introduced between 1970 and 1976 (Rodgers and Cox 1999). In field growth experiments, K. alvarezii has been found to grow as fast as 5.59 g d⁻¹, K. striatum as fast as 1.33 g d⁻¹, and E. denticulatum as fast as 1.99 g d⁻¹ (Glenn and Doty 1992). K. alvarezii and K. striatum have been estimated to cover the reef at a rate of 250 m yr⁻¹ in Kāne‘ohe Bay, Hawai‘i (Rodgers and Cox 1999). These algae have been found to cover up to 50% of a reef, growing in mats or mounds with a density as high as 30 kg m⁻² biomass (Conklin and Smith 2005) and have been implicated in killing corals (Conklin and Smith 2005, Woo 2000, Russell 1983). Feeding assays of Kappaphycus sp. with a native sea urchin Tripneustes gratilla (a major Hawaiian reef grazer) have found that the urchin can eat this alga; however it is less preferred than other native species (Stimpson et al. 2007).

Limited research on the physiological and chemical defense mechanisms of these species has been done. Often, E. denticulatum grows in shallow, subtidal, or patch reef environments in Hawai‘i, which routinely receive over 2,000 µmol photons m⁻² s⁻¹ of irradiance, but light saturation for this alga can be at 800 µmol photons m⁻² s⁻¹ (Mtolera et al. 1995). In laboratory experiments, Mtolera et al. (1995) found that E. denticulatum produced approximately 4 µmol H₂O₂ gDW⁻¹ h⁻¹ when catalase and peroxidase activities of the alga were active and when the enzymes were deactivated, the alga was capable of producing up to 15 µmol H₂O₂ gDW⁻¹ h⁻¹ at 800 µmol photons m⁻² s⁻¹ and increasing the pH of typical sea water from a pH of 8 to 9. Pedersen et al. (1996a) found comparable values of approximately 4.3 µmol H₂O₂ gDW⁻¹ h⁻¹ when catalase and peroxidase activities of the alga were active, and when the enzymes were deactivated the alga produced up to 15.1 µmol H₂O₂ gDW⁻¹ h⁻¹ at 800 µmol photons m⁻² s⁻¹ and 16.9 µmol H₂O₂ gDW⁻¹ h⁻¹ when pH was increased to 9. Mtolera et al. (1995) also found that pH of seawater increased to > 8.5 when E. denticulatum was grown in close proximity to Ulva reticulata and suggested that it was a competitive stress response. Collen et al. (1994)
found that mechanical stress to *E. platycladum* was also capable of inducing \( \text{H}_2\text{O}_2 \) release of approximately \( 4.2 \, \mu\text{mol} \, \text{H}_2\text{O}_2 \, \text{gWW}^{-1} \, \text{h}^{-1} \), or approximately \( 7 \, \mu\text{mol} \, \text{H}_2\text{O}_2 \) after 10 minutes of stress. Thus, \( \text{H}_2\text{O}_2 \) release may be a chemical defense against grazers.

Further, Mtolera et al. (1996) reported that \( \text{H}_2\text{O}_2 \) induced production of the primary VHOC metabolites, chloroform and bromoform. Pedersen et al. (1996) also detected a monocloramine produced by the same alga. It is striking to note that the reported production of \( \text{H}_2\text{O}_2 \) from *E. denticulatum* (up to 15 \( \mu\text{mol} \)) from previous studies is just short of the concentration required to inhibit bacterial growth and/or reduce bacterial colonies (16–26 \( \mu\text{moles} \)) as reported by Weinberger and Friedlander (2000). Additionally, the levels of bromoform that *E. denticulatum* is capable of making (100–300 ng gDW\(^{-1}\) \text{h}^{-1} or 1.67 - 5 ng gDW\(^{-1}\) min\(^{-1}\)) is well above the minimal amount found to eliminate epiphytic microalgae and diatoms (0.09 ng min\(^{-1}\) cm\(^2\) or \( \sim \) 3.1 ng min\(^{-1}\) gWW\(^{-1}\)) (Ohsawa et al. 2001).

**Gracilaria salicornia**

*Gracilaria salicornia* was introduced to Waikīkī and Kāne‘ohe Bay on the island of O‘ahu in 1971 and 1978 respectively (Smith et al. 2004). It was also taken to the Puko region of Moloka‘i from samples from Hawai‘i Island (Smith et al. 2004). Its growth rate has been estimated at 0.037 g day\(^{-1}\) ± 0.0026 SE and this plant can increase biomass by as much as 10% per day based on field growth experiments (Smith et al. 2004). This alga has been estimated to cover the reef at a rate of 280 m yr\(^{-1}\) in Kāne‘ohe Bay, Hawai‘i (Rodgers and Cox 1999), so it has the potential to spread across large areas of reef.

Feeding assays of five different species of acanthurid and scarid herbivorous reef fish suggest that *G. salicornia* is as much as eight times less preferred than native species of algae such as *Gracilaria coronopifolia* (Smith et al. 2004). Feeding assays of *G. salicornia* with the sea urchin, *Tripneustes gratilla*, have suggested that the urchin can eat this alga; however it is less preferred than other native species (Stimpson et al. 2007). In both cases, *G. salicornia* was less preferred over native algae, however it is unclear if there is a nutritional or biochemical property of the alga that renders it less palatable.
No information is available on the physiological defense mechanisms of this species and only limited data exist for other species of Gracilariales. Pedersen et al. (1996) found that *Gracilaria cornea* produced and released 3 nmol GFW⁻¹ h⁻¹ H₂O₂ at light levels of 600 µmol photons m⁻² s⁻¹ irradiance. Additionally, they found that when the algae was grown at a density of 14 kg 0.75 m⁻³, when compared to a lower density of 2 kg 0.75 m⁻³, that the pH of the 0.75 m⁻³ tank increased from 8.2 to 8.5 after just two hours. Oxidative bursts in response to pathogen response are also found for *Gracilaria cornea* J. Agardh, *Gracilaria bursa-pastoris* Gmelin Silva, *Gracilaria gracilis* Stackhouse Steentoft, Irvine et Farnham, *Gracilaria tikvahiae* McLachlan, *Graciliopsis lemaneiformis* Bory Dawson, Acleto et Foldvik (Weinberger et al. 1999), and *Gracilaria chilensis* (Weinberger et al. 2005). Evidence of oxidative stress and ROS metabolism in *Gracilaria tenuistipitata* was found in response to xenobiotic exposure (Collen et al. 2003). The alga, *Gracilaria verrucosa*, has been extensively researched because of a human poisoning in Japan, known as Ogonori disease, and the suspected bioactive compounds prostaglandins A₂ and E₂, arachidonic acid, and three chlorinated 12 carbon fatty acids have been identified (Shoab and Jaspars 2003). It is also interesting to note that only one species of *Gracilaria* has been tested for VHOCs and *G. cornea* produces far higher amounts (9.17 ng gDW⁻¹ min⁻¹) of bromoform (Pedersen et al. 1996a) than found to be effective in antifouling (0.09 ng min⁻¹ cm⁻² or ~ 3.1 ng gWW⁻¹ min⁻¹), (Ohsawa et al. 2001) and suppression of sporophyte formation (Denboh et al. 1997). There is substantial evidence of ecological chemical interactions by competitively dominant species of the red algae, *Eucheuma, Kappaphycus*, and *Gracilaria*. Species of these three genera are particularly successful invaders in Hawai‘i and individual investigation should be carried out to gauge the effectiveness of species-specific mechanisms.

*Other Invasive Algae in Hawai‘i*

The potential allelopathic impacts of *Hypnea musciformis* and *Avrainvillea amadelpha* are also of interest. *H. musciformis* is a red alien invasive macroalga that has been found to produce up to 29 ± 9 nmol H₂O₂ g⁻¹ fwt⁻¹ h⁻¹ from 600 µmol photons m⁻² s⁻¹ light (Pedersen et al. 1996b). *A. amadelpha* is a green siphonous macroalga, first noticed in
Hawaiʻi in 1980. Multiple species of *Avrainvillea*, including *A. longicaulis* (Sun et al. 1983), *A. rawsoni* (Carte et al. 1989), and *A. obtusa* (Meyer et al. 1994) have been found to produce a brominated diphenylmethane derivative termed, avrainvilleol. Meyer et al. (1994) found that chemically-extracted avrainvilleol deterred feeding behavior of the tropical herbivorous surgeonfish, *Naso lituratus*. Preliminary water-soluble extractions of *A. amadelpha* have inhibited coral larval metamorphosis and prevented calcification in laboratory exposure assays (Martinez unpub. data).

With the apparent lack of herbivory by native grazers on competitive dominant species, it is plausible that these algae may have physical or chemical properties that may render them unpalatable and deter herbivory. If so, these properties may also have negative effects on organisms living adjacent to these plants, including coral.

**Dissertation Questions and Hypotheses**

The following series of studies aim to understand these physiological competitive interactions at organismal and cellular levels using a series of physical and biological field, laboratory, physiological, biochemical, and larval ecological studies. Algal mats have the potential to impact the physical environment and thus modify habitats, which undoubtedly displace some organisms. An understanding of these physical impacts is important to develop an understanding of the potential biological impacts to native species.

The impacts of invasive species of algae are generally understudied (Coles and Eldredge 2002) and at present, there is very little information on the short-term physiological impacts of the overgrowth of invasive algae on coral health in Hawaiʻi. Such data are needed to guide management activities for control strategies. This can be accomplished through the development of new tools designed to investigate the biological impacts of the alga to coral recruitment and the direct physiological impacts to coral adults. Further, a fundamental void in coral reef restoration research is the lack of information on the success of restoration and management. The following research aims to investigate the impacts of algal overgrowth to coral health and test the success of algal removal efforts in
restoring coral health using physiological metrics. In addition, the reproductive biology and recruitment ecology of the Hawaiian indigenous coral, *Porites hawaiiensis*, is explored and described.

The research in this dissertation is focused around the following central questions and resulting hypotheses:

1. Do overgrowth and competition by alien invasive algal species adversely affect coral reef habitat quality and coral biology?

Chapter 1. Interactions Among Invasive Macroalgae and Benthic Reef Species: Insights into Chemically-Mediated Competition and Ecological Success of Invasive Algae

Chapter 2. Invasive Algal Mats Degrade Coral Reef Physical Habitat Quality

How do mound forming algae affect the physical conditions on the reef substratum such as sediment accumulation, light penetration, and water chemistry (O$_2$, CO$_2$, pH)?

_Hypothesis 2.1_: *G. salicornia* algal mats attenuate large quantities of light.

_Hypothesis 2.2_: *G. salicornia* algal mats exacerbate sediment accumulation.

_Hypothesis 2.3_: *G. salicornia* algal mounds nocturnally decrease O$_2$, nocturnally increase CO$_2$, and nocturnally decrease pH.

Chapter 3. The Reproductive Biology and Recruitment Ecology of *Porites hawaiiensis*

What are the reproductive and recruitment characteristics of the coral *P. hawaiiensis*?

_Hypothesis 3.1_: Larvae are released via brooding.

_Hypothesis 3.2_: Reproduction occurs monthly throughout the year.

_Hypothesis 3.3_: There is a positive relationship between coral adult size and increased reproductive output.
Hypothesis 3.4: Fertilization only occurs from conspecifics.
Hypothesis 3.5: Larval size is typical for Poritid larvae (5mm length).
Hypothesis 3.6: The preferred settlement substrate of larvae is crustose coralline algae.

Chapter 4. Physiological Impacts of Overgrowth by Gracilaria salicornia on Porites compressa and the Potential for Recovery

What are the impacts of alien algal overgrowth to coral recruitment?

Hypothesis 4.1: G. salicornia algal mat presence on a reef decreases the survivorship of coral larvae.

Hypothesis 4.2: G. salicornia algal mat presence on a reef decreases the settlement rate of coral larvae.

Chapter 5.

How does the overgrowth of G. salicornia affect coral health?

Can coral recover after the alga is removed?

Hypothesis 5.1: Coral overgrown by G. salicornia will exhibit a shading response by elevating pigments.

Hypothesis 5.2: Coral overgrown by G. salicornia will experience oxidative stress by elevated DNA damage.

Hypothesis 5.3: Coral overgrown by G. salicornia will undergo accelerated anaerobic respiration in response to periods of hypoxia.

Hypothesis 5.4: Live coral that has been overgrown by G. salicornia will recover from algal removal as measured by a reversal of altered physiological parameters.

Chapter 6. Gracilaria salicornia as a Competitive Dominant Invader and the Potential for Management Success (Synthesis and Recommendations)
Chapter 2. Invasive Algal Mats Degrade Coral Reef Physical Habitat Quality

Abstract
Invasive species alter the ecology of marine ecosystems through a variety of mechanisms or combination of mechanisms. This study documented critical physical parameters altered by the invasive red macroalga, *Gracilaria salicornia in situ*, including reduced irradiance, increased sedimentation, and marked variation in diurnal dissolved oxygen and pH cycles in Kāne‘ohe Bay, O‘ahu, Hawai‘i. Paired studies showed that algal mats reduced irradiance by 99% and doubled sediment accumulation. Several mats developed hypoxia and hyperoxia in the extreme minima and maxima, though there was no statistical difference detected in the mean or the variability of dissolved oxygen between different 30 min time points of 24 h cycles between algal mat-open reef pairs. The algal mat significantly acidified the water under the algal mat by decreasing pH by 0.10–0.13 pH units below open reef pH. A minimum of pH 7.47 occurred between 14–19 h after sunrise. Our combined results suggest that mats of *G. salicornia* can alter various physical parameters on a fine scale and time course not commonly detected. These changes in parameters give insight into the underlying basis for negative impact and suggest new ways in which the presence of invasive species leads to decline of coral reef ecosystems.

Keywords
Algae, *Gracilaria salicornia*, coral reef, diurnal respiration, acidification, pH

Introduction
Worldwide, algae compete with coral through a variety of mechanisms (Birrell et al. 2008, Jompa and McCook 2003, McCook et al. 2001) and algal dominance is maintained by typically intense herbivory and low nutrient additions (Sotka and Hay 2009, Smith et al. 2001, Littler and Littler 1984). In coastal O‘ahu waters, there are several species of
non-indigenous macroalgae, including *Gracilaria salicornia*, which spread across the benthos, overgrow native organisms (Smith et al. 2002), and are not palatable (Stimson et al. 2007). *Gracilaria salicornia* was introduced to O‘ahu in 1971 and 1978, and to Moloka‘i in the late 1980s from samples that originated from Hawai‘i Island (Smith et al. 2004).

The field growth rate of *G. salicornia* under ambient conditions has been estimated at 0.037 g d$^{-1}$; this alga can increase its biomass by as much as 10 % d$^{-1}$ based on field growth experiments (Smith et al. 2004). *G. salicornia* is found growing in very high sediment areas on soft bottom and hard bottom substrates from intertidal to 4 m deep (Abbott 1999) and can accumulate biomass of 5.193 kg m$^{-2}$ wet weight (Smith et al. 2004), which often grows in mounds and mats as thick as 8.9 cm (Beach et al. 1997). This proliferative alga has become well established and invasive, in some cases estimated to cover the reef at a rate of 280 m y$^{-1}$ (Rodgers and Cox 1999). Increases in algal cover have been associated with decreases in coral cover and hard substrate (Conklin 2007) as well as native algal species (Stimson et al. 2001). The mound morphology of *G. salicornia* creates an unusual three-dimensional open mesh of fused branches with space for a variety of residential organisms, most commonly filter feeders and detritivores (Rodolf Pan pers. comm.) as well as other epiphytes and epifauna. These algal mats effectively smother benthic sessile organisms, particularly the two most common coral species in Kāne‘ohe Bay, the branching coral (*Porites compressa*) and plating/foliose coral (*Montipora capitata*). Given the complex, but open mesh network of thalli that create the mound form of *G. salicornia*, accumulation of suspended sediment seems likely (Wolanski et al. 2009). To support this idea, evidence for hypoxic and hyperoxic oxygen flux can be observed in situ. For example, when this alga grows over sediment, the sediment underneath is frequently black (possibly hydrogen sulfide associated with sulfur reducing bacteria living in anoxic conditions) and yet, near the end of a day, mats can be found with bubbles loosely associated with the surface (supersaturation from thermal regime change, photosynthesis by canopy portions of *G. salicornia* or both).
Beyond the well-studied factors of nutrient enrichment and herbivory that regulate algal dominance on coral reef regions, a variety of more subtle mechanisms are also implicated in controlling algal-coral competition. These include active interactions such as pre-empting space, shading, smothering, abrasion, allelopathy (reviewed in McCook et al. 2001) by the alga as well as passive interactions such as increased sedimentation (Birkeland 1977). Further, an alga could have complex longer-term impacts by possibly promoting disease (Smith et al. 2006) as well as preventing coral recruitment (Birrell et al. 2008, Birrell 2005).

Little attention has been paid to the role of respiratory gases in affecting biogeochemistry at microscales and rapid time frames. Specifically, dissolved oxygen (DO), carbon dioxide (CO₂), and pH have physiochemical relationships, which allow these factors to vary substantially in reef settings. Biologically, DO is consumed and CO₂ is produced via respiration by marine organisms. When CO₂ concentration increases, CO₂ combines with H₂O to produce H₂CO₃⁻ (carbonic acid), which exists in equilibrium with H⁺ and HCO₃⁻. The increased availability of protons decreases the pH, thereby acidifying the water. Following this acidification, H⁺ react with CO₃ (carbonate) molecules thereby reducing carbonate, which in turn decreases the saturation states for aragonite and calcite in seawater (Doney 2010, Kleypas et al. 1999). At a low saturation state, calcification rates can decrease and CaCO₃ can dissolve. Hauri et al. (2010) found lower DO under algal mats than in reference settings above the mat for research sites on the Great Barrier Reef at a fixed time point in the day. However, DO and pH levels may differ between the benthos and several cm above an algal mat due to ‘background’ benthic respiration under conditions without macroalgal mats and with varying hydrodynamic processes. Further, very little is known about how the processes of photosynthesis and respiration affect the diurnal flux of DO and pH under algal mats and how these changes may compare with ‘natural’ benthic processes. The persistence of *G. salicornia* allows it to impose any one or combination of mechanisms of impact on other benthic organisms, especially the obligately sessile life form—coral.
The goal of this study was to understand how mats of *G. salicornia*, and the associated biological communities, can affect coral reef habitat quality at fine scales and in rapid time courses. We compared physical parameters under the algal mats with open reef benthic reference sites. Our goals were to assess the quantity of irradiance attenuated by algal mats, the quantity of sediment trapped by an algal mat, and to explore the relationship between these phenomena with algal mat morphometrics. To frame these interests, we tested a series of hypotheses: a) Does photosynthesis from the algal mats increase DO and pH under the algal mats to levels above the open benthic reef; b) Does nighttime respiration of the algal mat and associated species reduce DO and pH under the algal mats to levels below the open benthic reef; and c) Do extreme maxima, minima and variability imposed by these processes differ from the open benthic reef?

