ECOLOGY AND PHYSIOLOGY OF ANTARCTIC MACROPHYTES UNDER THE INFLUENCE OF ABIOTIC AND BIOTIC STRESSORS.

by

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ECOLOGY AND PHYSIOLOGY OF ANTARCTIC MACROPHYTES UNDER THE INFLUENCE OF ABIOTIC AND BIOTIC STRESSORS

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ABSTRACT

The physiology of many species of Antarctic marine algae was evaluated under a variety of biotic and abiotic factors found or predicted for the western Antarctic Peninsula. The potential biological stressors, filamentous algal endophytes, had few effects on their macrophyte host’s physiology which may be the cause for their persistence in this marine community. Adversely impacted hosts include Pachymenia sp. and Iridaea cordata. In areas of high endophyte infection Pachymenia sp. was significantly less resistant to forces that could penetrate their thalli. Maximum quantum yield near endophyte infection was significantly lower in I. cordata. Endophyte abundance is significantly lower in female gametophytes with carposporophytes than in unfertilized gametophytes and tetrasporophytes of I. cordata. The effects of potential abiotic stressors, near-future climate change conditions (including oceanic pH 7.8-7.6 and a temperature increase of 2 °C) were evaluated in the canopy-forming brown macroalgae Desmarestia anceps and D. menziesii, and the crustose calcified and fleshy red macroalgae Clathromorphum obtectulum and Hildenbrandia sp. The physiological responses of these macroalgae were measured through growth, photosynthetic parameters, chlorophyll a, calcium carbonate, and phlorotannin content. Species responded uniquely to each factor, notably Hildenbrandia sp. increased growth in high temperature * low pH treatments and D. menziesii increased phlorotannin content in low pH treatments and increased saturating irradiance in high temperature * low pH
treatments. The effects of crustose coralline algae on the settlement preferences of invertebrate larvae were examined. An interaction between coralline algae and invertebrate larvae would be particularly important for community dynamics if climate change impacted the physiology or dominance of these Antarctic coralline species, but no significant settling preference was observed for any coralline algal extract. In light of the competitive advantage *Hildenbrandia* sp. may have in near future crustose algal communities, this may be a positive finding. This dissertation shows that Antarctic algae are for the most part resilient to the biological and abiotic stressors assessed in these studies, and though macroalgae do not impact invertebrate settlement in the benthic environment, I provide a record for settlement patterns in this geographic region.

Keywords: Antarctic macroalgae, climate change, endophytes, recruitment, physiology
DEDICATION

This dissertation is dedicated to my extended family: Gamma, Ma, Randy, Davo, Dado, Stephanie, Sofie, Maggie, Reyna, Ami, Stepdawg, Megan, Elise, Kirk, Adam and Phil, Brendawg, Karl and Noah, Chris Voisard, Cindy and Bob, Mackenzie, Seanito and Kyle, Tarik and Alex, Port and Todd, Aunties Caffy, Alicia and Laurie, Uncas George Marco and Mark, Breen Hofmann, and of course Lakeesha, my Arctic Fox. Everyone has been incredibly supportive during my graduate studies.
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The Antarctic Environment

The western Antarctic Peninsula is characterized by an ice-free coastline for much of the year providing a unique subtidal environment on the Antarctic continent where photosynthetic organisms can flourish. The near-shore benthic community is home to dense macroalgal forests dominated by the endemic brown algae Desmarestia spp. and Himantothallus grandifolius, with Cystosphaera jacquinotii interspersed within deeper Desmarestia spp. canopy and Ascoseira mirabilis in shallower habitats/canopies (Wulff et al., 2009, Wiencke et al., in press). These canopies can reach biomass levels comparable to temperate kelp forests (Amsler et al., 2008) and structure the shallow habitat in the Antarctic benthos as kelps do in the Arctic and temperate marine communities (Clayton, 1994). Within Desmarestia canopies there is a robust sub-canopy of brown and red algae, including crustose morphologies, and an abundance of invertebrate fauna that includes mesograzers (grazer < 2.5 cm) and sessile invertebrates (personal observation).

The continent of Antarctica is isolated by the Southern Ocean and the continuous eastward movement of the Antarctic Circumpolar Current. This isolation combined with extreme environmental conditions (Zacher et al., 2009), including annual fluctuation in light levels and constantly low temperatures, has driven the adaptation of marine organisms in this marine community. One example of this is many invertebrates and marine algae are stenothermic (Peck, 2005, Wiencke et al., 2007). Ocean temperatures currently range from -1.8 to 2.5 °C on the
The western Antarctic Peninsula is particularly vulnerable to aspects of climate change and it is experiencing one of the fastest rates of change in the world (Clarke et al., 2007, Ducklow et al., 2013, IPCC, 2013, Turley, 2013). The average global increase in ocean temperature was 0.11 ± 0.02 °C per decade between 1971 and 2010 (IPCC, 2013), and in the last half century the oceans around the western Antarctic Peninsula have increased 1 °C in average temperature (Ducklow et al., 2013). Ocean temperatures are projected to increase by 0.6 to 2.0 °C by the end of the century, and deep water warming (>1000 m) will be most pronounced in the Southern Ocean. Changing sea ice duration and extent in this region due has been attributed to climate change, and this affects multiple trophic levels (Ducklow et al., 2013). Warming alone has been shown to severely impact species diversity in many ecosystems (Hillebrand et al., 2012), and temperature impacts may be more severe when coupled with other climate change factors.

The observed and projected increase in atmospheric $p$CO$_2$ (Tans & Keeling, 2013) not only causes warming but also reduces the pH of the world’s oceans. This shifts the saturation states of carbonate species (aragonite, calcite, and magnesium calcite (Mg-calcite)) and, because of their solubility increases in consistently cold seawater temperatures, the polar oceans are likely to become under-saturated in aragonite and Mg-calcite before other oceans (Orr et al., 2005), making it difficult for organisms to maintain calcification. Crustose coralline algae, which are key organisms in many benthic marine ecosystems, may be negatively impacted by these water changes, while fleshy macroalgae generally respond positively (Roleda & Hurd, 2012). If
lower pH and higher temperature do impact these marine communities, there is potential for a regime shift in both floral and faunal subtidal communities in the subtidal Antarctic.

**Antarctic Marine Algae**

The physiology of Antarctic marine algae is of great interest to phycologists because of their adaptations to extreme environmental conditions [summarized in Wiencke & Amsler (2012) and Wiencke et al. (2007)]. Approximately 124 algae species have been described from Antarctica but there are still cryptic morphologies that are hard to identify to species. Antarctic algae have many unique life history characteristics including growth periods that precede optimal light conditions, low optimal temperatures for such metabolic processes as photosynthesis, and many species elaborate secondary metabolites within their thalli (Lebar et al., 2007). In fact, 76% of algae in the benthos near Palmer Station elaborate chemical defenses against asteroid herbivores and 53% against fish herbivores (Amsler et al., 2005, Amsler et al., 2005, Amsler, 2008, Aumack, 2010, Amsler et al., 2014). The high abundance of chemical defenses in algae (and sessile invertebrates) of Antarctica is unique from a global perspective; selective pressures for defenses are expected to be higher at low latitudes because the sheer numbers and diversity of herbivores found in those habitats (Amsler et al., 2001).

Another unique aspect of the Antarctic phycobenthos is the paucity of free-living filamentous algae in the subtidal environment (Peters 2003), which are common in turf communities and as epiphytes on temperate and tropical reefs (Stephenson & Stephenson, 1972, Miller et al., 2009). Most filamentous algae in this geographic region are endophytic within the chemically defended macroalgae (Peters, 2003, Amsler et al., 2009). The regulation of the filamentous algal community is commandeered by the large mesograzer cohort which uses the
chemically defended macroalgal canopy as a refuge from visual predators, selecting for the abundance of filamentous algal endophytes (Fig.1, Amsler et al. 2014). The effect of endophytes on their macroalgal hosts was investigated in four macroalgal hosts from the region; *Gymnogongrus turquetii, Iridaea cordata, Myriogramme manginii*, and *Trematocarpus antarcticus*. High endophyte presence had pathogenic impacts on *I. cordata*, mild pathogenic impacts on *G. turquetii* and *T. antarcticus*, and did not impact *M. manginii* at all (Schoenrock et al., 2013). Many aspects of the Antarctic macroalgae and the benthic community remain to be investigated still, especially in light of global change in oceanic conditions.

Figure 1. Community interactions between mesograzers and algae in the subtidal western Antarctic Peninsula (taken from Amsler et al. 2014).
Objectives

The goals of this dissertation are to establish a baseline for many physiological parameters in a variety of Antarctic macroalgae and examine their response to physical and biological stressors including endophytes and climate change. Many species in this study can be cryptic, especially crustose coralline algae and endophyte species, and identification and morphological description of focal species is summarized in Appendix A. The first two chapters of this dissertation further examine the interaction between host and endophytic algae using the physiological properties photosynthetic parameters (chlorophyll a fluorescence), thallus toughness, and palatability in many host species, and fertility in I. cordata.