### Materials and Methods

#### Site Description and Experimental Design

The study site was the lagoonal portion of southern portion of the windward side of Moku o Loʻe, Kāneʻohe Bay, Oʻahu, Hawaiʻi (21° 25.874' N, 157° 47.257' W; Figure 2.1). This location was selected for the ubiquitous mats of *G. salicornia* growing over live coral along the reef at a similar low tide depth (0.4–1.8 m).
Figure 2.1 Map of the study area at Moku o Lo‘e, Kāneʻohe Bay, Oʻahu, Hawaiʻi. Replicate paired study sites were along a 100 m transect in the lagoonal portion of southern portion of the windward side of the island.

Ten replicate sites were chosen along a 100 m transect that met the following criteria: within 2 m depth interval, presence of an algal mat growing over carbonate reef areas with live coral, spaced at least 10 m from other research sites, and the presence of a suitable reference pair (without algae) at least 1 m from algal mat. A paired sampling design was used to test mean values for specified physical parameters between algal mat and reference sites.

**Irradiance Measurements**

Measurements of photosynthetically active radiation (PAR) were taken in water on November 15, 2010 at 1145 HST (Hawaiian Standard Time) with direct sunlight, directly under the center of the algal mat and then just above the algal mat for reference samples.
Measurements were taken with a LICOR LI-250A photometer with a flat plate cosine quantum sensor and corrected for underwater attenuation.

**Sediment Measurements**

To measure the amount of sediment accumulated under an algal mat and compare with reference values, sediment traps were deployed (Storlazzi et al. 2011). Two plastic 50 ml TB Falcon tubes were stacked with the bottom of one tube cut (to allow sediment to sink) and zip tied to small wooden stakes, which were secured inside crevices or sand pockets on the reef (Figure 2.2a). The total height of the trap was 20.5 cm and the surface area of the opening was 6.6 cm². One trap was placed under the horizontal center of an algal mat and oriented vertically at 90°. A reference trap was placed 1 m from that algal mat, for a total of 10 pairs of sediment traps. The traps were collected after eight days. Care was taken to cap the tubes underwater to not lose sediment. The trap contents were vacuum-filtered through pre-weighed Millipore 0.2 µm filter and dried in an oven for 2 d at 40°C. The filters were weighed and the difference was used to calculate the mass of dry sediment as g sediment d⁻¹.
Figure 2.2 a) Sediment traps were placed under algal mats and 1 m away for a reference site. b) Probes from data logging sondes were placed into mats of *Gracilaria salicornia* and 1 m away for a reference site.
Respiration, pH, and Salinity Measurements
Paired replicates in this study were sampled February 4 and 11, 2010; July 7 and 15, 2010; and August 6 and 18, 2010 to evaluate short and long day variation. We placed deployable data-logging sondes (YSI 6600 EDS V2, Yellow Springs, OH) with conductivity, temperature, turbidity, depth, dissolved oxygen, and pH probes in an algal mat and 1 m away (reference pair), each fixed the same distance from the bottom, which was approximately 5 cm. Each algal mat was carefully lifted and the sonde was placed underneath (Figure 2.2b). Data were logged every 5 min for several days. Six replicated observations using different algal mats and reference pairs were made over three to five days.

Algal Mat Size
After other measurements were taken, a six by six cm section of each algal mat was collected, the mat height was measured with a standard metric measure, the epifauna and flora were removed, the mat was rinsed to remove salt, dried at 80ºC in the laboratory for two days, and weighed for computation of biomass measurements.

Statistical Analyses
Minitab 16 statistical software was used to calculate tests of homogeneity of variances. PRISM GraphPad v. 5.0b was used to produce all plots. All remaining statistical analyses were performed using the SPSS statistical software 17.0.

Sizes of Algal Mats
Sizes of algal mat were calculated using several metrics: dry weight per area covered by mat (kg m\(^{-2}\)), thickness (cm), and density (g m\(^{-3}\)). Relationship between algal mat thickness and algal mat density as well as algal mat biomass and algal mat density were analyzed with linear regressions.
**Irradiance**

A Student’s paired t-test was used to compare the mean quanta of PAR transmitted on top of the algal mat and underneath it.

**Sediment**

The sediment trap rate (Storlazzi et al. 2011) was calculated as the mean sediment per day per area (g d\(^{-1}\) cm\(^{-2}\)) for each trap. Data did not meet the assumptions of a parametric paired test of means; non-parametric sign test was used to compare the mean sediment trap rates between the algal mat and reference group. Regression analyses were performed to find the best metric of algal mat size and quantify the relationship between algal size and the sediment trap rate.

**Dissolved Oxygen and pH**

Data for analyses were selected from time points at least 24 h after placement of sensors to allow the mound dynamics attain steady state rates after sensor deployment. Twenty-four hour cycles from six replicate-paired sites with data from 30 min intervals were normalized to time from sunrise with astronomical data from the US Naval Observatory Astronomical Data Services. This was necessary as the data varied over time of day associated with photosynthesis by *Gracilaria salicornia*. For data that were found to meet parametric statistical assumptions, the mean DO and pH levels for time intervals of 30 min throughout a 24 h cycle were tested between the algal mat and reference groups (48 tests per parameter) using a Student’s t-test. For data that did not meet the parametric statistical assumptions, the non-parametric sign test was used to test for differences with ranked medians. Spearman rho correlation analyses were conducted to examine the relationship between DO and pH for algal mats and controls with pooled data from a 24 hr cycle of five replicate pairs.
Results

Size of Algal Mats
The biomass of algal mats sampled ranged between 7.27 kg–20.20 kg dry weight m\(^{-2}\). Linear regression analyses tested the relationship between the algal size morphometrics of biomass (g m\(^{-2}\)) and height of mat (cm) with density (g m\(^{-3}\)). Both parameters were found to predict density, with biomass explaining 72% of the variation in density with a positive association and height explaining 42% of the variation with a negative association. Biomass, \(y=22.989x + 44.273, R^2=0.71, p<0.05, DF=9\). Height of mat, \(y=-3,455.625x + 29,375.761, R^2=0.42, p<0.05, DF=9\).

Irradiance
The Student’s paired t-test revealed significantly less photosynthetically active irradiance transmitted to the benthos under algal mats than for the reference group (\(p < 0.05, DF=9\)). The mean quantity of photosynthetically active irradiance transmitted under algal mats (n=10) was 1.17 ± 2.31 SE \(\mu\text{mol photons m}^{-2} \text{s}^{-1}\) and the mean for the reference group (n=10) was 1191.20 ± 60.201 SE \(\mu\text{mol photons m}^{-2} \text{s}^{-1}\). The mean ratio of light attenuated by algal mats was 99.3% ± 0.17 SE.

Sediment
The nonparametric sign test revealed a significantly higher sediment trap rate in the algal mat when compared with the reference group (\(p < 0.05, DF=8\)). The mean sediment trap rate for the algal mat was 25.28 ± 2.371 SE g m\(^{-2}\) d\(^{-1}\); the mean for the reference was approximately 50 % lower at 12.71 ± 2.435 SE g m\(^{-2}\) d\(^{-1}\). The majority of sediment collected appeared to be fine particulate. The sediment collected from within the mats was dark brown/black and had a hydrogen sulfide-like odor, whereas the sediment in controls was light brown or white.
Linear regression analyses tested the relationship between the algal biomass (kg m$^{-2}$) and morphometrics of height of mat (cm), and density (kg m$^{-2}$) as well as the rate of trapping sediment (kg m$^{-2}$ d$^{-1}$). Algal biomass and morphometric data explained little of the variation and did not predict the rate at which sediments were trapped (data not shown).

**Dissolved Oxygen**

Mean dissolved oxygen for five replicate algal mat pairs changed with time from sunrise. With appropriate paired sample statistical tests, no significant differences in mean DO levels were detected at particular 30 min intervals for algae and control groups, ($p > 0.05$, n=4) for all time points. However, nighttime minimum DO values (Table 2.1) in algal mats were found to be half those of reference values. Values of DO during daytime maximums were 47.25% higher under the algal mats than at the reference sites. Two mats had minimal DO values of 1.04–1.99 mg l$^{-1}$ and developed hypoxia (< 2 mg l$^{-1}$ or < 30% saturation) between time intervals 12 h 30 m from sunrise to 1 h 30 m after sunrise the next day or approximately 1830–0730 Hawaiian Standard Time (HST). Each instance of hypoxia lasted between 30–120 m. There were only near hypoxic values found for two reference sites with a minimum value of 2.12–2.99 mg l$^{-1}$ between 18 h 30 m from sunrise to 01 h 30 m after sunrise the next day or approximately 0030–0730 HST. The maximum value for the algal mat group reached 11.78 mg l$^{-1}$ (180 % saturation) and three mats maintained hyperoxia (> 100 % saturation) at various times of the day. The maximal value for the reference group was 8.00 mg l$^{-1}$ (108.9 % saturation) and one reference group site had hyperoxic DO levels between 2 h 30 m to 12 h from sunrise or approximately 0830–1800 HST. Though the overall standard deviation of the mean dissolved oxygen in the algal mat group was twice that of the reference group, the test of homogeneity of variances revealed no significant differences between the variability imposed on the oxygen levels at a 30 min interval ($p > 0.05$).
Table 2.1 Dissolved oxygen and pH extreme maxima and minima, mean and standard deviation data from six algal mats and six reference pairs over a 24 h diurnal cycle

<table>
<thead>
<tr>
<th></th>
<th>Maximum</th>
<th>Minimum</th>
<th>Mean</th>
<th>Std. Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>DO Algae (mg/l)</td>
<td>11.78</td>
<td>1.04</td>
<td>5.00</td>
<td>2.64</td>
</tr>
<tr>
<td>pH Algae</td>
<td>8.2</td>
<td>7.47</td>
<td>7.84</td>
<td>0.159</td>
</tr>
<tr>
<td>DO Reference (mg/l)</td>
<td>8</td>
<td>2.12</td>
<td>4.78</td>
<td>1.2</td>
</tr>
<tr>
<td>pH Reference</td>
<td>8.23</td>
<td>7.61</td>
<td>7.93</td>
<td>0.133</td>
</tr>
</tbody>
</table>

**pH**

Significant differences in pH were detected (0.1–0.13 pH units) in 14 of the 30 min intervals (just under 5% of all observations) when examining the algal mats against the reference groups at 14 h, 15 h 30 min, and between 19 h from sunrise to 2 h after sunrise the next day or approximately 2000, 2130, 2300–0200 hrs HST respectively. Extremely low pH values of 7.61 in the reference and 7.47 in the algal mats were also detected. Maximal values were pH 8.23 for the reference and pH 8.20 for the algal mats.

**Dissolved Oxygen and pH**

To test relationships between DO and pH, we performed Spearman rho correlation analyses for data points from the reference group and the algal mats. Tests found a moderately positive correlation between DO and pH in the reference group (0.674, p<0.000) and a stronger correlation in the algal mat treatment (0.749, p<0.000). Mats had a more diffuse relationship between DO and pH than that observed at the reference site (Figure 2.3). DO and pH levels for one of the sites that experienced hypoxia in a 24 hr cycle normalized for time from sunrise for the algal mat and reference pair respectively, are shown in Figures 2.4a and 2.4b. When normalized for time from sunrise for the algal mat and reference groups respectively (error bars removed for visualization), similar patterns were revealed (Figures 2.4c and 2.4d). For the reference group, DO and pH began to increase 2 h after sunrise (0800 HST). DO increased to its maximum mean value (6.00 mg l⁻¹), 7 h 30 m–9 h 30 m after sunrise (approximately 1330–1530 h HST).
pH reached a maximum mean value of 8.00 at 4 h 30 m–11 h 30 m after sunrise (1030–1730 HST). Near the end of daylight, DO and pH declined until they reached nighttime minimum mean values of 3.52 mg l\(^{-1}\) and pH 7.80 at 22 h 30 m from sunrise (approximately 0430 HST) respectively.

Figure 2.3 The relationship between dissolved oxygen and pH in a diurnal cycle for (a) six algal mats and (b) six reference pairs. Horizontal bars indicate the time from sunrise that these values occurred.

For algal mats, DO began to increase 1 h 30 m after sunrise with pH increasing 2 h after sunrise. DO increased to its maximum levels (6.80 mg l\(^{-1}\)) from 7 h 30 m–9 h 30 m after sunrise (approximately 1330–1530 HST), while pH increased to its maximum mean value of 7.95 between 7 h 30 m–10 h 30 m after sunrise (1300–1800 HST). Near the end of daylight, DO and pH declined until they reached night time minimum mean values of 3.40 mg l\(^{-1}\) and pH 7.71 at 23 h from sunrise (approximately 0500 HST) respectively.
Discussion

This study has demonstrated several important modifications of the benthic physical environment caused by algal mats of *G. salicornia*. Decreased irradiance, sediment trapping, and periods of hyperoxia, hypoxia, and acidification are all stressful conditions caused by the algal mats. We found significant changes in the timescale of 30 m, which underscore the need to take environmental measurements over a comprehensive timescale, as detailed in the next sections.
**Algal Size**

Algal biomass was found to be a strong predictor of algal density. Algal mat height was found to also predict algal density, but with a negative association with thicker mats being less dense. This finding was surprising as mat forms *in situ* appear homogeneous as presented in a 2D surface suggesting from casual observation that density of the mats did not vary. Though it is possible that water motion or herbivory could crop the mats or pull them loose.

**Irradiance**

The findings in this study corroborate with other studies (Dailer 2006, Beach et al. 1997), which have reported substantial attenuation of PAR by algal mats. Several physiological features of *G. salicornia* likely allow it to persist in such a high biomass state in both high light and low light environments. *G. salicornia* mats are known to self-shade, acclimating with a gradient of accessory pigments increasing from outside the mat to within (Beach et al. 1997). In addition, the alga’s photosynthetic saturating irradiance ($I_k$) can range from 134–633 µmol photons m$^{-2}$ s$^{-1}$ and compensating can range from 6–37 µmol photons m$^{-2}$ s$^{-1}$ in different habitats (Phooprong et al. 2007), which allows this alga to maintain high productivity and meet its basic metabolic needs under relatively low light conditions.

The consequences of reduced irradiance to coral have clear physiological implications. The near maximal levels of irradiance found under these algal mats (1.17 µmol photons m$^{-2}$ s$^{-1}$ ± 2.31 SE, n=10) are analogous to irradiance levels at greater than 45 m depths (Rivero-Calle et al. 2008) and are insufficient to achieve maximal photosynthesis in shallow reef corals living under either attenuated full sun (~231–586 µmol photons m$^{-2}$ s$^{-1}$ Langdon and Atkinson 2005) or deep shade (~31–89 µmol photons m$^{-2}$ s$^{-1}$ Porter et al. 1984). Further, these under-mat irradiances may not allow for either physiological group of corals to compensate for respiration (~150–350 µmol photons m$^{-2}$ s$^{-1}$ Langdon and Atkinson 2005, Levy et al. 2004, Falkowski and Dubinski 1981) or deep shade-adapted
coral (~21–50 μmol photons m$^{-2}$ s$^{-1}$, Porter et al. 1984, Falkowski and Dubinski 1981). While mesophotic communities can exist in extremely low irradiance, the taxa in those deep reefs are not common to shallow coastal zones (Rooney et al. 2010). A point of final concern is that under low irradiances, coral rates of calcification fall to low levels (Marubini et al. 2001).

**Sediment**

There was a significantly higher sediment trap rate for fine sediments in the algal mat treatment than for the reference group. As sediment fell or moved with water motion, the three dimensional structure of algal mats may allow multiple surfaces to baffle water flow, allowing sediment to fall out. It is also possible that waste, particulate organic matter, or decomposing tissue of the biological community inhabiting the algal mat contributes particulate material to the benthos below. Other studies have found either correlations between algae and sediment accumulation from field studies (Purcell 2000) or measured sediment previously accumulated on algae in the field (Stamski and Field 2006), but not actually transported to the benthos underneath. This study demonstrates enhanced sediment accumulation under algal mats. Sediment has several biological effects including preventing algal (Umar et al. 1998) and invertebrate larvae from recruiting (Birrell et al. 2005), inducing coral disease (Garrison et al. 2005), smothering and directly killing benthic organisms as well as increasing nutrients (Fabricius 2005). Algal mats like those of *G. salicornia* may further trap sediment, which may support algal growth and thus contribute to a positive feedback loop. Data from this study suggests that algal removal efforts, which have a secondary goal to reduce accumulated sediment, may be successful for both biomass removal and reduction in secondary impacts from sediment accumulation. Further, it might be expected that filter-feeding organisms might benefit from inhabiting the algal mat as there may be an increased opportunity for catching food; this corroborates with previous observations (Pan et al. 2008). The macroalga, *Dictyosphaeria cavernosa*, can take advantage of nutrients from sediment and waste from inhabiting animals when water column nutrient conditions are insufficient (Stimson et al. 1996) and this may be true for *G. salicornia* as well.
**Dissolved Oxygen**

There was a great deal of variability in the data from the algal mat and the reference groups; we did not detect a statistically significant difference for DO in mean or variances. Yet, water under algal mats reached extreme minimum hypoxic and maximum hyperoxic conditions in this study. There are several biological implications for an organism experiencing these phenomena. Low oxygen availability reduces the aerobic respiration capacity of an organism to lethal levels and can drive mobile species to evacuate an area (Grantham et al. 2004). At the other end of the spectrum, high concentrations of oxygen in water can produce reactive oxygen species (ROS). ROS such as superoxide, hydrogen peroxide, hydroxyl, and peroxyl molecules are all known to induce oxidative damage to biological organisms (Lesser 2006).