Chapters three and four investigate the effects of near-future oceanic conditions on the physiology of four common macroalgal species from the western Antarctic Peninsula. Chapter three focuses on the ecologically dominant species Desmarestia anceps and D. menziesii. Their responses to lower pH and higher ocean temperatures were measured through photosynthetic parameters, growth, chlorophyll a and phlorotannin content. In chapter four, two crustose red macroalgae (the calcified Clathromorphum obtectulum and fleshy Hildenbrandia sp.) were exposed to the same conditions. Physiological responses of these algae were measured through photosynthetic parameters, growth, chlorophyll a and calcium carbonate content (in the coralline algae only). Though climate change is likely to affect macroalgae species differentially due to specific life history characteristics, this research gives insights into how the community will respond to impending ocean conditions.

Lastly, chapter five investigates the relationship between two crustose coralline algal species (two life history stages of Clathromorphum obtectulum, and one stage of Hydrolithon subantarcticum) and settlement of benthic invertebrate larvae. In many marine communities
coralline algae or their associated microbiota can enhance invertebrate recruitment to the surrounding benthos (Heyward & Negri, 1999, Steinberg et al., 2002). To investigate whether such cues may 1) be present in Antarctic species and 2) may impact invertebrate recruitment, settlement plates with coralline algal extracts were deployed in the subtidal for one year to measure invertebrate settlement preferences.

Although broad in spectrum, this dissertation integrates important biotic and abiotic effects on members of the marine flora along the western Antarctic Peninsula. The interactions between algae, species responses to climate change, and the role of specific algae in the benthic community are examined. The western Antarctic Peninsula is unique in many ways, and forming a better physiological and ecological understanding of the species present can help ascertain how it is changing due to anthropogenic influences.
A COMPREHENSIVE STUDY OF ANTARCTIC ALGAL SYMBIOSES: MINIMAL IMPACTS OF ENDOPHYTE PRESENCE IN MOST SPECIES OF MACROALGAL HOSTS.

by

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ABSTRACT

Many species of macroalgae along the western Antarctic Peninsula have high coverage of filamentous algal endophytes. A previous field study showed that endophyte presence negatively impacts growth and survival in some Antarctic algae, but can have no impact on others. We examined nine species of common macroalgal hosts from the area surrounding Palmer Station, Antarctica, to examine fine-scale impacts of endophyte presence on host physiology. Physiological parameters were selected based on their potential to influence fitness of host algae. These included photosynthetic parameters, thallus toughness, and susceptibility to grazers in those species previously known to be chemically defended. We found that few macroalgal hosts are impacted by the presence of endophytes and that these impacts are not consistent across all physiological parameters. *Iridaea cordata* was the only species among the nine examined that demonstrated physiological stress in the presence of endophytes. Out of four species in the previous study, *I. cordata* was also the most heavily impacted by endophyte presence.
INTRODUCTION

The relationships formed between endophytes and their host macrophytes can be complex (Correa, 1994, Potin, 2012) and impacted by their environment (Russell et al., 2005, Eggert et al., 2010). Classification of these symbioses range from mutualistic to pathogenic depending on the species involved (Correa, 1994, Potin, 2012), therefore defining these relationships is important to understanding ecosystem processes. The high frequency of endophyte presence in many species of Antarctic macroalgae was first noted by Peters (2003) and subsequently quantified by Amsler et al. (2009). These endophytes are filamentous algae which are interstitial in the cortical cells of their host alga, rarely penetrating the medulla (personal observation). These algae are not host specific; they are present in seasonally adapted species, rhodophytes, phaeophytes, both calcified and fleshy algae (observation, Peters, 2003, Amsler et al., 2009) as well as invertebrates (Hommersand et al., 2009). In contrast, there are few incidences of free living filamentous algae in the benthic subtidal environment along the western Antarctic Peninsula. These include the filamentous brown algae Geminocarpus geminatus on senescent Desmarestia antarctica R.L. Moe & P.C. Silva and of Elachista antarctica on Palmaria decipiens (Wiencke & Clayton, 2002, Peters, 2003).

Filamentous endophytes in Antarctic macroalgae have been shown to grow freely out of the host thallus when relieved of mesograzier presence (Amsler et al., 2009, Aumack et al., 2011b). Peters (2003) hypothesized that the dearth of free-living filamentous algae and high incidence of endophytic filamentous algae in this region is the result of intense mesograzier pressure. Macroalgae in subtidal areas of the western Antarctic Peninsula support very high densities of amphipod mesograzers (Richardson, 1971, 1977, Huang et al., 2007, Aumack et al., 2011a) and a subsequent study confirmed that mesograzers associated with the chemically
defended macroalgal species in the Antarctic subtidal do indeed ablate free-living filamentous algae, selecting for their endophytic habit (Amsler et al., 2012). Worldwide, research on endophyte-macroalgal symbioses has shown that endophytes have varied effects on their hosts (Potin, 2012, Schoenrock et al., 2013). In Antarctic macroalgae, high occurrence of endophyte presence throughout a host thallus has a negative impact on growth in three species and survival in one species of macroalgae, but no impact on another (Schoenrock et al., 2013). This infers that pathogenicity of endophyte presence may be host or endophyte specific, but few studies have examined these symbioses.