Extreme fluctuations in these variables can impose a great deal of stress into a habitat, which could drive the community composition based on organisms that can tolerate living in these conditions. Crustaceans are known to have significantly higher lethal hypoxia oxygen thresholds than other groups (making them the most sensitive), though fish have the highest sublethal hypoxia oxygen threshold with Crustaceans and Molluscs next and Polychaetes, Echinoderms, and Cnidarians with the lowest (Vaquer-Sunyer and Duarte 2008). If hypoxia induced by the algal mats was an issue on the reef, it might be expected that mobile organisms such as fish, echinoderms, crustaceans, and gastropods may be able to temporarily inhabit these algal mats in the daytime and then move as DO conditions go hypoxic. In contrast it might be expected that benthic sessile organisms such as coral, algae, tubeworms, snails, and sponge may be most susceptible to the extreme DO minima.

Kuhl et al. (1995) measured DO in coral tissue under different flow and light regimes and found that % saturation of DO fall from 100 % to \( \leq 2\% \) in stagnant and 5–20% in low flow conditions with thick boundary layers. Under moderate irradiance, photosynthesis can increase DO to as much as 240% saturation under high flow conditions with a thin boundary layer. These ranges are well below and above the minima and maxima found in this study. The measurements taken in this study were near the center of the
microhabitat under the algal mat and not at the coral surface. The levels of oxygen that corals experienced under these algal mats are unknown, but it is possible that DO levels are much lower than what we measured in this study. While some algae and coral cope with daily hypoxic and hyperoxic conditions with enzymatic and non-enzymatic antioxidant defenses (Lesser 2006), this tolerance might allow them to survive under oxidative damaging conditions at least temporarily. *G. salicornia* imposes these conditions on itself when it grows in large biomass. Portions of the algae are frequently buried in mud and are still pigmented and alive, and thus one of the keys to this alga’s success may likely be a well-developed and effective oxidative damage response physiology.

**Respiration and pH**

Levels of DO can be used as a proxy measure for respiration (Clavier et al. 2008). As the algal mat community respires, O$_2$ is consumed and CO$_2$ is released. The variability imposed on the relationship between DO and pH in the algal mat group could likely be from respiratory acidification in the daytime while photosynthetic basification (O$_2$ production) was occurring. The relationship between DO and pH can be further interpreted from their corresponding diurnal cyclicity, as was seen in our data. As O$_2$ production increased, there was a point at which pH peaks in both data sets. This may be a reflection of community respiration in the day, where CO$_2$ and O$_2$ production are both occurring. It is also interesting to note that it takes about 1.5 h until production meets the compensation point for respiration (where DO begins to increase).

The mean and minimum pH under the algal mats, were found to be less than that for the reference groups, though the maximum pH was similar. The daily increase in pH may be explained by the consumption of CO$_2$ in photosynthesis and the production of hyperoxic water with ROS, which could accept hydrogen ions and thus increase the pH. A corresponding pH maximum for the algal group was not detected above the reference group, but instead stayed maximally at 8.2. It is possible that CO$_2$ produced from diurnal metabolic respiration and photorespiration (from the algal mat and epiphytes) was concurrently consumed by carbon fixation reactions of photosynthesis.
The impacts of decreased pH to coral and coral reef invertebrates are now just beginning to be understood. Minimal pH levels found in this study under the algae are comparable to published values that showed impacts to coral health, such as skeleton dissolution, decreased settlement, fertilization success, and growth. pH ranges of 7.3–7.6 have been found to dissolve coral skeletons (Fine and Tchernov 2007). pH conditions of 7.7–7.9 decrease several biological processes in coral, such as fertilization success, gamete viability, settlement of larvae, and growth in juvenile recruits (Albright et al. 2008, Albright et al. 2010). pH conditions of 7.3–7.6 delay the algal infection rate of recruits (Suwa et al. 2010). The abundance of red calcareous algae has also been found to decrease under low pH conditions with shifts to increased abundance of non-calcareous algae (Fabricius et al. 2011, Kuffner et al. 2007).

It is still uncertain how the global process of ocean acidification will affect these kinds of microscale processes. With estimates of global surface acidification of 0.14–0.35 pH units by the 21st century (IPCC), microscale processes like nocturnal benthic respiration could affect or exacerbate detrimental effects on corals. While DO and pH can vary in these algal microhabitats, the implications to organisms like coral when considered in synergy with other factors remain important but uncertain. Such concerns become more important as non-palatable invasive species, like *G. salicornia*, spread at ~ 280 m y–1 in Kāne‘ohe Bay, O‘ahu (Rogers and Cox 1999) as well as outside (Abbott 1999), and if it remains unchecked will impact macro-scale classes of benthic habitat on at least two islands.

At present conditions, coral reefs can undergo a small dissolution at night (Suzuki et al. 1995), but light and dark calcification can more than compensate for that (Yates and Halley 2006). It is possible that the lower pH found under invasive algal mats may decrease the aragonite saturation states at night and accelerate dissolution of CaCO3 reef material, biological skeletons, and reduce the ‘dark calcification rate’ of calcifying organisms. For confirmation, calcification, alkalinity, and carbonate chemistry
measurements should be taken diurnally under the algal mats to understand the direct influence to the processes of calcification and dissolution.

Because DO and pH minima in the algal mats are lower than those observed in open reef conditions, flow in the algal mat is likely to be reduced and the boundary layer above coral overgrown by the algal mats would be expected to be much larger than open reef regions. Several studies have shown that flow (Escartin and Aubrey 1995, Carpenter and Williams 1993) as well as advection (Worcester 1995) can be reduced by macrophytes. Water flow is important for aerobic benthic heterotrophic organisms for food and to reduce the boundary layer thus increasing diffusion of respiratory gases. Additionally, for autotrophic benthic organisms flow increases the diffusion of nutrients and can reduce photorespiration and photoinhibition (Nakamura 2010).

**Conclusions**

Mats of *Gracilaria salicornia* impose microscale changes in water quality to the living community under these mats. These modifications include changes in physical processes of sediment accumulation and attenuation of irradiance. Additionally, as a function of respiration with nightly periods of hypoxia and accelerated acidification, mats impose single and synergistic combinations of stress on organisms living under mats. These data suggest that the presence of large biomass stands of *G. salicornia* mats have the capability to drastically change benthic habitat quality, that several mat morphometrics be considered when measuring physical parameters in and under the alga, that comprehensive diurnal sampling be performed when trying to understand microscale biogeochemical processes, and that ocean acidification models at the appropriate scale include these variables, especially when invasive algal biomass reaches the large scale of reefs.
Chapter 3. The Reproductive Biology and Recruitment Ecology of *Porites (Synaræa) hawaiensis*

**Abstract**

*Porites hawaiensis* (Vaughan 1907) is a small, shallow-water zooxanthellate scleractinian coral endemic to the Hawaiian Archipelago. This species is a brooding coral, releasing zooxanthellate planula larvae daily through the year. Colony size and reproductive output are positively correlated with colonies 0.882 cm$^2$ or larger being reproductively mature. The larvae of *P. hawaiensis* were negatively buoyant upon release and did not swim in the water column under laboratory conditions, but rather "crawled" on substrata. Larval metamorphosis and settlement were induced by the presence of the crustose coralline algae *Hydrolithon onkodes*, usually within 48 h of initial exposure. The ecology of *P. hawaiensis* and the behavior of its larvae may limit the dispersal potential and geographic range of this endemic taxon. The availability of *P. hawaiensis* planulae through the year and the observed larval response to metamorphic inducers makes this a good model organism for studies to test the effect of various parameters on larval recruitment over its geographical range.

**Keywords**

*Porites hawaiensis*, coral reproduction, planulae, coral recruitment

**Introduction**

Coral reefs persist through the linked processes of reproduction, the formation of new individuals from prior stock, and recruitment, the process through which these new individual become part of a population. Corals have the ability to reproduce both sexually, through the formation of gametes that can fuse and develop into larvae, and asexually, through fragmentation or the formation of propagules in the absence of gamete fusion. Corals display diverse life history strategies, with the majority of species of coral for which detailed reproductive data are available identified as hermaphroditic broadcast
spawners, with notably fewer species exhibiting brooding behavior (Baird et al. 2009; Richmond and Hunter 1990; Harrison and Wallace 1990). In Hawai‘i, only 25% of the coral species for which data are available are known to brood larvae, while the majority spawn gametes (Kolinski and Cox 2003).

Of the species of Poritidae for which reproductive data are available, most have been observed to be gonochoric broadcast spawners (Baird et al. 2009; Kolinski and Cox 2003; Richmond and Hunter 1990; Harrison and Wallace 1990). Porites brighami and Porites lichen, are the only known brooding Poritids in Hawai‘i and they represent 1/3 of the family Poritidae in Hawai‘i for which reproductive data are available (Baird et al. 2009). These corals, like other brooding Poritids, grow to a relatively small size. Porites furcata in the Caribbean and Stylarea punctata (the smallest Poritid coral) in Guam have been found to planulate monthly, year round (Schloeder and Guzman 2008; Golbuu and Richmond 2007).

Porites hawaiiensis, also referred to as P.cf. bernardi by Fenner (2005), is typified by flat, encrusting colonies that grow to a size less than 10 cm in diameter and thus far has been found only in Hawai‘i. This specie’s small size and inconspicuous nature have contributed to a history of taxonomic confusion in the scientific literature (Forsman et al. 2010) and has thus far not been found in areas surrounding the Hawaiian Archipelago (Line Islands, Phoenix Islands, Johnston Atoll) despite specific searches (J.E. Maragos pers. comm.). The only published data on its distribution are from Honolulu, Hawai‘i (Vaughan 1907) and the Northwestern Hawaiian Islands in the Papahānaumokuākea Marine National Monument (Maragos et al. 2004). Colonies of this species have been observed to live at depths ranging from the shallow subtidal to a depth of 5 m (Fenner 2005 referring to P.cf. bernardi; Maragos et al. 2004). The reproductive mode, reproductive behavior, and larval and recruitment ecology of P. hawaiiensis were previously unknown and are described here.

The goal of this study was to investigate key biological and ecological attributes of reproduction and recruitment in Porites hawaiiensis, including the mode of reproduction,
reproductive frequency, the relationship between coral size and fecundity, larval behavior, and responses to putative settlement cues. These data are compared with those of other species of corals from Hawai‘i and within the family Poritidae and demonstrate a similar pattern for several small species.

**Materials and Methods**

Data on reproduction were collected using a combination of adult colony dissections, real-time observations of planulation as well as planula catchment. Reproductive patterns were determined from two larval catchment study periods: Period one (June 2007) and Period two (September 2007–February 2008). Recruitment studies were performed in the laboratory using the collected larvae with the subsequent presentation of various substrata under controlled conditions.

**Field Collection and Size Measurements**

Colonies of *P. hawaiiensis* were collected from the subtidal-intertidal coast (0–3 m deep) adjacent to the Kewalo Marine Laboratory (KML), Honolulu, Hawai‘i (21°17′35.4″ N, 157°51′53.7″ W). Additional colonies were collected from within a large (ca. 3,000 l) flow-through sea water tank maintained at the KML, which likely originated from larvae entrained from the areas surrounding the seawater intake and which are likely to be of the same population. Crusts of non-articulated crustose coralline algae (CCA) found with the coral were removed from rocks using a hammer and chisel (Fig. 3.1).
A total of eight colonies were collected from the seawater tanks from January through March 2007 for dissection and larval collection. For these studies, three colonies of *Porites hawaiiensis* were collected from the sides of the seawater tanks and 10 were collected from the field site in June 2007 for study period one and an additional 10 were collected from the field site in September 2007 for study period two. Corals used in this study were immediately photographed following collection for measurement purposes with a high resolution digital camera, and each colony was placed into a separate 600 ml Nalgene plastic beaker maintained in an outdoor seawater table with individual ambient flow-through seawater, and were covered with an 80% light reduction shade cloth. Temperatures ranged from 25–30 °C throughout each study. Surface area (cm²) was measured using the NIH Imaging Software ImageJ (http://rsb.info.nih.gov/ij/) from the high-resolution photos taken the day of collection, which were calibrated with a standard metric measure.
**Colony Dissections**

Colonies of *P. hawaiiensis* were taken into the laboratory and dissected into longitudinal sections (~ 1 cm) with a stainless steel dissection razor blade. Polyps and mesenteries were visually inspected for presence of larvae using a Zeiss Stemi SV11 stereomicroscope (Germany). This technique proved to be an efficient means of identifying colonies containing fully developed larvae.

**Larval Collection and Measurements**

The outgoing water from the collection beakers was inspected and no larvae were obtained from the discharge. The content of each beaker was inspected under a Zeiss Stereo microscope, collected using glass Pasteur transfer pipettes, and larvae were stored in 0.20 µm Millepore-filtered seawater in covered 200 ml glass Pyrex beakers.

For study period one, the beakers were inspected daily for motile larvae; for study period two the beakers were inspected 2–3 times a week (data are presented as cumulative planulation between dates of inspection). Following larval collection, the parent corals were cleared of sediments and filamentous algae and the beakers cleaned.

Mean larval release (data pooled across all colonies per day) versus sampling dates and relative daily planulation versus lunar age days (pooled across four complete lunar cycles of October 2007 to January 2008) were graphed on scatterplots. Relative daily planulation is the mean number of larvae released per lunar day, pooled across all nine reproductive colonies, and normalized as a proportion of the maximum number released, as per Richmond and Jokiel (1984). Mean larval release pooled across all planulation dates versus coral size for both study periods was also graphed on a scatterplot. Linear regression analyses were performed using SPSS Statistical software version 17.0.

Seawater quality was reduced (low flow and higher sediment) for study period two, due to a seawater intake pipe malfunction and subsequent repairs. Corals sampled during study period one were maintained in seawater from an intake that was 250 m from shore.
at a depth of 10 m, while during study period two, seawater intake repairs required the use of seawater from a pipe in the Kewalo Channel 6 m from shore and in 3 m water depth.

**Larval Biology**

Larvae were observed under a microscope for locomotive behavior. Larval size was measured within 24 hrs of release for n=189 larvae using a Zeiss Stereomicroscope with an ocular scale calibrated with a standard stage micrometer. Linear measurements of length and width were taken and fitted to equations for calculating the volume of a prolate spheroid according to Isomura and Nishihira (2001) using the following equation:

\[ V = \frac{4}{3} \pi ab^2 \]

where \( a = \text{length}/2 \) and \( b = \text{width}/2 \).

There were no observed changes in size or contraction behavior of larvae during measurements.

**Larval Settlement**

A series of settlement assays were designed for assessing the settlement preferences of *P. hawaiiensis* larvae, similar to those used by Golbuu and Richmond (2007). After larvae were collected from adult colonies, they were transferred to 200 ml beakers filled with 0.20 µm Millepore filtered seawater (FSW) in the laboratory. Maintained under these conditions for over two months, the larvae did not settle. An assay was performed to test for preferred substrata and assess variability in settlement behavior. An array of deep well glass Pyrex dishes (90 x 50 cm) covered with plastic lids, to reduce evaporation, was used with 10–15 larvae each in FSW along with a piece of coral rubble of similar size (~ 8 cm\(^3\)) with an assemblage of crustose coralline algae (CCA), turf algae, and natural biofilms collected from the site from which the adults were obtained.
A settlement cue assay was conducted with a similar design using 10 larvae in n=3 replicate glass dishes for each treatment, which included a control treatment of FSW, a ~1.5 cm² chip of *Hydrolithon sp.* (CCA) and FSW, and a 48 hr old microbial biofilm conditioned glass slide cover slip conditioned in flowing sea water tables at the Kewalo Marine Laboratory. Because small numbers of larvae were released from individual colonies during a single brooding event, all of the larvae used for the settlement assay were pooled from a collection of up to 10 days from 3–12 different coral colonies. All larvae were stored together upon collection and were chosen haphazardly for use in the settlement experiments. Assays were run for a period of 2–12 days and were maintained in seawater tables with ambient seawater temperatures and under 80% light reduction shade cloth. Settlement assays were inspected every day for the duration of the experiment and the numbers of larvae that had settled and not settled were recorded. Settlement was defined as the firm attachment of a planula to a surface determined visually by gently pipetting water over the settled larva.

Data are presented as the proportion of settlement over time in various treatments. Differences in the mean percent settlement at 48 hrs were analyzed using a Student’s t-test with SPSS Statistical software version 17.0.

**Results**

**Reproductive Frequency**

*P. hawaiensis* was observed to either contain or release planulae every month of the year. In addition to the formal study periods one and two, planulae were observed during the months of March–May and July–August (Table 3.1). Individual colonies were only inspected and observed to release planulae between 11:00–18:00 hrs (Fig. 3.2). Colonies exhibited an average range of 0–6 larvae released per day (Figure 3.3).
Table 3.1 Observations of planulae occurring throughout the months of January–May and July–August for *Porites hawaiiensis*.

<table>
<thead>
<tr>
<th>Date</th>
<th>Method</th>
<th>Number of Colonies</th>
<th>Number of Larvae</th>
</tr>
</thead>
<tbody>
<tr>
<td>16 January 2007</td>
<td>Dissection</td>
<td>2</td>
<td>1 and 14</td>
</tr>
<tr>
<td>23 January 2007</td>
<td>Dissection</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>2 February 2007</td>
<td>Dissection</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>16 March 2007</td>
<td>Dissection</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>2 April 2007</td>
<td>Catchment</td>
<td>1</td>
<td>15</td>
</tr>
<tr>
<td>29 April–2 May 2007</td>
<td>Catchment</td>
<td>1–3</td>
<td>155</td>
</tr>
<tr>
<td>22–29 July 2007</td>
<td>Catchment</td>
<td>1–3</td>
<td>150</td>
</tr>
<tr>
<td>6 August 2009</td>
<td>Catchment</td>
<td>6</td>
<td>37</td>
</tr>
<tr>
<td>7 August 2009</td>
<td>Catchment</td>
<td>6</td>
<td>63</td>
</tr>
<tr>
<td>8 August 2009</td>
<td>Catchment</td>
<td>6</td>
<td>52</td>
</tr>
<tr>
<td>9–15 August 2009</td>
<td>Catchment</td>
<td>6</td>
<td>31</td>
</tr>
<tr>
<td>16–19 August 2009</td>
<td>Catchment</td>
<td>6</td>
<td>65</td>
</tr>
<tr>
<td>20–24 August 2009</td>
<td>Catchment</td>
<td>6</td>
<td>24</td>
</tr>
<tr>
<td>25–28 August 2009</td>
<td>Catchment</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>29–31 August 2009</td>
<td>Catchment</td>
<td>6</td>
<td>1</td>
</tr>
</tbody>
</table>
Figure 3.2 *Porites hawaiiensis* releasing, zooxanthellate planula larvae. Due to lack of buoyancy and weak swimming characteristics, once the larvae are released they scoot from the parent colony or are carried away by ciliary or water motion.

Figure 3.3 Mean number of planulae released per day (cumulative) by *Porites hawaiiensis* colonies during the study periods one (June 2007) and two (Sept 2007 to Feb 2008).
For study period one, individual corals were observed to release planulae daily and cumulatively over 2–3 day periods. A plot of mean number of planulae per reproductive coral observed versus lunar day does not demonstrate a lunar periodicity; however a slight trend of planulation peaking between the lunar 3rd quarter and the new moon was observed (Fig. 3.3). Interestingly, the peak of larval release for study period one was September 19, 2007, just one day before the summer solstice. The peak during study period two (November 19–December 10, 2007) was a few weeks before the winter solstice (December 21, 2007). This trend can be observed from a plot of the relative daily planula count from reproductive corals observed on sampling dates versus lunar age for the four complete lunar cycles of September 2007 to January 2008 within study period two (Fig. 3.4). There were fluctuations in the mean number of larvae released for both study period one and study period two with a decrease in larval release over the time parent colonies were maintained in the collectors.

![Graph showing planula release normalized to maximum daily planula production versus lunar day, for 4 complete lunar cycles for 9 reproductive colonies (October 2007 to January 2008).](image)

**Figure 3.4** Planula release normalized to maximum daily planula production versus lunar day, for 4 complete lunar cycles for 9 reproductive colonies (October 2007 to January 2008).
Reproduction in Relation to Colony Size

Linear regression models were used to test the relationship between coral size and reproductive output. The smallest colony that was observed to release any planulae was 0.882 cm$^2$ in area and the next smallest in the study (0.812 cm$^2$) did not release any. When examining the mean number of larvae released per day as a function of initial size for corals, regression analyses for each study period analyzed independently indicated a positive linear relationship between colony size and fecundity (Figure 3.5). Further, differing reproductive activities for these two periods were detected. For study period one (n=13), the relationship can be described by the regression line $y=0.186 + 0.433x$ with $R^2=0.60$ (1, 12), $p < 0.05$. For study period two (n=13), the relationship can be described by the regression line $y=-0.245 + 0.119x$ with $R^2=0.60$ (1, 12), $p < 0.05$.

![Figure 3.5 Average number of planulae released per day (cumulative) per coral regressed against initial size of the coral colony (n=26). Open circles and the solid line for the June 2007 study and open squares with the dashed line for the Sept 2007 to Feb 2008 study.](image-url)
**Larval Size and Motility**

The larvae of *P. hawaiiensis* contained zooxanthellae from the parent colony and were immediately competent to settle. Larval dimensions were measured and sizes were calculated for 189 larvae. Their mean length was $0.81 \pm 0.019$ mm. Their mean volume was $0.043 \pm 0.0025$ mm$^3$. These planulae were negatively buoyant and sank to the bottom of the collection vessel upon release. Larvae were seen using ciliary motion to crawl along the substratum, and were never observed to swim up in the water column when maintained inside the laboratory or under conditions in shaded or unshaded conditions in outside water tables. Of interesting note, the larvae were relatively viscous when in contact with a physical surface. Larvae easily and effectively adhered to the sides of glass pipettes during transfer to a degree above that of other coral larvae.

**Substrate Selection and Settlement Variability Assay**

The settlement assays were performed for 12 days with maximum settlement of $92.0\% \pm 3.41$ SE observed at 48 hrs. There was no change in the settlement behavior of unsettled larvae when offered the same cues and substratum for up to 12 days. The larvae that settled did not settle on the sides or the top of the substrata, but rather the majority settled on the bottom of the glass dish within a few cm of the substrata. A few larvae did settle on the bottom of the substrata; none settled any higher than 2 cm from the bottom of the dish.

For the settlement cue assay, there was no settlement observed in any replicate of the FSW control. Maximum settlement rates (Fig. 3.6) were observed at 48 hrs with the majority of settlement in the *Hydrolithon sp.* treatment ($96.7 \% \pm 3.33$ SE) and minimal settlement in the microbial biofilm treatment ($20.0\% \pm 10$ SE). After finding that these data met the assumptions, a Student’s t-test was run and revealed a statistically significant difference between mean settlement for each treatment with $p < 0.05$ and $t\text{-stat}=7.273$. As in the previous experiment, few larvae settled on the bottom of the CCA and the majority settled on the bottom of the glass dish itself near the substrata.
Discussion

Reproductive Behavior

Data from planula collections and dissections indicated that *P. hawaiiensis* planulates almost every day of the month, year-round. There was no distinct pattern of lunar periodicity for planulation though the highest monthly planulation events were observed just after the 3\textsuperscript{rd} quarter of the lunar cycle. The lower mean larval production over time from the September 2007 to February 2008 study may be associated with yet unknown biological patterns of reproduction or may have been partially due to an effect of reduced water quality. From this data, it is unclear if *P. hawaiiensis* has seasonal differences in fecundity.

Individual corals were segregated for over four months and were still capable of consistently producing larvae, demonstrating that *P. hawaiiensis* does not require sperm from conspecifics for larval production. It is possible that *P. hawaiiensis* either produces larvae via self-fertilization or asexually via parthenogenesis. This situation calls for histological and or molecular studies for resolution.
Reproduction and Size

The observation that *P. hawaiiensis* reproduces consistently with an unusual frequency for corals in the family Poritidae and has a small encrusting growth form, uncommon for poritid corals, poses a question of an energetic trade off with reproduction and growth. While other species of scleractinian corals also grow to a comparable maximum small size, frequent reproduction has only been observed in a few species smaller than 1 cm in diameter (Harrison and Wallace 1990).

Similar ecological distribution and reproductive attributes can be found in different genera of the Poritidae, such as *Stylarea punctata*, which also grows to a relatively small maximum size and is capable of producing planulae multiple days a month, year-round in Guam (Golbuu and Richmond 2007). These reproductive strategies are characteristic of opportunistic species with limited dispersal ranges.

Larval Dynamics, Size, and Motility

The larval size of *P. hawaiiensis* planulae (0.81 ± 0.019 mm) is characteristic of Poritid planulae, compared to 0.4 mm ± 0.098 SD for *Porites furcata* (Schloeder and Guzman 2008), 0.322 ± 0.011 mm for *P. panamensis* (Glynn et al. 1994), 1 mm (Szmant 1986), and 0.5–1 mm (Edmunds et al. 2001) for *Porites astreoides*. As such, larger-sized planulae for *P. hawaiiensis* may allow for longer larval competency period (Isomura and Nishihira 2001) as other species with larger planulae contain more lipid energy reserves (Richmond 1987). For example, the large larvae of the coral *P. damicornis* (Isomura and Nishihira 2001), which are more than eight times larger than those of *P. hawaiiensis*, are competent to settle and thus disperse for up to 103 days (Richmond 1987). Though photosynthetic activity can contribute to larval energetics (Isomura and Nishihira 2001), the size of the larvae overall in combination with behavior seem to enhance the capacity for longer distance dispersal of larvae.
The locomotory mode of *P. hawaiiensis* is unusual for scleractinian coral as the larvae of most other species exhibit relatively strong swimming behavior. However, larvae of at least four species of scleractinian corals in the family Dendrophylliidae completely or partially lack swimming behavior (Harrison and Wallace 1990). Those species are either solitary or colonial azooxanthellate scleractinian species. *P. hawaiiensis* is the only zooxanthellate scleractinian coral observed to date with benthic crawling larvae. This lack of swimming behavior may provide further insight into the apparent limited dispersal of this species. Dispersal mechanisms of coral larvae are thought to be largely dependent on the physical characteristics of wave motion and ocean currents. In addition, a strong swimming larva is able to more efficiently select appropriate substrate by swimming and searching. *P. hawaiiensis* larvae appear to have minimal success in this ability, suggesting several other compensatory traits such as rapid responses to appropriate cues, more generic settlement specificity as well as more flexible settlement behaviors may be important.

**Settlement Dynamics**

The settlement response observed in the presence of CCA was pronounced. Maximum settlement measured over 12 days occurred at 48 hrs and the variability among replicates was relatively low. This suggests relatively fast settlement responses with suitable habitat availability, supporting a hypothesis of a dispersal limited species. In settlement assays where the larvae were offered CCA (*Hydrolithon* sp.) and a biofilm, CCA were highly preferred. The response to CCA was statistically significant and although the larvae rarely settled directly on the algae (possibly due to an inability to swim onto it), they mostly settled within a few cm of the CCA. Larvae were never observed to settle when maintained in 0.20 µm FSW in the absence of any putative inducers for up to two months, suggesting that a cue is necessary to induce settlement. It is still uncertain if the cue to which the larvae responded was of algal or microbial origin.

The small size of *P. hawaiiensis* larvae, lack of a strong swimming behavior, viscous nature, negative buoyancy, and the expediency of settlement in the presence of an appropriate settlement cue and substrata suggest that *P. hawaiiensis* may not be as well
suited for long distance dispersal as other species of coral larvae. It is common for brooded larvae to settle in a similar habitat near the parent and this is likely for *P. hawaiiensis*. Certainly, there would be an ecological advantage to having several individuals in proximity if fertilization through spawning was an important mechanism for reproduction. Further, these traits could also serve to keep the coral in a particular zone on coral reefs. The adults collected from this study were found encrusting in the intertidal zone and very shallow subtidal zone in an area with very high water motion. Coral are rarely found in such a stressful place, presumably due to unfavorable conditions, such as high irradiance, air exposure, and high wave energy. Biological characteristics, like those of *P. hawaiiensis* larvae, may favor the retention of this coral to habitats like this, which presumably don’t have much competition from other coral. Additionally, the small size of adult *P. hawaiiensis* would likely not be as well suited to compete for space and resources in a calmer habitat, such as a lagoon or reef flat, which are often dominated by larger or massive species. Detailed field surveys should be done to understand the exact range of *P. hawaiiensis*. Further, field dispersal studies could be done to understand the transportation of *P. hawaiiensis* larvae. Particles the same size and density of *P. hawaiiensis* could be produced and released in the habitats where *P. hawaiiensis* colonies live in order to understand how larvae of this species can be transported.

This study provides the first data on reproduction, larval biology, and recruitment ecology of the endemic scleractinian coral, *Porites hawaiiensis*. In addition to the basic value of such research, studies of coral life history provide tools that can be applied to the conservation of coral reefs and related natural resources. Fecundity, reproduction, and recruitment success are all quantitative metrics that can be studied as indictors of coral reef health and resilience. The observed differences in reproductive behavior, which were noted in this study, may serve as an example of how decreased water quality can affect coral physiology at the level of reproductive function. Further, the nature and consistency of the reproductive biology and settlement dynamics of *P. hawaiiensis* larvae make them an ideal tool for testing the impacts of non-natural stressors (e.g., anthropogenic stressors) may have on this crucial part of the coral lifecycle. The
consistent settlement behavior, rapidity of settlement in the presence of a suitable cue, tendency to settle on flat (glass) substrates, low variability, and consistent year-round reproduction in the laboratory are all very useful characteristics for these types of larval assays. This study demonstrates that larvae can be sustainably “farmed” in a laboratory setting and that *P. hawaiiensis* is an excellent candidate model organism for larval biology studies.
Chapter 4. The Impacts of *Gracilaria salicornia* on Coral Recruitment

**Abstract**

Coral-algal competition is a critical ecological interaction, especially for tropical reefs, worldwide. While most studies have focused on this interaction in the adult stage of both organisms, very few have aimed to study the interaction in the highly sensitive coral larval stage with fleshy macroalgae. This study assessed the impacts of the invasive red macroalga *Gracilaria salicornia* to the recruitment of the Scleractinian coral, *Porites hawaiiensis*, in a laboratory setting and to the common reef coral, *Pocillopora damicornis*, in the field in Kāneʻohe Bay, Hawai‘i. In laboratory studies that tested for an effect of *G. salicornia*, significantly fewer larvae of *P. hawaiiensis* survived to four days into the assay compared to a control. No differences were detected in settlement. Paired field studies showed no difference in survivorship or settlement up to six days for *P. damicornis*. Our results suggest that when larval pre-emption, water motion, algal abrasion, predation, or sedimentation of coarse particulates are excluded, *G. salicornia* mats may affect species of coral larvae differently when light, sedimentation of fine particulates, and water chemistry are factors.

**Keywords**

Algae, *Gracilaria salicornia*, coral reef, planula, invasive algae, coral settlement, coral recruitment, *Porites hawaiiensis*, *Pocillopora damicornis*

**Introduction**

Coral reefs are important worldwide as precious natural, cultural, and economic resources. Scleractinian reef building coral are one of the fundamental components of coral reefs and provide much of the physical structure and ecological habitat for coral reef organisms. Coral recruitment and replenishment are extremely important ecological processes, which are paramount for the maintenance of coral reef structure and ecological
habitat (Hughes and Tanner 2000, Richmond 1997). Coral reef physical structure is
dynamic and weighs heavily on the balance of calcification, accretion, and dissolution.
Bioerosion and dissolution of carbonate occur on coral reefs at the same time that
calcifying organisms like coral are contributing carbonate with growth (Yates and Halley
2006, Suzuki et al. 1995). On a healthy coral reef, coral die from natural processes such
as disease and predation as well as anthropogenic processes, while new recruits are
replacing them. Healthy coral reefs, with an even balance of accretion and degradation
from biological processes, have an ecological loss if no new recruits are replacing
calcifiers being lost.

Fleshy macroalgae are known to exacerbate several key properties of coral reef habitats
including reductions in irradiance and water motion as well as enhancement in
sedimentation, physical abrasion, disease-causing bacteria, water chemistry, and
2001). Over 65 studies examine the impact that different functional types of macroalgae
have on coral in the adult stage, which were investigated using direct and indirect
manipulative studies as well as indirect correlative studies (McCook et al. 2001).

Of the many fewer studies that have aimed to understand how algae impact coral
recruitment, the majority have studied the role of crustose coralline algae (CCA) (Morse
et al. 1996, Morse et al. 1994, Morse and Morse 1991, Morse et al. 1988) or associated
bacterial biofilms (Tran and Hadfield 2011, Webster et al. 2004, Negri et al. 2001,
Heyward and Negri 1999) in producing cues that coral larvae use to settle. By
comparison only a handful of studies have attempted to understand how fleshy
macroalgae impact coral recruitment (Vermeij et al. 2009, Kuffner et al. 2006, Nugues
and Szmant 2006, Maypa and Raymundo 2004). To date, the fleshy macroalgae that
have been studied for impacts are primarily brown algae, a few green algae, with only
two species of red algae, Acanthophora spicifera (Vermeij et al. 2009) and Laurencia
papillosa (Maypa and Raymundo 2004) investigated.
Any one or combination of the impacted variables mentioned have the potential to impact coral. The summation of previous studies suggests that macroalgae may have different interactions with different species of coral larvae. In such a situation, case studies that focus on critical taxa comparisons are warranted.

In Hawai‘i, *G. salicornia* is an invasive red alga that overgrows coral reef benthic habitats on three islands (Smith et al. 2004). This proliferative alga has become invasive, in some regions by becoming nearly 100% cover and dominating large sections of reef. This marine plant can expand at a rate of 280 m y$^{-1}$ (Rodgers and Cox 1999) and is known to increase its mass by as much as 10 % d$^{-1}$ (Smith et al. 2004). Increases in algal cover have been associated with decreases in coral cover and hard substrate (Conklin 2007) as well as native algal species (Stimson et al. 2001). There are no data that examine physiological impact(s) of *G. salicornia* to coral and specifically, coral larvae. Thus, an important gap in information needed by the management community is the nature of this algal-coral interaction and the long-term impacts to regional reef ecology. This information gap puts the resilience of coral reefs in Hawai‘i at risk. With recent data (Chapter 2) suggesting that *G. salicornia* mounds impact the physical environment of coral reefs by imposing periods of hypoxia, sediment accumulation, and nightly acidification, the next step is to understand if the alga reduces coral contribution to the reef, by somehow disrupting sensitive coral larval settlement.

The goal of this study was to understand how *G. salicornia* mats and the associated biological communities can affect coral larval settlement by testing settlement and survivorship of coral larvae exposed to algae in both laboratory and field assays. The goals were to test the influence of algal mats of *G. salicornia* to two common species of coral larvae when presented with a suitable substrate and when larval pre-emption, sedimentation, and algal abrasion factors were excluded.

In laboratory and field assays it was hypothesized: a) survivorship would be lower under algal mats of *G. salicornia* compared to a reference condition b) planula settlement would be lower under algal mats of *G. salicornia* compared to a reference condition.
Materials and Methods

A lab survivorship and settlement assay and a field survivorship and settlement assay were performed to test the effects of *G. salicornia* to coral larvae.

**Lab Survivorship and Settlement Assay**

Colonies of the coral, *Porites hawaiiensis*, were collected from the subtidal-intertidal coast (0–3 m deep) adjacent to the Kewalo Marine Laboratory (KML), Honolulu, Hawai‘i (21°17’35.4” N, 157°51’53.7” W), placed in funnels fitting in 600 ml Nalgene Beakers with flow through seawater in seawater tables at the Kewalo Marine Lab, and were monitored for larvae over 10 days. When enough larvae were collected they were pooled and placed in settlement dishes. Algal mats of *G. salicornia* and seawater were collected from Kaimana Beach on the south shore of O‘ahu, Hawai‘i (21° 15’44.75” N, 157° 49’18.63” W) and transported to the Kewalo Marine Laboratory, Honolulu, Hawai‘i. The seawater was filtered with a glass fiber filter and the algae were sorted from fouling organisms and thoroughly washed with seawater from the site to remove detritus and particulates.