Algal physiology can be measured in a variety of ways; the present study targets parameters which directly affect the fitness of an alga, more specifically its ability to grow and survive in order to genetically contribute to future generations. The first goal of the present study is to determine the effect of endophyte presence on palatability of the host to sympatric herbivores, specifically common amphipod mesograzers. In previous studies endophytes have been shown to make the host more palatable to grazers (Correa & McLachlan, 1992, Craigie & Correa, 1996) and associated algae can make a host more desirable (Karez et al., 2000). Many Antarctic algae are known to elaborate chemical defenses against herbivory (Amsler et al., 2005), but presence of palatable endophytes could make thalli more appetizing, compromising the advantage of chemical defenses. Brown and green endophytes grown out of thallus material in culture are significantly more palatable to herbivores than their hosts (Amsler et al., 2009). Alternatively, hosts may elaborate defenses in response to endophyte presence, thereby deterring herbivory as a side effect when infected with endophytes. For example fungal endophytes are responsible for production of a chemical anti-herbivore compound in terrestrial grasses (Clay, 1996).
The second goal of this study is to determine the effects of endophyte infection on host thallus toughness. Thallus toughness is a defense against grazer pressure and/or environmental stressors (Littler & Littler, 1980). Previous studies have shown that endophyte infection weakens the host thalli and decreases resistance to environmental stressors (Schaffelke et al., 1996, Ellertsdottir & Peters, 1997). Epiphytic algae associated with a host can increase drag in current or wave action and cause the thallus to break more frequently (D'Antonio, 1985, Hemmi et al., 2005, Anderson & Martone, 2014). Environmental stressors can act in two different ways on thalli: the force from wave action or current may damage a thallus by ripping it, tearing it from its substrate, or causing scour on crustose morphologies, while grazer pressure may puncture or scrape a thallus. Consequently we looked at both types of physical stress and expect that presence of endophytic algae will weaken the host thallus to both. Any weakening to grazer pressure may make that alga more accessible as a food source to local herbivores, especially if endophytes make the thallus material more palatable in that specific species.

The third goal of this study is to determine the effect of endophyte infection on photosynthetic parameters of the host. All known algal endophytes found along the western Antarctic Peninsula are pigmented and presumably provide their own energy resources through photosynthesis. By measuring chlorophyll a fluorescence one can define photosynthetic performance in algae and changes therein under the influence of stressors. If endophyte presence results in physiological stress to the host, the photo centers of the host adjacent to endophyte infection could be expected to be less efficient than those further away from infection (photosynthetic parameters would be negatively impacted). In previous studies pulse amplitude modulated (PAM) fluorometry has been used successfully with Antarctic algae to generate rapid light curves (RLCs) and measure maximum quantum yield (MQY) (Runcie & Riddle, 2006,
2012). From these measurements one can get an accurate calculation of maximum electron transport rate (ETR$_{\text{max}}$), saturating irradiance (E$_k$), and the slope to saturation of photo centers ($\alpha$).

The host macroalgae used in this study were chosen using information from a previous endophyte survey (Amsler et al., 2009) and field observations. Two conspicuous brown algae are included: *Himantothallus grandifolius* (A. Gepp & E.S. Gepp) Zanova and *D. antarctica*. *H. grandifolius* has an endophyte load that ranges from 0-10% cover of the thallus (Amsler et al., 2009) and is kelp-like in morphology, growing to > 15 m in length and is one of the more ecologically important macroalgae in Antarctica (Wiencke & Amsler, 2012). *D. antarctica* is a pseudo-perennial species that can grow in dense beds in the subtidal, but is more commonly found sporadically interspersed within the algal canopy. The age of individuals is distinguished by size and condition of thallus material and endophyte loads range from 0-60% cover (Amsler et al., 2009). High endophyte occurrence is more common in the second year individuals which were used in this experiment. Both species share three brown algal endophytes (genotypes) including *G. geminatus* and genotypes C and D from Amsler et al. (2009). Each has one distinct endophyte genotype; *H. grandifolius* genotype B and *D. antarctica* genotype H (Amsler et al., 2009).