An assay was performed to test for preferred settlement substrata and assess variability in settlement behavior. Four deep-well glass Pyrex dishes (90 x 50 cm) covered with plastic lids (to reduce evaporation) were used. Each dish contained 10–15 larvae each in FSW along with a piece of coral rubble of similar size (~ 8 cm$^3$) with an assemblage of crustose coralline algae (CCA), turf algae, and natural biofilms collected from the site from which the adults were obtained. The substrate was placed in the dish with 150 ml of filtered seawater and a 20.4 ± 0.1 g wet weight piece of alga was submerged in the water in the dish. The larvae were introduced with a glass Pasteur pipette to the top of the algae and were allowed to sink to the bottom. Due to the small numbers of larvae released from individual colonies during a single brooding event, all of the larvae used for the settlement assay were pooled from a collection of up to 10 days from 3–12 different coral colonies. All larvae were stored together upon collection and were chosen haphazardly for use in the settlement experiments. The assay was run and maintained in seawater tables with ambient seawater temperatures and lighting. Dishes were inspected everyday for four days and the number of larvae that were attached and not attached to a substrate
was recorded. Attachment was defined as the firm attachment of a planula to a surface (Golbuu and Richmond 2007, Harrison 2006). Settlement was defined as a planula attached to the substrate and completely metamorphosed into a polyp (Golbuu and Richmond 2007, Harrison 2006). Data are presented as the proportion of attached larvae over time in various groups.

**Field Survivorship and Settlement Assay**

The study site was the lagoonal portion of southern portion of the windward side of Moku o Lo'e, Kāne'ohe Bay, O'ahu, Hawai'i (21° 25.874' N, 157° 47.257' W, (see Figure 4.1a). This location was selected because there are ubiquitous mats of *G. salicornia* growing over live coral along the reef at relatively the same 0.4–1.8 m depth at low tide. Ten replicate sites were chosen for monitoring along a 100 m transect that met the following conditions: within 2 m depth interval, presence of algal mat growing over carbonate reef with live coral, location was at least 10 meters from another site, and presence of a reference pair site without algae at least 1 m from algal mat (Figure 4.1a). The intent of implementing these criteria was to allow each site to be independent from the other from a microscale perspective, but still allow for local oceanographic conditions to be similar. Paired studies were constructed to test the mean proportion of surviving and settled larvae between the algal mat group and the reference group over time.
Figure 4.1 a) Map of the study area at Moku o Loʻe, Kāneʻohe Bay, Oʻahu, Hawaiʻi, b) The experimental design consisted of nine paired sites along a 1 m transect parallel to the shore, algal mat and reference sites were at least 1 m apart.
To compare the effect of an algal mat covering a reef substrate with a reference reef with no algal mat, field settlement chambers with larvae from the coral *Pocillopora damicornis* were deployed under the center or thickest part of algal mats of *G. salicornia* with an open reef reference (coral substrate with no algal mat) 1 m away, replicated for nine mats for a duration of one week. Six colonies of *P. damicornis* were collected from the field site reef and were monitored for larvae for a period of six days near the full moon at the Hawai‘i Institute of Marine Biology. The settlement chambers were constructed from glass pyrex dishes (90 x 50 cm) sealed with plastic Nitex mesh 100 µm which were secured with zip ties. The design consists of 10 larvae per dish and one dish per mat and reference for each of the replicate pairs. To account for the effect of the available substrate at each site (substrate effects, chemicals, light, sediment, bacteria) round pieces of carbonate rubble from each location were placed into the settlement chamber and put in with the larvae. The settlement chambers were sampled after two and six days and the number of larvae unsettled, settled, metamorphosed, and the location of settlement (the glass chamber near the top or bottom and the substrate near the top or bottom) were counted visually using a dissection microscope after ferrying the chambers to the shore submerged in water.

**Statistical Analyses**

SAS Enterprise Guide v. 4.3 was used to perform the mixed model analysis of variance (ANOVA) tests with the procedure “PROC MIXED.” Plots were made using GraphPad Prism version 5.0b for Mac OS X, GraphPad Software, San Diego, California, USA, (www.graphpad.com). Several mixed model ANOVAs were performed to analyze the data. A mixed model ANOVA is essentially a factorial ANOVA, which allows for random variables, repeated measures, unbalanced data, and the ability to specify a particular covariance matrix. The test uses an iterative process of a restricted maximum likelihood function, which is calculated from the probability distribution of contrasts that replace the original data to produce unbiased estimates of variance and covariance parameters.
**Lab Survivorship and Settlement Assay**
Survivorship of coral larvae was calculated as the proportion of larvae counted as a proportion of total larvae initially added to the chamber. Survivorship was the dependent variable, site was the random variable, day was the repeated measures variable with subject site by group, and day, group, and day by group were fixed factors. A mixed model ANOVA was performed to analyze differences in mean survivorship over time, between groups and between groups over time. The data was arcsinsqrtx transformed to meet assumptions of the model.

Another mixed model ANOVA was performed to analyze mean settlement over time, between groups and between groups over time.

**Field Survivorship and Settlement Assay**
Survivorship of coral larvae was calculated as the proportion of larvae counted as a proportion of total larvae initially added to the chamber. The larval settlement proportion for each possible position (top and bottom) was calculated three ways: 1) as the mean count of settled larvae as a proportion of the total initial larvae, 2) as the mean count of settled larvae as a proportion of the total surviving larvae by day, and 3) the mean count of settled larvae as a proportion of the total settled larvae.

Because this experiment was performed at paired sites across the reef, the variable ‘site’ was identified as a random variable and the interaction group by site as the subject to account for the influence in the model and assumptions of independence. In the model, survivorship was the dependent variable, site was the random variable, day was the repeated measures variable with subject site by group, day, group, day by group, position, day by position, group by position, and day by group by position were fixed factors. Four mixed model ANOVAs were performed to examine differences among variables. One analysis tested mean survivorship between groups, over time, and between groups over time. The data in this model were arcsinsqrtx transformed to meet assumptions of the test. Another analysis tested mean settlement position of initial larvae in the chamber (top or bottom) over time, between groups, position, day by groups, day by position,
group by position, and day by group by position. Another analysis tested mean settlement position of the proportion of larvae recovered by day in the chamber (top or bottom) over time, between groups, position, day by groups, day by position, group by position, and day by group by position. The data in this model were arcsinsqrt transformed to meet assumptions of the test. The last analysis tested mean settlement position of the proportion of total settled larvae by day in the chamber (top or bottom) over time, between groups, position, day by groups, day by position, group by position, and day by group by position.

Results

Lab Tests for Survivorship and Settlement
The analysis of survivorship detected significant differences in mean survivorship over time, between groups, and between groups over time (Table 4.1). A posteriori comparisons with Bonferroni correction indicated that mean survivorship (100–90%) for the control group did not differ between successive days. Mean survivorship for the group exposed to algae was not significantly different from day one (96.67%) to day two (93.33%) or day two to day three (83.33%), but the mean survivorship on day four (43.33%) was significantly lower than that on day three. There were no significant differences in mean survivorship between the control and algal treatment pairs when comparing days one, two, or three, but the mean survivorship in the algal treatment on day four was significantly lower than for the control group by less than half (Figure 4.2a). Analysis of settlement patterns did not detect any differences in mean settlement of larvae over time, between groups, and between groups over time. By day four, settlement ranged between 30–90% for the control group and 20–60% for the group with algal exposure (Figure 4.2b).
<table>
<thead>
<tr>
<th>Survivorship</th>
<th>DF</th>
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<th>p Value</th>
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<tbody>
<tr>
<td>Group</td>
<td>1.4</td>
<td>10.28</td>
<td>0.0327</td>
</tr>
<tr>
<td>Day</td>
<td>3.12</td>
<td>27.58</td>
<td>&lt;0.0001</td>
</tr>
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<td>Group x Day</td>
<td>3.12</td>
<td>6.21</td>
<td>0.0087</td>
</tr>
<tr>
<td>Settlement</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group</td>
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<td>0.05</td>
<td>0.8319</td>
</tr>
<tr>
<td>Day</td>
<td>3.12</td>
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<td>0.7956</td>
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<tr>
<td>Group x Day</td>
<td>3.12</td>
<td>0.46</td>
<td>0.7143</td>
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</tbody>
</table>
Figure 4.2 The mean percentages of a) larval survivorship, b) larval settlement, over time in the laboratory assay. Reference in white bars and algal mat group in shaded bars. Error bars are SD.
In-situ Survivorship and Settlement

Mean survivorship and settlement were analyzed to compare differences between the algal mat group with the control group overall, the day in the assay overall, and the interaction between the difference in groups on each day.

Mean survivorship as well as the proportion of settlement by location of initial larvae, proportion of surviving larvae that settle, and numbers of larvae that settled during the field assay can be found in Figure 4.3. A significant difference was detected for survivorship between the second and sixth days in the field assay overall as well as between groups, but not between groups over time. Overall, mean survivorship from the algal treatment (66.3%) was higher than for the control group (50%). Mean survivorship was also higher for the algal treatment (23.3%) and for the control group (10%) on the sixth day.
Figure 4.3 The mean percentages of a) larval survivorship for reference and algal groups at two and six days, b) overall larval settlement for reference and algal groups at two and four days of initial larvae, c) larvae settled on the top or bottom of the settlement chamber or substrate of initial larvae at two days, d) larvae settled on the top or bottom of the settlement chamber or substrate by proportion of initial larvae at six days, e) larvae settled on the top or bottom of the settlement chamber or substrate by proportion of survival at two days, f) larvae settled on the top or bottom of the settlement chamber or substrate by proportion of survival at six days, g) larvae settled on the top or bottom of the settlement chamber or substrate by proportion of total settled at two days, and h) larvae settled on the top or bottom of the settlement chamber or substrate by proportion total settled larvae at six days of deployment in the field assay. Reference in white bars and algal mat group in shaded bars. Error bars are SD.
A significantly higher proportion of larvae settled on the bottom of the chamber overall and significantly higher proportion of larvae settled on the second day of the assay than the sixth for total larvae (Table 4.2).

<table>
<thead>
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<th>Survivorship</th>
<th>DF</th>
<th>F stat</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>1.16</td>
<td>5.61</td>
<td>0.0307</td>
</tr>
<tr>
<td>Day</td>
<td>1.15</td>
<td>55.71</td>
<td>0.0001</td>
</tr>
<tr>
<td>Group x Day</td>
<td>1.15</td>
<td>0.35</td>
<td>0.5611</td>
</tr>
</tbody>
</table>

**Overall Settlement**

| Group              | 1.16 | 0.65   | 0.4305  |
| Day                | 1.46 | 14.11  | 0.0005  |
| Group x Day        | 1.46 | 3.21   | 0.0797  |
| Position           | 1.46 | 4.19   | 0.0463  |
| Group x Position   | 1.46 | 3.69   | 0.0609  |
| Day x Position     | 1.46 | 1.13   | 0.2933  |
| Group x Position x Day | 1.46 | 0.42   | 0.5209  |

**Settlement of Surviving**

| Group              | 1.16 | 0      | 0.9669  |
| Day                | 1.46 | 0      | 0.9446  |
| Group x Day        | 1.46 | 2.47   | 0.1227  |
| Position           | 1.46 | 3.31   | 0.0754  |
| Group x Position   | 1.46 | 1.24   | 0.2716  |
| Day x Position     | 1.46 | 0.39   | 0.5361  |
| Group x Position x Day | 1.46 | 0.15   | 0.6996  |

**Location of Settled**

| Group              | 1.16 | 0.29   | 0.5975  |
| Day                | 1.46 | 1.24   | 0.2714  |
| Group x Day        | 1.46 | 1.4    | 0.2421  |
| Position           | 1.46 | 4.2    | 0.046   |
| Group x Position   | 1.46 | 1.08   | 0.3035  |
| Day x Position     | 1.46 | 0.02   | 0.8926  |
| Group x Position x Day | 1.46 | 0.24   | 0.6254  |

A significantly higher proportion of larvae settled on the bottom of the chamber overall, as a proportion of the total larvae settled by day.

Overall, the settlement rate was low for both groups, with sizeable variability. It is important to mention that of the nine replicate sites on the sixth day there were only
surviving settled larvae at one site for the control group and the rest of the sites had no surviving settled larvae. In some cases larvae settled on substrates with *G. salicornia* recruits (Figure 4c).

Figure 4.4 a) Settlement chambers at two days. Left side is the reference group and the right side is the algal mat group, which had accumulated fine sediment. b) Close up of settled larvae on top of the substrate and the bottom of the settlement dish for the reference group. c) Close up of settled larvae on top of the substrate and adjacent to *Gracilaria salicornia* recruits.
Discussion

Lab Tests for Survivorship and Settlement

Detection of algal impacts requires close examination of altered behavior or even death of sensitive larval stages, as was set up in this research. Larval survivorship was expected to be lower when in close contact to the alga *G. salicornia*, as detected. By day four, about half of the larvae were still alive in the algal treatment when compared with the control group. This data suggested that *Gracilaria salicornia* impacts the viability of coral larvae in lab settings. Other studies have found that algal extracts or proximity to live thalli (Kuffner et al. 2006, Smith et al. 2006, Kuffner et al. 2004) can impact coral larvae. Those algae are regarded to be well defended chemically while *Gracilaria* species in general are held to be poorly defended, chemically (Higa and Kuniyoshi 2000). A mechanistic basis for this mortality remains to be elucidated as water motion, algal abrasions from water motion, predation, and sedimentation were excluded from this assay. Impacts associated with light and water chemistry were possible, including lack of water circulation that may have concentrated potential allelopathic compounds and/or microflora.

Three earlier studies detect macroalgal compounds conditioning the bathing medium in experimental water. This conditioned water in turn enhances bacterial populations (Kline et al. 2006, Smith et al. 2006) resulting in transfer to coral and promotes detrimental impacts, such as disease (Nugues et al. 2004) and mortality (Smith et al. 2006). Using *P. damicornis*, Maypa and Raymundo (2004) tested the impact of three species of fleshy rhodophyte macroalgae to larval survivorship, settlement, and growth in the Philippines. They found that survivorship declined below control levels over time up to about eight weeks for larvae exposed to the algae while survivorship in controls remained stable; survivorship in that study was proportional to the distance larvae had settled from the algae. Additionally, they also found no influence to settlement from conditioned water extracts of two species and increased settlement from one species of algae. Using larvae from a Hawaiian species of coral (*Montipora capitata*) and four species of Hawaiian algae, Vermeij et al. (2009) found an inverse relationship between settlement density and fleshy algal cover in field surveys, significantly lower survivorship in the presence of
algae and antibiotics, and lower survivorship in the presence of algae (*Ulva fasciata* syn. *Ulva lactuca*) without antibiotics. The investigators also did not detect an impact of presence of *U. lactuca* to coral settlement with bacteria, though they did detect an impact to larval settlement between *U. lactuca* and three other species of fleshy macroalgae. Though no microbiological analyses were done for this assay, microbial activity is a distinct possibility.

**In-situ Field Survivorship and Settlement**

A coral reef is a very stress-filled and competitive place for a larva to recruit. It seems plausible that a multitude of factors may affect pre- and post-settlement survivorship. Just about half of the control larvae survived these manipulations a proportion comparable to results of other studies with similar field methodology. Similar patterns of survival (69%) have been found with *P. damicornis* in the same bay (Kāne‘ohe Bay), (Kuffner 2001) and others have higher but comparable recoveries ranging from 70–80% (Kuffner et al. 2006) of coral larvae and 25–30% gorgonian larvae (Kuffner and Paul 2004). Further, *P. damicornis* has been found to settle to only 4–56% on a coral reef in Kāne‘ohe in direct sun due to a multitude of factors, including UV (Kuffner 2001). The reference group was exposed to full light, which sometimes can reach levels up to 2,000 μmol photons m⁻² s⁻¹ which certainly may stress larvae. There was a substantial amount of sediment on the bottom of the dishes in the algal mat group (Figure 4.4a). Larvae settled both on the substrates and on the settlement dish (Figure 4.3b).

There was no difference in the number of larvae settled between groups on either day. In both groups, there were fewer surviving settled-larvae on the sixth day than the second day. Of the initial larvae, only 13.6% survived and settled in the reference and 35% in the algal groups on day two; 6.7% in the reference and 18.9% in the algal groups on day six; most of those larvae settled on the bottom. Of the total amount of larvae surviving, there was no difference between the settlement proportion at location of the algal and reference groups, which may suggest that settling on the top or bottom of a substrate or dish had no advantage on survivorship on either day. Of the total number of larvae that did settle, more were settled on the bottom than the top, which suggests again that
settlement may be preferred underneath a substrate or on the bottom of the dish. This might suggest that settlement at the bottom is either a behavioral preference or more advantageous. The bottom of the dish has the substrate with potential chemical cues; however, more larvae settled on the bottom than the top of the substrate or dish. The top of the substrate and dish had more access to irradiance, especially for the reference group. The exact position that a coral larva settles on a substrate can be very important for its successful recruitment. A larva that settles in direct sunlight is at risk of being visible and accessible to predators and must be able to tolerate the large quantity of energy that can be damaging or induce photoinhibition. On the contrary, a larva must not settle too far away from light energy or the efficiency of photosynthate production may be impacted.

The field settlement experiment suggests that given the parameters of the experimental design, (excluding larval pre-emption, water motion, algal abrasion, and predation; and including parameters of light, sedimentation and exposure to water chemistry including dissolved respiratory gases) that if a larva is under an algal mat of *G. salicornia*, that survivorship and settlement is not affected up to six days. The unavoidable physical constraints of the experimental design and particularly the limitations of the settlement chamber (reduced water flow capacity, sediment size, debris, predator pre-emption, and prevention of algal abrasion) may have influenced the results of this study. The algal mat group collected more fine sediment than the control group and in some cases the sediment turned black, which was possibly hydrogen sulfide from anoxic conditions. In previous work we estimated that algal mats of *G. salicornia* exacerbate fine sediment accumulation by at least double and that nightly periods of anoxia can occur (see Chapter 2). We also estimated that the algal mats block up to 99% of irradiances, which make it a difficult environment for any photosynthetic organism. Larvae settled both on top of the substrate, underneath it, and on the settlement dish itself. It is interesting to note that across the groups, the majority of settlement occurred on the bottom of the substrate or dish. It seems most likely that if a larva had the ability to select a spot for settlement in a limited light environment that it would select a site in the light. Several species of coral larvae are known to prefer to settle in response to the quality of irradiances (Mundy and
Babcock 1998) and UV has been found to reduce settlement of Hawaiian *P. damicornis* on coral reefs in Kāneʻohe Bay, Hawaiʻi by 6–35% with larvae from colonies collected at shallow depths showing more sensitivity by preferring to settle in indirect irradiance than direct irradiance (Kuffner 2001) and patterns of settlement in shaded areas on a reef have been observed.

Additionally, water circulation and exchange were likely minimal in the settlement chamber if not underneath the entirety of the mat. In a natural reef setting, a larva is both carried by water currents and motile larvae swim to search for a suitable substrate (Richmond 1997). Coral larvae by and large are ciliated and have the ability to actively survey substrates along a depth gradient. It does seem unlikely, though not improbable, that a larva can navigate the labyrinth of a mesh network of branching macroalgal thalli and reach a substrate below. Future research in a flume either with larvae or particles the same size and density could be tested under a passive motion model.

**Conclusions**

A comprehensive understanding of the impacts of *G. salicornia* to coral reef organisms, such as coral, is crucial to understand the broader ecosystem interactions and impacts. The larval phase of coral is a very important and understudied part of the coral life cycle. The logistical constraints from field survivorship and settlement assays with coral larvae impose unavoidable confounding factors and limitations to data interpretation, particularly in a system such as under an algal mat. The data from the research presented here suggests that: 1) when algae is exposed to larvae in a semi-controlled system that larval survivorship may decrease with no effect to settlement by as much as four days if the larvae remain in that location and water flow becomes stagnant; 2) when larvae have proximity to and no physical contact with *G. salicornia*, that larval survivorship and settlement may not be effected; and 3) larvae of *P. damicornis* prefer to settle at the bottom of a substrate in field assays. This issue is fundamentally important to understanding the long-term effect of *G. salicornia* on coral larval recruitment and there are many potential mechanisms of effect left to be investigated.
Chapter 5. Physiological Impacts of Overgrowth by *Gracilaria salicornia* on *Porites compressa*.

Abstract

Invasive species can seriously impact marine ecosystems and the biology of native species. In many cases, the impacts as well as the underlying mechanisms of impact to native species remain unclear. This study assessed the impacts of the invasive red macroalga, *Gracilaria salicornia*, to the physiology of the scleractinian coral, *Porites compressa*, in Kāʻeʻohe Bay, Hawai‘i. Paired studies showed that coral did not die when covered by the algae for as long as seven weeks. Yet, corals were visually bleached within two weeks of being covered by algal mats. In response to shading, chlorophyll *a*, *c*₂ and total carotenoids increased in remaining zooxanthellae. There was no evidence of oxidative stress or anaerobic respiration despite diurnally fluctuating hypoxic and hyperoxic conditions. When management was simulated by removal of algal mats, pigment quantity recovered in coral tissues after three weeks; zooxanthellar density recovered within five weeks. Our results suggest that *G. salicornia* mats imposed substantial physiological stresses on the symbiosis and photosynthetic capacity of the coral *Porites compressa* and that recovery can be detected within three weeks if the alga is managed. Further, we demonstrated that the removal of mats is an effective management action leading to recovery from short-term stress. Thus, short-term management efforts that include removal of algal biomass are likely to be successful in restoring the health of *P. compressa* in Kāʻeʻohe Bay.

Keywords

Invasive algae, *Gracilaria salicornia*, coral reef, biomarker, coral biology, coral health, *Porites compressa*
**Introduction**

Coral reefs provide valuable physical coastal, ecological, socioeconomic, and cultural services worldwide. Scleractinian coral are the building blocks of much of the physical structure and raw material, which comprise coral reefs. Marine ecosystems are comprised of a diverse assemblage of organisms including microorganisms, plants, algae, and animals. Often in coral reef ecosystems, resources such as space and nutrients are limited, which fosters competition between benthic organisms such as coral and macroalgae. Ecological factors can affect dominance as well, for instance in coral reefs where nutrient availability is low and herbivory is high, coral may dominate, and when nutrients are high and herbivory low, algae may dominate (Smith et al. 2001, Littler and Littler 1984). Several studies have aimed to describe the complex dynamics and mechanisms by which coral and macroalgae directly and indirectly compete as reviewed in Fong and Paul (2011), Chadwick and Morrow (2010), Ritson-Williams et al. (2009), Birrell et al. (2008), and McCook et al. (2001). Several mechanisms and combination of mechanisms have been identified including space pre-emption, shading, smothering, abrasion, sedimentation, allelopathy (reviewed in McCook et al. 2001), promoting disease (Smith et al. 2006), and preventing recruitment (Birrell et al. 2008 and 2005). The common themes of these comparisons are that generalizations across taxa cannot be made and different species of algae have different interactions with different species of coral, which necessitates individual investigation.

In Hawai‘i, several species of non-indigenous macroalgae have become invasive by spreading across the benthos, overgrowing native organisms, and competing for resources (Smith et al. 2002). *Gracilaria salicornia* was introduced to the islands of O‘ahu (1971 and 1978) and Molokai from samples from Hawai‘i Island from which the origins are uncertain (Smith et al. 2004). This alga can increase its biomass by as much as 10% a day with a growth rate estimated at 37 mg d$^{-1}$ (Smith et al. 2004). *G. salicornia* often grows in mounds or mats as thick as 8.9 cm (Beach et al. 1997), accumulating biomass of up to 5.19 kg m$^{-2}$ wet weight (Smith et al. 2004). In Kāne‘ohe Bay, Hawai‘i this alga has been estimated to cover spread across the reef at a rate of 280 m y$^{-1}$ (Rodgers and Cox...
where increases in algal cover have been associated with decreases in coral and hard substrate cover (Conklin 2007) and cover of other species of algae (Stimson et al. 2001). These algal mats create three-dimensional habitats that support a variety of fouling organisms.

Previously we found that *G. salicornia* mats in Kāne‘ohe Bay, Hawai‘i reduce transmittance of photosynthetically active radiation (PAR) to the benthos by as much as 99%, effectively double the accumulation of fine sediment and particulates to the reef area below, can create periods of hypoxia to the benthos below lasting from 30–90 min at night while simultaneously amplifying acidification of seawater diurnally from respiration, and can impose periods of hyperoxia from photosynthesis. In addition, some research has suggested that species of Gracilariales can produce and release reactive oxygen species (ROS) as a stress response (Weinberger et al. 2005, Collen et al. 2003 Weinberger et al. 1999, Pedersen et al. 1996a) and volatile halogenated organic chemicals (VHOC) (Pedersen et al. 1996b), which have been found to have allelopathic effects (Ohsawa et al. 2001). Extracts of *G. salicornia* were also found to have antimicrobial properties (Vijayavel and Martinez 2010).

The most common species of coral in Kāne‘ohe Bay, is *Porites compressa* (73% total cover) (Hunter and Evans 1995) and it is the primary coral species being overgrown by *G. salicornia* in Kāne‘ohe Bay. *P. compressa* is a common, endemic species of scleractinian coral that forms multiple blunt < 6 cm finger-like branches. At present, there is very little information on the short-term physiological impacts of the overgrowth of invasive algae on coral health. Specifically, it is unknown if the alga induces mortality of coral. Further, it is important to gain some understanding on whether management (removal) of the alga will promote coral health and survivorship.

The goal of this study was to understand how *G. salicornia* mats and the associated biological communities can affect coral biology by comparing physiological health parameters of coral under the algal mats with open reef benthic references. Our goals were to simulate algal overgrowth on fragments of coral and to assess the impact to coral
health over time by inspecting and monitoring for: mortality, lesions, zooxanthellae density, pigment level, development of oxidative stress, and metabolites of anaerobic respiration. Further, we aimed to assess if simulating management action and removing the algae could restore coral health.

The following were hypothesized: a) coral would develop a physiological response to decreased irradiance by increasing its pigment levels, b) coral would undergo and exhibit evidence of oxidative stress, and c) exhibit signs of anaerobic respiration.

**Materials and Methods**

**Site Description and Experimental Design**

The study site was the lagoonal portion of southern portion of the windward side of Moku o Lo‘e, Kāne‘ohe Bay, Hawai‘i (21° 25.874'N, 157° 47.257'W) (see Figure 5.1). This location was selected because there are ubiquitous mats of *G. salicornia* growing over live coral along the reef at relatively the same 0.4–1.8 m depth at low tide. Ten replicate sites were chosen for monitoring along a 100 m transect that met the following conditions: within 2 m depth interval, presence of algal mat growing over carbonate reef with live coral, location was at least 10 meters from another site, and presence of a reference pair site without algae at least 1 m from algal mat. The intent of implementing these criteria was to allow each site to be independent from the other from a microscale perspective, but still allow for regional oceanographic conditions to be similar.
Figure 5.1 a) Map of the study area at Moku o Lo‘e, Kāne‘ohe Bay, O‘ahu, Hawai‘i, b) The experimental design consisted of nine paired sites along a 1 m transect parallel to the shore, algal mat and reference sites were at least 1 m apart.
**Field Transplant Experiment**

Two hundred ~3 cm length branches of *P. compressa* were collected from one individual colony from the study site. The fragments were glued with Z-spar marine epoxy to 5 x 5 cm ceramic tiles. The fragments were allowed to recover for five weeks in a plastic chicken wire cage on the reef. The tiles were inspected and cleaned weekly and recovery was judged based on new coral growth over the marine epoxy. On September 3, 2009, after recovery, each fragment was inspected for lesions or signs of disease and if none were found, then the fragment was haphazardly distributed and grouped with each of 20 locations for the field study.

Six fragments were placed as replicates under the 10 algal mats used for the study and four fragments were placed as replicates at the reference pair locations for each algal mat. For the algal group, the algal mat was gently lifted up and the fragments were placed in the center. A small 16 cm$^2$ mesh net was tented over the algal mat to secure it. For the reference group, the fragments were placed a small 16 x 16 x 11 cm plastic chicken wire cage with 1 cm$^2$ mesh to prevent confounding physical damage. The fragments were inspected weekly for lesions and development of disease by removing them from their group locations and taking photographs underwater for seven weeks. Coral fragments were sampled on four sampling periods: September 10, 2009 (one week covered by the mat), September 17, 2009 (two weeks covered by the mat), October 9, 2009 (three weeks uncovered), and September 23, 2009 (five weeks uncovered). Coral was sampled underwater by detaching the fragment from the tile and placing it in a plastic bag underwater. The samples were ferried to the shore in a dark container and the water was immediately drained, the bag sealed and the whole sample was immersed in dry ice and eventually frozen at -80 °C. Algal mats ranged from 7.27 kg m$^{-2}$ to 20.20 kg m$^{-2}$ dry wt.

**Biochemical Analyses**

While still frozen, coral samples were measured with calipers and surface area was calculated according to Naumann et al. (2009) as the sum of the surface area of cylinders. This proved to be an effective technique to estimate the surface area of this branching
coral. Each coral fragment was ground with a mortar and pestle in liquid N\textsubscript{2} into a fine powder, weighed, and stored at -80 °C for further analyses.

**Zooxanthellae Density**

Zooxanthellae were extracted from the host and skeleton by a homogenization, washing, and centrifugation technique. Approximately 300–400 mg of coral powder was weighed and placed in 1.5 ml Eppendorf tubes with 1 ml ice cold 0.2 µm filtered seawater (FSW). The tissue was homogenized with a Tissue Tearor tissue homogenizer (Biospec Products, INC) on ice for 1 m. The homogenate was vortexed for 30 s and the supernatant was transferred to a 15 ml tube on ice. This homogenization method was found to be effective in breaking up the coral animal cells, but leaving the much smaller zooxanthellae cells intact. Subsequently, the remaining homogenate was washed with 1 ml ice cold FSW, vortexed, and the supernatant transferred as above. The wash step was completed 3 times. To pellet the zooxanthellae, supernatant was centrifuged at 3,000 g in an Eppendorf refrigerated centrifuge for 5 m. The supernatant was discarded and the pellet was resuspended in 1 ml FSW. Resuspended zooxanthellae were aliquotted into clear and amber 1.5 ml Eppendorf tubes and centrifuged in a microcentrifuge at 3,000 g for 5 m. The supernatant was discarded and the samples in the amber tube were frozen at -80 °C for pigment analysis. The samples in the clear tube were resuspended in 100 µl 4% formalin and stored in the dark at 4°C for cell density counts. Cells fixed in 4% formalin were counted using a Neubauer Hemacytometer and the density of the total extract as well as the portion stored for pigment analysis were calculated.

**Algal Pigment Extraction**

The frozen zooxanthellae pellets were resuspended, vortexed, and the pigments were extracted with 90% acetone at 4 °C for 24 hrs. Extracts were analyzed for absorbance with a Molecular Devices M5 Spectramax spectrophotometer and contents of chlorophyll \(a\) and \(c_2\) were calculated using the equations of Jeffrey and Humphrey (1975) in Jeffrey and Welschmeyer (1997). Total carotenoids were calculated according to equations by
Strickland and Parsons (1972) and treated similarly. Spectral profiles of absorbance were read in 1 nm increments from 350–750 nm on representative samples.

**Oxidative Stress**

The samples were assayed for DNA damage as evidence of oxidative stress following methods used in Downs et al. (2011) and Kurochkin (2009). DNA was extracted with the Dojindo Get pure DNA tissue kit (GK03-20) and resuspended in 10 mM Tris buffer pH 8. DNA was quantified using the Invitrogen High Sensitivity DNA quant-it kit (Q33120). DNA was labeled with ARP probe and quantified using the Dojindo DNA AP site kit (DK02-10). The DNA AP site assay was modified for use with chemiluminescence by diluting the DNA sample 1:10 according to the methods.

**Metabolic Condition**

The S9 or cytosolic fraction of coral animal cells was obtained following methods of Rougee (2011). Approximately 300–400 mg of coral powder was weighed and placed in 1.5 ml Eppendorf tubes with 1 ml ice cold FSW and 1mM Phenylmethysulfonylfluoride (PMSF) was added. The tissue was homogenized with a tissue homogenizer as described above on ice for 1 m. The homogenate was vortexed for 30 s and then centrifuged at 3,000 g in an Eppendorf refrigerated centrifuge for 5 m to pellet and remove the zooxanthellae. The supernatant was transferred to a new tube and spun at 10,000 g for 10 min at 4 °C. The supernatant containing microsomes and cytosol were aliquoted to new tubes and frozen at -80 °C until use.

To quantify the cellular material, protein was quantified using the Bicinchinonic Acid method described by Smith et al. 1987 using bovine serum albumin as the protein standard. L-lactate concentration of a sample concentration representing 1 mg ml⁻¹ protein was assayed using Biovision (Mountain View, CA, USA) Lactate Assay Kit II #K627-100.
**Statistical Analyses**

SAS Enterprise Guide v. 4.3 was used to perform the mixed model analysis of variance (ANOVA) tests with the procedure “PROC MIXED.” Plots were made using GraphPad Prism version 5.0b for Mac OS X, GraphPad Software, San Diego, California, USA (www.graphpad.com). Several mixed model ANOVAs were performed to analyze the data. A mixed model ANOVA is essentially a factorial ANOVA, which allows for random variables, repeated measures, unbalanced data, and the ability to specify a particular covariance matrix. The test uses an iterative process of a restricted maximum likelihood function, which is calculated from the probability distribution of contrasts that replace the original data to produce unbiased estimates of variance and covariance parameters.

In the case of the analyses performed from this study, a mixed model ANOVA was performed to analyze differences in mean quantity of the variable investigated between groups, by week, between groups by week, by exposure period and recovery period, and between groups by exposure period and recovery period. The quantity of the parameter investigated was the dependent variable, site was the random variable, week was the repeated measures variable with subject identified as “site by group,” and week, group, week by group, period, and group by period were fixed factors. To meet assumptions of the model, some data were transformed and assigned repeated measures covariance matrices (CM).

**Zooxanthellae Density**

The quantity zooxanthellae (number of cells) was calculated as a proportion of surface area (cells cm\(^{-2}\)). To meet assumptions of the model, the data were log (x+1) transformed and assigned a CM of unstructured.

**Algal Pigment Extraction**

The quantity algal pigments, contents were calculated as a proportion of surface area (ug cm\(^{-2}\)) and per zooxanthella cell (ug pigment cell\(^{-1}\)). For the analysis of Chlorophyll \(a\)
(Chl a) \( \mu g \ cm^{-2} \) and Chlorophyll \( c_2 \) (Chl \( c_2 \)) \( \mu g \ cm^{-2} \), data were log (x+1) transformed and assigned a CM of unstructured. For the analysis of total carotenoids \( \mu g \ cm^{-2} \), the data were sqrt (x) transformed and assigned a CM of variance components.

For the analysis of Chl a \( \mu g \ cell^{-1} \), the data were sqrt (x) transformed and assigned a CM of variance components. For the analysis of Chl \( c_2 \) \( \mu g \ cell^{-1} \) and total carotenoids \( \mu g \ cell^{-1} \), data were log (x+1) transformed and assigned a CM of unstructured.

For the analysis of Chl \( a:c \) ratio, data were not transformed and were assigned a CM of unstructured. For the analysis of DNA AP sites per \( 10^5 \) bp, the data were log (x+1) transformed and assigned a CM of unstructured. For the analysis of L-lactate \( \mu m \ mg^{-1} \) protein, the data were not transformed and assigned a CM of variance components.

**Oxidative Stress**

The quantity of DNA damage was calculated as the mean proportion of total DNA in \( 10^5 \) bp against a standard curve with the following concentrations: 0, 0.5, 1.0, 2.5, 5.0, 10.0, 20.0, and 40.0 AP sites per \( 10^5 \) bp.

**Metabolic Condition**

The quantity of L-lactate was calculated as concentration \( \mu m \ mg^{-1} \ mL^{-1} \) total soluble protein against a standard curve with the following concentrations: 0, 2, 4, 6, 8, and 10 mmol.

**Results**

Mean values for zooxanthellae density, pigment concentrations, DNA AP sites, and concentration of L-lactate within groups over time are reported in Table 5.1. The results of the statistical analyses can be found in Table 5.2.
Table 5.1 Descriptive statistics for health variables.

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### Table 5.2 Results of tests of significance for health parameter analyses.

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There were no obvious signs of lesions or the development of disease in any of the coral in either the algal mat group or the reference group. During weeks one and two, the coral in the algal mat group became pale and exhibited signs of a bleaching effect (Figure 5.2). At week two, the recovery phase of the experiment was initiated and the coral were allowed to recover. At three weeks into recovery, the coral from the algal mat began to become darker, and by five weeks into recovery the color was nearly indistinguishable from the reference coral. There was little to no change observed in the reference coral over time. The coral that stayed under the algal mat survived up to seven weeks and stayed white in color.
Figure 5.2 *P. compressa* at different timepoints of the experiment: a) one week under the algal mats, left to right reference and algal mat groups; b) two weeks under the algal mats, left to right reference and algal mat groups; c) three weeks into recovery, left to right, reference, algal exposure recovery transplant, algal mat group; d) five weeks into recovery, left to right, reference, algal exposure recovery transplant and algal mat groups; e) Coral under algal mats for seven weeks, the dark rings inside are coral tissue.
Zooxanthellae Density

There were significant differences in mean cell density cm\(^2\) overall between groups (with lower density in the algal mat group), but not between groups by week, the exposure period and after, or groups between exposure period and after (Table 5.2). Post-hoc tests suggests that mean zooxanthellae density decreased from week one to week two and increased from week five to week seven.