Seven species of red macroalgae were included in this study: *Iridaea cordata* (Turner) Bory de Saint-Vincent, *Myriogramme manginii* (Gain) Skottsberg, *Gymnogongrus turquetii* Hariot, *Curdiae racovitzae* Hariot, *Gigartina skottsbergii* Setchell & N.L. Gardner, *Pachymenia* sp., and *Trematocarpus antarcticus* (Hariot) Fredricq & R.L. Moe. *I. cordata* has endophyte loads ranging from 0-75% cover (Amsler et al., 2009) and inhabits the shallow subtidal. *M. manginii* has endophyte loads ranging from 0-40% cover (Amsler et al., 2009) and generally
inhabits the shallow subtidal. *G. turquetii* has endophyte loads ranging from 0-35% (Amsler *et al.*, 2009) and inhabits the shallow subtidal, sometimes in dense patches. *G. skottsbergii* has endophyte loads ranging from 0-5% cover (observation, Amsler *et al.*, 2009) and can be very large (~ 3 m in diameter) inhabiting a wide depth range. *C. racovitzae* has endophyte loads ranging from 0-25% cover (Amsler *et al.*, 2009) and is generally found in the shallow subtidal. *Pachymenia* sp. has endophyte loads ranging from 0-20% (observation) and is generally found ~ 5 m depth growing to 1 m in length alongside *I. cordata*. Lastly *T. antarcticus* has endophyte loads ranging from 0-40% cover (Amsler *et al.*, 2009) and grows in large patches in the shallow subtidal, but can be found in deeper environments among the *Desmarestia* spp. canopy. Endophytes in all species include common brown genotypes as well as genotype G in *I. cordata*, genotype F in *T. antarcticus*, genotype E in *C. racovitzae* (unique to these species) and both green endophyte morphologies (Amsler *et al.* 2009). Figure 1 illustrates the range in size and morphology of the species used in this study. Not all species were used consistently throughout the experiments because of morphological or physiological differences (e.g. *M. manginii* is too brittle for tensometry) and frequency and location of endophyte presence in certain species/populations.

**METHODS**

All algae were collected using SCUBA from the archipelago surrounding Palmer Station, Antarctica (64°46' S, 64°03' W, see Amsler *et al.* 2009 for map), and placed in flow through aquaria under ~22 μmol photons m⁻² s⁻¹ irradiance (~ 12h: 12h day: night cycle) in ambient temperatures until used in experiments.
Palatability

Two different feeding studies were used to assess variance in palatability of the host thallus caused by endophyte infection. A common amphipod from the western Antarctica Peninsula, *Gondogeneia antarctica*, was used as the herbivore in these studies. To determine whether the endophyte itself makes the host more or less palatable a choice feeding assay was performed with thallus material with and without endophyte presence from the same individual (n = 30). To evaluate whether the chemistry of the host is altered by endophyte infection a choice feeding assay was performed with thallus material not containing endophyte material from two hosts: one with endophyte presence and one without endophyte presence (n = 10). *T. antarcticus*, *G. turquetii, I. cordata, H. grandifolius, M. manginii, D. antarctica* and *Pachymenia* sp. were used in the first study, and *G. turquetii, C. racovitzae, M. manginii, I. cordata* were used in the second study.

Feeding assays were run for ~96 h (or until a significant portion of the thallus material was missing) in 125 ml Nalgene bottles with filtered seawater (FSW), which was partially changed daily. Each assay used individual *G. antarctica* and included two thallus fragments as described above. A paired autogenic control in a separate bottle was used to correct for weight change in thallus material not due to herbivory. Thallus fragments 2-10 mg in mass were cut into different shapes in order to differentiate between them. At the end of the experiment, all thallus fragments were reweighed with the amphipod and change in mass of fragments exposed to herbivory were corrected using % change in autogenic controls. Feeding rate was recorded as Δ μg thallus mg amphipod⁻¹ time (h)⁻¹. Mean feeding rates were log transformed and compared using a student’s t-test and a paired student’s t-test, accordingly (SPSS). One sample t-tests were used to compare corrected feeding rates with an expected feeding rate of 0 μg thallus mg
amphipod$^{-1}$ h$^{-1}$ to determine whether fragments were significantly consumed throughout the assay. A p-value of $\leq 0.05$ was used to determine significant differences in these and all following tests.

**Thallus toughness**

Thallus toughness was measured in two ways: penetrometry following the methods of Duffy & Hay (1991) and tensometry following the methods of Carrington et al. (2001). *Pachymenia* sp., *I. cordata*, *H. grandifolius*, *M. manginii*, *D. antarctica*, *G. turquetii*, *C. racovitzae*, *T. antarcticus*, and *Gigartina skottsbergii* were used in penetrometry measurements. The force (N) required to puncture the thallus was measured in 3 locations across individual thalli (n = 10 per species) with and without endophyte presence (6 locations per individual). Repeated measures were averaged for a thallus and all data were log transformed before analysis. The force to puncture a thallus was compared between areas of endophyte presence and absence using a paired t-test (SPSS).

*Pachymenia* sp., *I. cordata*, *M. manginii*, *H. grandifolius*, *C. racovitzae*, and *T. antarcticus* were used in tensometry measurements. Locations with and without endophyte presence on the blade of an individual were cut from the thallus in a t-bone shape (Carrington et al., 2001). Each t-bone (n = 5 per species) was videotaped being broken by a Force Gauge Model M4-200 (Mark-10, Copiague NY) mounted on a Palmer stand in ambient seawater (~2 °C). Dimensions of the t-bone (thickness, width, and length between grips) were measured (cm) prior to testing, and length between grips was recorded at increasing force values. These values were used to create a stress-strain curve which calculates toughness (strain energy density, J/m$^3$) in the thallus fragments (Denny, 1988). All data were log transformed and mean toughness was
compared using a paired t-test (SPSS) for each species. All measurements were done within 48 h of collection to ensure that aquarium conditions did not change the properties of the thallus material, although such effects are probably miniscule (Harder et al., 2006).

*Photosynthetic Characteristics*

A Diving-PAM fluorometer (WALZ, Germany) was used to measure photosynthetic parameters in thallus material with and without endophyte presence. *I. cordata, M. manginii, Pachymenia* sp., *G. skottsbergii, C. racovitzae, T. antarcticus, and G. turquetii* were used in this experiment (n = 3-8 depending on species). Absorbance of the photosystems in each species was measured using the PAM light diode and a LI-COR sensor. Areas adjacent to endophyte infection and areas furthest away from infection on each thallus were dark adapted for 30 min using dark adapting clips (WALZ, Germany). Areas adjacent to endophytes were chosen so that the photo centers of the endophyte species did not contribute to the fluorescence signal of the host. MQY (Fv/Fm) was measured after ~15 min of dark adaptation followed shortly by RLCs (30 s increments, actinic light from 0-200 μmol m⁻² s⁻¹ irradiance). Eₖ, α, and ETRₘₐₓ were calculated from the RLCs which were fit to a hyperbolic tangent function outlined in Jassby & Platt (1976). All data were log transformed and paired t-tests (SPSS) were used to compare average MQY, Eₖ, α, and ETRₘᵢₙ in areas adjacent to and furthest from endophyte presence in all species. Species were grouped and a paired t-test was used to compare areas adjacent to and far from endophyte presence to determine whether a larger sample size teases out significant differences in rhodophytes.
RESULTS

Palatability

Palatability assays using thallus material from the same individual found no significant differences in amphipod feeding rate between material with and without endophyte presence (Figure 2). Palatability assays using thallus material from hosts with and without endophyte presence (test for primed defenses) also showed no significant differences in amphipod feeding rate (Figure 3). One sample t-tests showed thallus consumption was significantly greater than the expected 0 μg thallus mg amphipod$^{-1}$ h$^{-1}$ in the first assay in *I. cordata*, *H. grandifolius* (no endophyte), *D. antarctica*, and *M. manginii* (endophyte). In the second assay consumption was significantly greater than 0 μg thallus mg amphipod$^{-1}$ h$^{-1}$ in *Pachymenia* sp., but not in *I. cordata*, giving us differential results in that species.

Thallus Toughness

Penetrometry assays revealed a significant difference in the force required to puncture the thallus in one species, *Pachymenia* sp., where thallus material with endophyte presence was weaker than material without (Figure 4). All other host species showed no significant difference in the force required to puncture the thallus with and without endophyte presence. Tensometry assays revealed no significant difference in toughness of thallus tissue with or without endophyte presence in any of the species tested (Figure 5).

Photosynthetic characteristics

Analysis of photosynthetic parameters in most host species showed no significant differences between areas adjacent to and furthest away from endophyte presence. The one
exception was *I. cordata*, where MQY significantly decreased in areas adjacent to endophyte presence (Figure 7). Paired t-tests showed no significant trends across species in all parameters, however there was a general depression in photosynthetic parameters adjacent to endophyte presence in most species (Figure 6-9).

**DISCUSSION**

Antarctica is a characteristically harsh environment: low light levels, extreme physical conditions (Wiencke *et al.*, 2007), and large mesograzer populations (Amsler *et al.*, 2008) make it an extreme environment for the phycobenthos. Notably, the large mesograzer cohort is thought to be responsible for high levels of endophytic algae along the western Antarctic Peninsula (Peters, 2003, Amsler *et al.*, 2014). However, from an evolutionary perspective the persistence of filamentous algal endophytes in Antarctic macroalgae may be credited to their benign impacts on many algal hosts, shown in most species in this study. Weakness in host physiology caused by endophyte presence, or pathogenicity, could reduce growth and survival of ‘infected’ individuals in a community, effectively reducing the fitness of that species. Loss of these individuals would include any symbionts, potentially reducing fitness of the filamentous endophytes as well.