Although there was no significant difference detected between groups by week, mean data varied (Figure 5.3) and zooxanthellae density appeared to decrease after one week under the algal mat when the mean density of zooxanthellae decreased to 2/3 that of the reference, and again by week two when it was less than half of the reference group (Table 4.1). By three weeks into recovery, the mean zooxanthellae density of the algal mat coral increased to roughly 2/3 of the reference group, and by the seventh week it was 87% of the reference group.
Figure 5.3 a) Mean chlorophyll a cm$^{-2}$, b) Mean chlorophyll c$_2$ cm$^{-2}$, c) Mean carotenoids cm$^{-2}$, d) Mean chlorophyll a cell$^{-1}$, e) Mean chlorophyll c$_2$ cell$^{-1}$, f) Mean carotenoids cell$^{-1}$, g) Mean chl a:c ratio, h) Mean zooxanthellae density cm$^2$, i) Mean DNA AP sites per $10^5$ base pairs over time, j) concentration of L-lactate in samples on weeks one, two and five. Shaded bars represent the algal group and white bars represent the reference group; Shaded bars represent the algal group and white bars represent the reference group.
Algal Pigments

Chlorophyll \( a \) (\( \mu g \ \text{cm}^2 \))
There were significant differences in mean content of Chl \( a \) \( \mu g \ \text{cm}^2 \) overall between groups (with more in the reference group), between weeks, between groups by week, but not between the exposure period and after, or groups between exposure period and after. Post-hoc tests revealed significantly less Chl \( a \) in the algal groups than the reference at weeks two and five. For the algal group, there was a significant decrease in Chl \( a \) from week one to week two and week five to week seven, but no difference between week two to week five and there were no changes in Chl \( a \) level over time for the reference group. Figure 4.3 shows how data varied between groups and over time.

By week two, mean Chl \( a \) in the algal group was 60% of the reference group and by week five, Chl \( a \) in the algal group was less than half of the reference group.

Chlorophyll \( c_2 \) (\( \mu g \ \text{cm}^2 \))
There were significant differences in mean Chl \( c_2 \) \( \mu g \ \text{cm}^2 \) overall between groups (higher in the reference group), between weeks, and between groups by week. This trend was not found between the exposure period and after, or groups between exposure period and after. Post-hoc tests revealed an overall decrease in Chl \( c_2 \) from week one to week two but no differences between successive weeks. There was significantly less pigment in the algal group than the reference group during weeks two and five. For the algal group, there was a decrease in Chl \( c_2 \) from week one to week two and week five to week seven, but not from week two to week five. There were no changes in the Chl \( c_2 \) level for the reference group over time. There was significantly less Chl \( c_2 \) in the algal group than the reference group during both the exposure period and the recovery period. There was no difference between Chl \( c_2 \) levels during the exposure and recovery periods within the algae or reference groups.
By week two, Chl $c_2$ in the algal group was 52% of the reference group and by week five, Chl $c_2$ in the algal group was approximately 50% of the reference group. By week seven, the Chl $c_2$ level had increased to nearly the same amount as the reference group.

**Total Carotenoid Content ($\mu$g cm$^{-2}$)**
There were significant differences in mean content of carotenoids ($\mu$g cm$^{-2}$) overall between groups (more in the reference), between groups by week, and groups between exposure period and after, but not between weeks, or the exposure period and after. Post-hoc tests revealed significantly less pigment in the algal groups than the reference groups during week five, but not during other weeks or within groups over successive weeks. There was significantly less pigment in the algal groups than the reference groups only during the recovery period, but not the exposure period or within groups between periods. By week five, pigment in the algal group was 45% of the reference group.

**Chlorophyll $a$ Content ($\mu$g cell$^{-1}$)**
There were significant differences in mean chlorophyll a $\mu$g cell$^{-1}$ between weeks, and between groups by exposure period and after, but not between groups, groups by week, or the exposure period and after overall. Post-hoc tests revealed an increase of Chl $a$ from week one to week two, significantly more Chl $a$ in the algal group during the exposure period, and in the reference group there was significantly more Chl $a$ in the recovery period.

**Chlorophyll $c_2$ Content ($\mu$g cell$^{-1}$)**
There were significant differences in mean Chlorophyll $c_2$ in terms of $\mu$g cell$^{-1}$ between weeks, and between groups by exposure period and after, but not between groups, groups by week, or the exposure period and after. Post-hoc tests revealed an increase of pigment from week one to week two, and in the reference group there was significantly more Chl $c_2$ in the recovery period.
**Total Carotenoid Content (µg cell⁻¹)**

There were significant differences in mean content of carotenoids when normalized per cell (µg cell⁻¹) between weeks, between groups by week, between groups by exposure period and after, but not between groups overall or the exposure period and after. Post-hoc tests revealed an increase of carotenoids from week one to week two and a decrease from week two to week five. There were significantly more carotenoids in the algal group than the reference group on week two and significantly less carotenoids in the algal group than the reference group on week five.

In both the algal and reference groups, carotenoids increased from week one to week two and from week two to week five with no change in week five to week seven. There were significantly more pigments in the algal group than the reference group during the exposure period and significantly less pigment in the algal group than the reference group during the recovery period. Also, there were marginally significantly more carotenoids in the recovery period than the exposure period for the reference group with no differences in the algae group between the two periods.

Carotenoid content (µg cell⁻¹) increased in the algal group by as much as 2.8 x between week one and week two and decreased by as much as 3.2 x between weeks two and week five. The level of pigment on week two was 1.8 x the amount of pigments for the reference group.

**Chlorophyll a:c Ratio**

There were significant differences in the mean chlorophyll a:c ratio overall between groups (where the algae group had higher ratios), between weeks, between groups by week, between groups by exposure period and after, but not between the exposure period and after. Post-hoc tests revealed a higher pigment ratio in week two than week one, a significantly lower pigment ratio from week two to week five in the algae group, and significantly higher pigment ratio in the algal group than the reference group at week two. In addition, there was a significantly higher pigment ratio in the algal group than the reference group during the exposure period and a significantly higher pigment ratio in the
exposure period than the recovery period for the algae group, but no differences in the ratio during the recovery period between groups or time periods for the reference group.

The algal group increased its Chl $a/c$ ratio by as much as 10% between weeks one and two and then returned to levels comparable with week one by week five.

**Oxidative Stress**

There were no significant differences in mean DNA AP sites between groups, between weeks, group by week, between the exposure period and after, or groups between exposure period and after. The mean quantity of DNA AP sites was $302 \pm 32 \text{ SE } 10^5$ base pairs (bp) across all time points across all groups.

**Metabolic Condition**

There were significant differences in mean L-lactate concentration overall between weeks, between the exposure period and after, but not between groups, between groups by week, or between groups by exposure period and after. Post hoc analyses suggested that overall lactate concentration marginally increased from week one to week two and that overall, lactate concentration was higher on week five than during the first two weeks.

**Discussion**

This field transplant study attempted to investigate the impacts of *G. salicornia* to the coral *P. compressa* in mortality and physiologically when it is overgrown by the alga and determine if the coral, once affected, could recover if management was simulated and the algae were removed. The field transplant study yielded 97.5% survivorship with the loss of one sample of each group. Of interesting note, during weekly inspection, the sea slug *Phestilla sibogae* was found underneath the tile of two separate nubbins, which were missing tissue in both an algal mat group and a reference group from two different sites at two different times. This slug is known to have a strong chemosensory response to this species of coral, which is a primary prey (Hadfield and Pennington 1990). In this case,
the presence of the algal mat did not prevent the detection of the coral by the slug. Both of these nubbins were not sampled and by the end of the field experiment (seven weeks) they had completely died. Otherwise, no visible lesions or obvious signs of disease developed in the coral. Coral under the algal mats frequently had mucus, particulate matter, and sediment attached, though there was live tissue visible on the coral.

**Zooxanthellae Density**

It was hypothesized that the coral would exhibit a physiological response to shading by the algal mat over time by increasing pigments. Figure 4.2 shows the physical appearance of the coral in the algal mat group and reference groups over time. One of the unanticipated responses that was detected was a bleaching effect. At one week of exposure to the algal mat, the coral appeared pale; then at two weeks the coral appeared very white, with a scarce smattering of lightly colored spots on some individuals. Due to this unanticipated effect, and because coral bleaching is a very serious health issue, at this point the recovery phase of the experiment was implemented because it was uncertain if the coral would survive in these conditions.

Unlike most studies, which subsample a small portion of a whole coral, in this study the whole coral was sampled and homogenized to calculate a mean zooxanthellae density. This was the best way to account for the heterogeneity of color (presumably more dense zooxanthellae in some locations than others with more pigments in the areas with some light) across the coral when considering the entire potential photosynthetic capacity of the whole coral. There was a good deal of variability in the physical conformation of each mat and though branches were tightly woven, there were a few spaces between the branches that likely allowed some light to come through to the bottom of the mat at different angles over time (especially with wave action), which may account for the variable color shading patterns that formed on the coral in the algal group. Zooxanthellae density can vary between different species, different depths, and even different parts of a single colony (Helmuth et al. 1997). Though possible, it is unclear if the zooxanthellae were more concentrated in areas that may have received light. Titlyanov et al. (2001) found that *Stylophora pistillata* acclimating to light levels of 30–8% PAR increased both
zooxanthellae density and pigments. In contrast, they found that, coral acclimating to extremely low light such as 0.8% PAR lost zooxanthellae and propose that it was via degradation, while the retained zooxanthellae accumulated high concentrations of chlorophyll. Previously we measured transmitted light levels of <1% PAR under algal mats that were used in this study (Chapter 2). These results are in agreement with the Titlyanov et al. (2001) study.

It is likely that what occurred was dark bleaching. Dark bleaching has been reported a handful of times in the literature (Downs et al. 2009, Koren et al. 2008, Franzisket 1970, Smith 1939). Smith (1939) found that dark bleaching in anemone’s resulted as an exocytosis of the algal cell from the animal, where Downs et al. (2009) found evidence of significant vacuolization in the gastrodermal cells as well as microvacualization occurring after 18 h in the dark, which may have been a precursor to cell autophagy as was seen in coral that were held in the dark under high temperatures. Without histological examination, it is unclear what happened to the symbionts in this study. It is possible the symbiont cells were separated via exocytosis (Smith 1939), digested as host cells starved (Downs et al. 2009, Titlyanov et al. 2001), or both (Titlyanov et al. 1996).

Just one week under the algal mat was sufficient to induce a change in color that could be visually scored as bleaching. Coral bleaching is a serious health impairment as the delicate relationship of animal and alga is interrupted. The consequences of coral bleaching to the coral animal include reduced energy and metabolite transfer from the alga to the animal and lack of photoprotective and antioxidant properties of the alga, which can result in mortality (Brown 1997) and the following recruitment of algae (Diaz-Pulido and McCook 2002). The fact that there was significantly less zooxanthellae in coral of the algae group than the reference group, suggests that zooxanthellae density decreased and never recovered when monitored up to five weeks.
**Algal Pigments**

**Pigment by Surface Area**
By weeks two and five there were significantly less Chl $a$ and $c_2$ per surface area in the algal group than the reference group. Further, the total carotenoids were significantly less per surface area in the algal group than the reference group at week five. Additionally, there was a decrease in Chl $c_2$ from week one to week two and week five to week seven, suggesting a partial decrease in Chl $c_2$ over time in the algal group, while there was no change in the reference group. It is likely that there were either less symbionts and/or symbionts were more dispersed. Reduced pigment per surface area generally could suggest either less light harvesting capability, resulting in less photosynthate available for both the alga and the animal, or sun acclimation, which can be ruled out by the experimental treatments. Additionally, if zooxanthellae became more dispersed, self-shading could have been reduced perhaps making light harvesting more efficient (Stambler and Dubinsky 2005, Crossland and Barnes 1977). There was no difference in the amount of pigment per surface area between groups by week seven (five weeks in recovery), suggesting that the light harvesting capability, when assessed by surface area, had recovered.

**Pigment by Zooxanthella Cell**
During the exposure period the algae group had significantly more Chl $a$ per zooxanthella cell than the reference group. In both groups, there was more Chl $a$, Chl $c_2$ and total carotenoid contents per cell in week two than week one. There was more overall Chl $c_2$ on week two that week one. Amongst both groups there were more carotenoid contents at week two than week one or five. At week two the algal group had more total carotenoid content than the reference group, then at week five the algal group had lower levels of total carotenoids than the reference group. It was likely that the zooxanthellae that remained in the coral increased their pigments in response to lower irradiance.

There was a significant difference between amounts of Chl $a$ between the groups during the exposure period, which suggests that pigment per cell was increased during the
exposure. Chl $a$ is the major pigment involved in photosynthesis and the pigment of highest quantity in chloroplasts; an increase in this pigment suggests an increased light harvesting adaptation. There was no difference between the amounts of Chl $c_2$ between groups overall, between groups on any single week, or between periods. Chl $c_2$ increased from the exposure period to the recovery period for the reference and any concurrent signal from the algal group may have been lost in this comparison during the analysis. Further, the Chl $a:c$ ratio was higher in the algal group than the reference group on week two, which supports the increase in Chl $a$ and lack of increase in Chl $c_2$. Carotenoids such as peridinin and beta-carotene function both as accessory pigments, which aid in light harvesting, and as antioxidants (i.e., reducing agents), where xanthophylls also function as antioxidants. The fact that carotenoids were elevated on week two may suggest a shading acclimation response and/or an antioxidant response. Also of interesting note, the reference group had more Chl $a$ and $c_2$ throughout the recovery period than the initial period, which underscores the necessity to have a paired reference when studying organisms with dynamic physiologies such as coral. The mean Chl $a$, Chl $c_2$, and carotenoids for the reference group were approximately in agreement with data from a study by Kuffner (2005) on $P. compressa$ for the time of year in the same general location. The amount of pigment for the algal group during the algal mat exposure was less than reported in the Kuffner (2005) study. Appril et al. (2007) studied $Porites lobata$, a related coral near the same location over a range of depths to 21 m, to find varying basal levels of pigmentation, which are also comparable to those, found in this experiment.

The increase in pigment per zooxanthella cell during the algal mat exposure under less than 1% PAR was in agreement with findings of the Titlyanov et al. (2001) study. Further, Falkowski and Dubinsky (1981) performed a reciprocal high light and low light adapted coral transplant with $S. pistillata$ in the Red Sea and found that both groups acclimated to the different light regimes.
Oxidative Stress

There was no difference in DNA AP sites between groups or over time. This suggests either there was no impact of DNA damage from ROS caused by the algal mats or that there was a chemical or physical effect that occurred homogenously along the reef. If there were any elevated ROS produced by the algae or by the coral in response to a toxicant effect or nightly hypoxia, it is possible that internal antioxidant compounds reduced damage from these molecules. Zooxanthellae have a number of antioxidant pigment compounds including peridinin, b, b carotene, and xanthophylls diatoxanthin and dinoxanthin. Peridinin is a major accessory pigment and more abundant in dinoflagellates than other pigments (Appril et al. 2007) and although it quenches ROS five-fold less efficiently as beta-carotene, it may be the main functioning antioxidant in coral (Pinto et al. 2000). Peridinin also absorbs light energy in the 470–550 nm ranges and to assess carotenoids in the study we read absorbance at 480 nm, so it is very likely that the major carotenoid detected in this study was peridinin. If coral were experiencing elevated ROS, the increase in accessory pigments likely would have had antioxidant activity and thus could partly explain a lack of evidence of oxidative stress. Coral animals have non-enzymatic antioxidants such as ascorbic acid and glutathione (Lesser 2006), green fluorescent proteins (Bou-Abdallah et al. 2006), mycosporine-like amino acids (Shick and Dunlap 2002), carotenoids, and tocopherols (Furla et al. 2011). However, many host carotenoids such as beta-carotene are derived from zooxanthellae in the coral animal (Mobley and Gleason 2003). In addition, the coral animal and zooxanthellae also have enzymatic antioxidants including Cu/Zn and Mn superoxide dismutase, catalase, and peroxidases, to protect them from oxidative damage (Furla et al. 2011; Lesser 2011; Downs et al. 2007, 2006, 2005a; Rougée et al. 2006).

The range of AP sites found in *P. compressa* (270–334 per $10^5$ bp) during its time on this reef may be a reflection of the basal amount present in the genetic individual and/or the species that was used for this experiment. Coral living in a range of environments on coral reefs in the Atlantic, Caribbean, and Pacific generally have a range of 26–462 AP sites per $10^5$ bp, which appears to be consistent for individual species (Cheryl Woodley and Lisa May unpublished data). Coral experiencing oxidative stress often have DNA
AP sites a few hundred if not thousands higher than healthy coral (Downs et al. 2011). This research establishes the first baseline for *P. compressa*. It is also possible that a stressor such as a chemical agent was present homogenously at the study site and affected both groups in the same way.

**Metabolic Condition**

There was no significant difference in L-lactate between groups. It is possible that any L-lactate produced from anaerobic respiration during periods of hypoxia that may have occurred at night were metabolized by the time the coral was sampled. It is also likely that L-lactate was not the primary metabolite of anaerobic respiration in coral. There is almost no information on anaerobic respiration in coral and little more available on the entire Cnidarian phylum. Organisms most commonly produce lactate in either of two isomers, L-lactate and D-lactate. Some organisms, like humans, produce both isomers but predominantly one over the other. Cnidarians (inferred from data on anemones) are regarded to possess the L-lactate isoform and while several other invertebrate species have the D-lactate isoform as the primary metabolite of anaerobic respiration, individual investigation may be warranted because different classes of the same invertebrate Phyla have been known to possess both L and D isoforms (Long and Kaplan 1968). Navarro and Ortega (1984) surveyed several metabolites when an anemone was subjected to hypoxia and while they did find that lactate (isoform not distinguished) increased 10-fold, they also found the amino acid, alanine, increased 20-fold, suggesting that metabolism was undergoing transamination processes. In addition to lactate dehydrogenase, there are several other specialized pathways that some animals, including Cnidarians, use for metabolism in anaerobic respiration including octopine, alanopine, and strombine dehydrogenases (Livingstone 1991). These opine pathways involve the reductive condensation of pyruvate with an amino acid to form an imino acid derivative. There can be several distinct reasons why we did not detect elevated levels of L-lactate in coral samples that were exposed to the algal mats: 1) there was no anaerobic response and thus metabolite to detect; 2) L-lactate was produced during periods of hypoxia but by the time the coral was sampled (~ 4 h after sunrise) L-lactate had been metabolized; 3) another
metabolite was produced via an opine pathway; or 4) the isomer of lactate that the coral produce was D-lactate instead of L-lactate.