A previous study showed high levels of endophyte presence within the hosts *G. turquetii*, *T. antarcticus* and *I. cordata* decreased growth late in the growing season (Schoenrock *et al.*, 2013). Survival also decreased in individuals with high endophyte levels in the hosts *G. turquetii* and *T. antarcticus* (Schoenrock *et al.*, 2013) showing high endophyte presence probably hastens senescence. Still, evidence of whether endophyte presence directly impacts fitness of any of these host species is unspecified. Early senescence could only be detrimental to fitness of a species if it occurs before spore/gamete dispersal or somehow reduces reproductive potential of
an individual. The measurements used in this study targeted aspects of algal physiology that are fundamental to growth and survival in the subtidal, and therefore directly impact reproductive potential (De Wreede & Klinger, 1988). However, the impact of endophyte presence on fertility and spore dispersal were not directly measured.

In this study endophyte presence had no significant impact on palatability or consumption rate in any of the species assayed. *I. cordata, H. grandifolius, D. antarctica, M. manginii* and *Pachymenia* sp. were consumed by *G. antarctica* in very low quantities (Figure 2-3). Consumption rates of *D. antarctica* were probably highest because individuals used in this study were second year perennials that have the largest cover of endophytes and are potentially senescent. In a previous study (Amsler *et al.*, 2009), *D. antarctica* was less palatable to herbivores likely due to the fact that they were first year individuals. Regardless, the absence of preferential consumption between thallus material in assays shows that endophyte presence will not have any effect on grazer pressure in situ.

The mechanical measurements used in this study test two aspects of thallus toughness; resistance to puncture and breakage of the thalli. All species tested required less energy to puncture their thalli than previously recorded (Amsler *et al.*, 2005), which may be related to time of year experiments took place (November-December versus March-April) or because endophytes often infect the older portions of thalli which may be deteriorating (specifically in *D. antarctica* and *H. grandifolius*) but there were few significant impacts on thallus toughness of host species. Strain energy density has never before been measured in algae from Antarctica but breaking energy for these species was within the normal range for intertidal temperate species (Harder *et al.*, 2006). Endophyte presence had no significant impact on toughness in any species with the exception of *Pachymenia* sp., specifically its resistance to puncture force (Figure 4).
*Pachymenia* sp. is often found at depths above 5 m (observation) which is a high energy environment with fewer grazers. These results indicate that this species may be resilient only to the most aggressive stressors in its environment, however we did not evaluate materials properties to test this. In the future, evaluation of materials responsible for thallus toughness would be valuable in understanding the vulnerability of *Pachymenia* sp. to puncture force, as well as the mechanical properties of Antarctic algae in general. Overall, consumption and breakage of individuals should not be impacted by endophyte presence in situ, therefore would have no impact on fitness.

In pigmented organisms, one might expect the presence of photosymbiont to be a stressor on the photophysiology of the host due to shading or another physical/chemical mechanism (Enriquez and Borowitzka 2010). Measured photosynthetic parameters differed slightly from previous studies on photophysiology of Antarctic macrophytes (Runcie & Riddle, 2006, Runcie & Riddle, 2012, Huovinen & Gomez, 2013), most likely due to individuals acclimation to aquarium conditions and a consistent source of light. Despite a slight trend in a negative impact in many photo-parameters due to endophyte presence, only *I. cordata* showed a significant reduction in MQY in thallus material adjacent to endophyte presence (Figure 7). No other photo-parameters were significantly impacted in this alga or any other species, nor were observed trends across species significant. Antarctic algae are well adapted to their environment and have very low optimal irradiance requirements (Wiencke et al., 2007) which may explain the low impact of a photosymbiont on most host species. *I. cordata* is the species where high levels of endophyte presence negatively impacted host growth the most (Schoenrock et al., 2013). Though palatability and thallus toughness of *I. cordata* were not impacted by endophytes, reduced MQY,
or utilization of photon irradiance, indicates stress to host photosystems which could negatively affect fitness in this species.

In conclusion, Antarctic endophytes should be classified as innocuous or harmful depending on their host alga. Thorough molecular identification has yet to be done on the green algal endophytes of these hosts, but most hosts do share brown genotypes and similar green endophyte morphologies (Amsler et al. 2009), so endophyte species is probably not a driver in mixed effects on examined hosts. Future work should focus on nutritional aspects of the endophytes (carbon sequestration, etc.), high resolution information on biomechanical properties of the algae, and microscale characterization of photosynthetic properties and palatability of the algae. Studies should also examine the effect of endophyte presence on reproductive potential and gamete dispersal in these algae, maybe the ultimate measure of fitness. Therefore this study provides a springboard for future investigations into the innocuous, sometimes pathogenic, relationships between many Antarctic macroalgae and their endophytes.