This research showed dramatic impacts to the biology of the coral holobiont. Dark bleaching and photosynthetic pigment response to low light were the major detected effects. It is alarming to discover that when the algae cover coral for just two weeks, these dramatic changes can take place. It is unclear that if the exposure period was longer if the coral would have suffered any other detrimental effects. Coral overgrown for as much as seven weeks did not suffer mortality and pigmented tissue was found concentrated inside the coral, with endolithic algae sometimes visible through the paleness of the coral. Fine and Loya (2002) have suggested that endolithic algae can be a source of energy for coral during bleaching and it is unclear what role (if any) they played here. Franzisket (1970) found that Hawaiian P. compressa held in darkness with ambient water flow (with plankton food) survived over 60 days after bleaching and suffering tissue atrophy. He found that the coral recovered with exposure to light in filtered seawater, within three weeks. This reference gives some information as to the resilience of P. compressa when light is excluded; however, the recovery process from Franzisket’s experiment utilized filtered seawater, which may have filtered out possible sediment and fouling propagules of algae and invertebrates, which makes it difficult to apply to the case of smothering by G. salicornia in the field. It is unclear whether or not P. compressa in the dark for 60 days can compete with potential algal fouling organisms in a stressed condition. Franzisket suggests that during extended periods of darkness and subsequent coral bleaching, that coral supplement nutrition with heterotrophy and in mats of G. salicornia there are an abundance of fouling organisms and fine particulates, which may act as a source of food.

Conclusions
Mats of G. salicornia appear to impose physiological stresses to the coral P. compressa by inducing bleaching despite low light levels and yet requiring enhanced photosynthetic pigments. Impacts of oxidative stress and hypoxia were not detected with the methods used. P. compressa covered with mats of G. salicornia recovered after five weeks.
Nonetheless, the results from this study are encouraging because they suggest that *P. compressa* smothered by *G. salicornia* may not suffer immediate mortality and that the potential for recovery if management action is taken is promising. Further, we suggest that environmental resource management techniques be investigated for efficacy by using physiological metrics in addition to ecological metrics.
Chapter 6: *Gracilaria salicornia* as a Competitive Dominant Invader and the Potential for Management Success

Summary

The red alga, *Gracilaria salicornia*, is an invasive species in Hawai‘i, which overgrows native organisms and substrates (Conklin 2007, Smith et al. 2002, Stimson et al. 2001). Increases in algal cover have been associated with decreases in coral cover (Conklin 2007), and it was unknown how the algae affected coral reef habitat quality and coral biology.

The research presented in this dissertation describes fine-scale, short-term impacts of the mat-forming alga, *Gracilaria salicornia*, on the physical habitat quality of coral reefs, on aspects of coral health in different life stages, and on the resilience of coral overgrown by this alga. Further, the first and only data are presented on the reproductive biology and recruitment ecology of the indigenous coral, *Porites hawaiiensis*.

Algal mats of *G. salicornia* attenuated as much as 99% of irradiance. Competitive dominant mat forming algae impose a substantial loss of light energy and resulting photosynthetic energy to benthic photosynthetic organisms like coral. That energy is important for growth, reproduction, and basic metabolism and presumably the loss of that energy results in impacts to at least one, if not all, energy requiring processes. *G. salicornia* directly accelerates sediment and particulate accumulation to the substrate below and organisms overgrown by this alga also experience accelerated sediment deposition. Organisms that inhabit algal mats in minimally flushed conditions may experience conditions of hypoxia, high CO$_2$, and enhanced acidification, which can induce death, oxidative stress, or less efficient metabolism. These altered conditions may shift the community dynamics from naturally formed community assemblages to those in favor of organisms that are tolerant of *G. salicornia* overgrowth.
For the important coral recruitment processes of larval settlement and survivorship no impacts were found to larval settlement of *Porites hawaiensis* and *Pocillopora damicornis* larvae with no effect on survivorship of *P. damicornis* larvae in the field assay and reduced survivorship of *P. hawaiensis* in the lab assay. The precise impacts of this alga to coral recruitment processes are difficult to tease from potential confounding variables and variability in species. Further, logistical constraints limit the ability to research this important issue.

When algal overgrowth of the most common species of coral in Kāne‘ohe Bay, *Porites compressa*, was simulated, the coral exhibited signs of darkness and low light induced stress. When management was simulated and the algae were removed, the coral recovered, suggesting that this species is resilient and that algal control efforts will be successful in restoring the health of coral overgrown by this alga. In spite of prominent opportunities for oxidative damage to occur to coral overgrown by the algae and again when the coral were recovering, there was no evidence of damage, suggesting that the coral had sufficient internal defenses to oxidative stress. In addition, the lack of evidence of elevated anaerobic respiration through lactate dehydrogenase and l-lactate metabolites in coral overgrown by the algal mats experiencing low oxygen levels suggests that other pathways may be involved in this process in coral. The synthesis of these data strongly suggests that the invasive alga, *G. salicornia*, is a source of disturbance on coral reefs in Hawai‘i, which must be controlled and that further work is needed to understand physiological processes in coral. These results provide a wealth of information that can be helpful for the justification and development of management strategies and future research. In areas targeted for management, attempts to save live coral under *G. salicornia* are not a lost cause. Based on this research, it is recommended that locations with live coral living underneath or bordering mats of invasive algae should be prioritized for algal removal. Further, the coral species from the field study was a branching perforate coral; this form is the most resistant of coral morphologies to sediment accumulation because it has less horizontal surface area and tissue can live several mm into the skeleton. Encrusting, plating, and other morphologies that grow at a horizontal axis (such as for the second most common coral in Kāne‘ohe Bay, *Montipora capitata*),
are extremely more sensitive to the impacts of sediment accumulation from *G. salicornia* and coral species of these growth forms should be prioritized first in restoration.

The research in this dissertation also describes for the first time, the reproductive biology and recruitment ecology of the coral, *Porites hawaiiensis*. This study provides rich insights into coral biology by describing an impressive reproductive output for a small coral and describing the first known Scleractinian zooxanthellate coral with non-swimming larvae. It is necessary to understand a species in order to implement proper management. Based on this data it is suggested that coral 0.9 cm$^2$ and smaller not be damaged or collected, so they can reach reproductive size.

**G. salicornia as a Successful Invader**

*G. salicornia* persists as a perennial species at sites on O‘ahu. This marine plant has a suite of physiological characteristics, which allow it to competitively dominate in a variety of hard and soft bottom substrate habitats. This marine plant grows with small, creeping, one layer thick branches in areas of high water motion to mats as thick as 5–9 cm (Ch. 2; Beach et al. 1997), which grow on hard and soft bottom sediment substrates. This marine plant has an extremely efficient light harvesting ability, which allows it to maximize photosynthesis in relatively low irradiance (Phooprong et al. 2007) and forms gradients of both photoprotective and light harvesting pigments (Beach et al. 1997) so it can minimize damage and maximize the absorbance of irradiance through its thick, self-shading morphology. Branches can live fully buried in mud while the alga simultaneously accelerates the sedimentation of itself, which contributes sources of nutrients that may support its own growth (Larned 1998). The respiring biomass of the biological communities that the mats support can cause periods of anoxia and acidification at night and still the alga persists. *G. salicornia* has a suite of active antioxidant defenses (Vijayavel and Martinez 2010), which may allow it to survive in the harsh conditions in which it lives. Several species of Gracilariales are known to produce bursts of reactive oxygen species (ROS) in response to stress and pathogen defense as well as volatile halogenated organic compounds (VHOC) and these processes likely also
occur in *G. salicornia*. Further, ROS and VHOC may reduce palatability and thus grazing, further protecting *G. salicornia*.

The success of *G. salicornia* may yet improve with the oncoming pressures of climate change. The consequent negative effects of increasing temperature, ocean acidification, and sea level rise on coral resulting in death could open up a significant amount of substrate for macroalgae (Fong and Paul 2011), such as *G. salicornia*. Rising sea level, increased coastal erosion, and increased storm intensity could all lead to increased sedimentation on coral reefs and the deposition could be further enhanced be the presence of algal mats. Macroalgae such as *G. salicornia* may thrive with the infusion of nutrients that comes along with runoff and sediments. Finally, increased levels of CO₂ through industrial emission may accelerate the growth of species of *Gracilaria* as has been shown in culture (Israel et al. 2005, Friedlander and Levy 1995).

**Organisms Living with *G. salicornia***

The conditions that the algal mats impose can create distinct microhabitats, which may suit some species more than others. Organisms that can live successfully under or in an algal mat must either be mobile and thus leave the algal mat or possess a physiology that can tolerate this altered microhabitat. Sessile autotrophic organisms must have extremely efficient light harvesting abilities and photosynthetic efficiencies to sustain in low light conditions. Detritivore and filter-feeding invertebrates likely capitalize on the rain of particulates and possible plankton accumulating in the algal mats. Further, organisms must be resistant to sediment stress, particularly in areas of low water motion where sediment may settle out. Encrusting and plating morphologies could likely get buried in sediment. The organism must be able to tolerate periods of hypoxia, hyperoxia, and lower pH. These conditions are consistent with production of reactive oxygen species near or in organisms both extracellularly and intracellularly. Organisms that don’t require high water motion and can be resistant to algal abrasion, such as organisms with hard skeletons and shells may survive in the algal mats better than soft and delicate growth forms. Finally, organisms that live in the algal mats must not produce large and passive reproductive propagules that heavily depend on large-scale synchronized
dispersal for reproduction because the algal mats may restrain these propagules. Detritivores and filter feeders such as crabs, sea cucumbers, sponge, fan worms, and occasionally small goby fish can be found living amongst or underneath the algal mat. Most of these organisms are either mobile, tolerant of low oxygen conditions, algal abrasion, and sediment or spawn relatively small gametes.

Lessons Learned and Future Research
This research has shed light on a number of aspects of research and has equally created numerous follow-up questions. It is unclear how patterns of community respiration occurring in the algal mats affect the process of calcification. There is no evidence of calcification occurring in the daytime or of carbonate dissolution occurring at nighttime in response to enhanced acidification and low light levels. Follow-up studies that incorporate measurements of alkalinity and calcification and dissolution should be done to further understand these processes. It is unclear if larvae, when considered either as passive or motile objects, can even reach the substrate under an algal mat in order to recruit. Follow-up studies with larvae or objects the same size and density should be done in a flume to understand if they can enter the dense interwoven net of branches, which comprise an algal mat. To gain a better perspective on the entire length of time that *P. compressa* and other species of coral can survive under an algal mat, a field transplant study should be repeated with several species over much longer time scales on the order of months to determine the resilience of different species. Also, when dark ‘induced’ bleaching is observed, samples should be fixed for histology to investigate the fate of the zooxanthellae and the additive impacts of other stressors should be determined. The use of new techniques in molecular biology, such as quantification of stress protein expression, could be utilized to investigate changes in coral health over time.

Strategies and Challenges of Management in Hawai‘i
The accumulation of large biomass of macroalgae on coral reefs is thought to be mediated by several mechanisms including a combination of increased nutrients and
decreased herbivory (Fong and Paul 2011, Smith et al. 2010, 2001, Littler and Littler 1984), reduction in topographic complexity, and natural disturbance (McCook 1999). It has been suggested that coral reef management priorities, as they relate to macroalgae and coral, should include the protection and/or restoration of herbivore populations (Mumby and Steneck 2011), managing adjacent watersheds (Richmond et al. 2007, Jokiel and Naughton 2001), and minimizing terrestrial run-off and nutrient inputs (Richmond et al. 2007, McCook 1999). In some cases, conservation and protection are preferred to restoration of impacted reefs (Jokiel et al. 2006).

Management through mitigation and restoration when considering alien species has always been challenging. Up until now, management strategy has been primarily prevention through public awareness and management practices, direct mitigation through removal of the invasive species, attempts to enhance native grazers, and the reintroduction of native algal or other benthos to appropriate areas (State of Hawai’i 2003).

**Short-Term Control Measures: Manual Removal**

The manual removal of *G. salicornia* in coastal waters of Honolulu, Hawai‘i has been attempted and has yielded mixed results. In the Smith et al. (2004) study, manual removal was found to temporarily reduce percent cover of *G. salicornia*; however the alga was found to be able to nearly double in percent cover in four weeks after clearing. In separate efforts, 20,000 kg of biomass was removed after wide-scale manual removal using 400 volunteers and 2,000 person hours to physically remove algae (Smith et al 2004), yet the algae continued to accumulate. Smith et al. (2004) also tested the influence of physical and chemical control agents and found that high temperature, high salinity, and algicide as well as herbicide were effective in reducing the algal growth rate. However, it is not particularly feasible to implement wide-scale invasive control measures, especially the introduction of chemically toxic materials on the scale of an entire reef.
A platform vessel fitted with an underwater vacuum hose called, “The Super Sucker,” has been developed to assist in efficient removal of invasive species in Hawai‘i (Conklin 2007). This procedure is successful in removing large quantities of algae over relatively short periods of time and has been adopted by the State of Hawai‘i as a sanctioned management method.

**Long-Term Control Measures: Enhancing Grazing**

Manual removal is extremely laborious and primarily a short-term solution; a more sustainable approach is to enhance grazing by herbivores. A few studies have attempted to gather information on the feasibility of enhancing herbivores as well as attempted the implementation. Conklin and Stimpson (2004) attempted to increase herbivorous fish on an affected reef in Kāne‘ohe Bay. Most of the fish did not remain on the reef very long and the result was no change in grazing activity. The authors suggested that there was not suitable shelter habitat, possibly due to degradation. Palatability tests with grazing sea urchins, *Tripneustes gratilla*, showed that naïve urchins could eat as much of *G. salicornia* as three other species of macroalgae, but urchins that had been exposed to solely *G. salicornia* when given a choice of four other species, ate the other species (Stimson et al. 2007). Smith et al. (2004) showed that amongst several species of acanthurid fish that the native *Gracilaria coronopifolia* was preferred to *G. salicornia*. As of yet, efforts to enhance grazers by protection from fishing have been slow to develop.

It is clear that more efforts should be taken to investigate management techniques of invasive algae. With the knowledge that the coral most commonly overgrown in Kāne‘ohe Bay by *G. salicornia* is capable of recovery, the motivation for successful restoration should be high.

**The Future of Research for Management**

Gaining an understanding through research of the consequences of not managing this species is essential. Further, having methods that provide short-term information can
prove vital to understanding how best to focus resources and adapt strategies if needed during management. Historically, ecological metrics such as percent cover, species diversity, and abundance were used to measure management success. Ecological dynamics are often regarded to be the most sensitive parameters to disturbance (Goatley and Bellwood 2011, Houk et al. 2010, Viehman et al. 2009, Nystrom 2006, Wilson et al. 2006). Monitoring ecological parameters is important for the long-term assessment of ecosystem health and restoration success. The drawback of using these as the primary metrics to assess the success of a management action is that these processes take time, often on the scale of years, until any measurable unit of change can be detected. For a long-term restoration action, years may pass before enough data can be collected to assess whether a particular action is effective and opportunities for adaptive management would also become delayed. Biological and physiological metrics, such as those used in these studies, have the benefit of producing usable data in the matter of days to weeks and are more sensitive than ecological metrics in the short-term. The two tests for the impact of *G. salicornia* to larval settlement were accomplished with 1–3 weeks preparation and 2–4 days of actual study. The field coral transplant study was accomplished from start to finish in less than three months. The following laboratory work took an additional 6–8 months after training, methods, and resources were gathered and developed. At the other end of the spectrum, if one was to wait for reproductive seasons or use coral growth, percent cover, or benthic community dynamics as indices of management success, the time scale could be much longer before any result could be detected. In the case of coral research, parameters such as zooxanthellae density, efficiency of electron transport, pigment concentrations, DNA damage, and stress protein expression are all short-term indices of coral health. The drawback to advanced physiological methodologies is that they require some specialized expertise and equipment and so capacity and resources should be balanced with the needs for short-term data, while leveraging strategic partnerships to accomplish goals. Restoration biology should incorporate physiological metrics as at least intermediate indices of success.
Additionally, proper thought should be given to experimental design; to include an appropriate reference and to minimize confounding variables and opportunities for excessive assumptions. If solely ecological methods from a descriptive study rather than a manipulative study were used to investigate the impact of *G. salicornia* on coral biology, erroneous conclusions from field data could have occurred. For example, a field survey that lifts up algal mats and records the substance under the algal mats may have revealed some live or bleached coral, but more than likely it would have revealed carbonate substrate with no live coral. In the case of *P. compressa*, the branches are distinct and one may have drawn the conclusion that *P. compressa* was previously alive in that location and that the algal mat caused its death because the coral was not alive. The fact of the matter is that without direct data it is impossible to know if the coral had died before or after the algal mat attached to the substrate. Further, one could survey live coral bordering algal mats of *G. salicornia* and analyze the frequency and incidence of disease; however direct causation is still difficult to infer. Finally, if one monitored several areas of live coral in proximity to algal mats over time and documented the change in coral health over time, similar to methods used for the field transplant experiment from this research, one may have to wait several months to years for the algae to naturally invade those locations with no assurance that enough locations would be invaded to give enough replication for statistical analyses. Any of these three hypothetical studies would have given weak information and would be extremely inefficient.

**Final Conclusions**

With only a handful of studies describing the physiology and ecology of the invasive alga, *Gracilaria salicornia*, it is clear that more work needs to be done and that this alga needs to be managed in Hawai‘i. It is most likely that a combination of both short-term control measures (e.g., manual removal) and long-term sustainable control measures (e.g., increasing grazing activity by enhancing grazers) would be most effective in controlling invasive macroalgae such as *G. salicornia* in Hawai‘i. Though it may be unlikely that *G. salicornia* will ever become eradicated completely, and with the
increased potential for an enhanced competitive advantage in the future from climate change, the need to set sustainable control measures in motion is ever more critical.


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123


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