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REFERENCES


FIGURES

Figure 1. Line drawings of size and morphology of the nine species used in this study.
Figure 2. *G. antarctica* feeding assay results using thallus material from the same individual (means ± SE). 3. *G. antarctica* feeding assay results using thallus material without endophyte presence from individuals with and without endophyte presence (means ± SE).
**Figure 4.** Force required to penetrate thallus material with or without endophyte presence in nine species of Antarctic macroalgae (paired t-test, means ± SE).
**Figure 5.** Force required to tear thallus material with and without endophyte presence in six species of Antarctic macroalgae (paired t-test, means ± SE). Significant differences are noted by asterisk.
Figures 6. $E_k$ 7. MQY in species adjacent to and far from endophyte infection. Asterisks indicate significant differences (Paired t-test, means ± SE).
Figure 8. α 9. ETR$_{\text{max}}$ in species adjacent to and far from endophyte infection. Asterisks indicate significant differences (Paired t-test, means ± SE).
LIFE HISTORY BIAS IN ENDOPHYTE INFECTION OF THE ANTARCTIC RHODOPHYTE, *IRIDAEA CORDATA*

by

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ABSTRACT

Endophytic organisms are known to have varied effects on their host organism in terrestrial and marine environments. In studies on marine algae these symbioses can range from innocuous to pathogenic depending on host and endophyte species. This study targeted the relationship between filamentous algal endophytes and one red algal host found along the western Antarctic Peninsula in which endophytes were shown to be pathogenic in two previous studies. We analyzed endophyte presence in the three life history stages of *Iridaea cordata* and endophyte impact on fertility in female gametophytes and tetrasporophytes. We found endophytes proliferate throughout significantly more thallus area in tetrasporophyte and sterile gametophyte hosts than in carposporophyte hosts, but there was no correlation between endophyte coverage and fertility. This study also provides a demographic analysis of *I. cordata* populations surrounding Palmer Station, Antarctica, showing these populations are haploid dominated (~78% individuals). These results indicate that the pathogenic impacts of endophytes on *I. cordata* will not affect the products of sexual recombination in carposporophytes as much as they will the unfertilized haploid gametophytes and tetrasporophytes.
INTRODUCTION

The shallow coastline along the western Antarctic Peninsula is often dominated by large algal communities (Wiencke and Amsler, 2012). *Iridaea cordata* (Turner) Bory de Saint-Vincent is a member of the Gigartinaceae and common in shallow subtidal communities but can be found down to 30 m depth (Amsler et al., 1995, Wiencke and Clayton, 2002, Wiencke and Amsler, 2012). This is a dominant species in shallow basins and newly exposed substrata (Quartino et al., 2013) suggesting it is a good colonizer. The life history of *I. cordata* is characterized by triphasic isomorphic alternation of generations and both sporophytes and gametophytes have been described, though males may be rare (Wiencke and Clayton, 2002). *I. cordata* is a pseudo-perennial seasonal responder: growth initiates with lengthening photoperiod and a portion of its thallus is shed every year (Wiencke and Clayton, 2002). The Antarctic *I. cordata* resembles *I. cordata* from South America (type locality) morphologically but there is greater than 3% dissimilarity in rbcL genes, indicating the Antarctic entity is a different species (Hommersand and Fredericq, 2003, Hommersand et al., 2011). However, until a formal taxonomic reappraisal is done, "*I. cordata*" is the appropriate name for the Antarctic entity.

Many of the algae in this geographic region are host to filamentous algal endophytes (Peters, 2003, Amsler et al., 2009). The prevalence of these endophytes is probably a result of intense mesograzer pressure coupled with the widespread presence of chemically defended macroalgal refuges (Amsler et al., 2014). The effect of these algal endophytes on their algal hosts has been investigated using various parameters of the host algae in nine common macroalgae from the area (Schoenrock et al. 2013, unpublished). Species that exhibited negative impacts of endophyte infection were differentially affected: *Pachymenia* sp. showed a decrease in thallus toughness, *Trematocarpus antarcticus* and *Gymnogongrus turquetii* showed decreased growth.
when endophyte infection was widespread throughout the thallus, and *I. cordata* showed both decreased growth and maximum quantum yield (MQY) due to endophyte presence (Schoenrock et al., 2013, unpublished). Many species were not affected however, highlighting variability in the symbioses between host algae and endophyte (Schoenrock et al., 2013, unpublished). Parameters measured in previous studies were those that directly contribute to the fitness of the host, but actual impact on reproduction was not quantified in any species.

In *I. cordata*, endophyte filaments are located throughout the thallus but rarely penetrate the medulla in any life-history stage (Figure 1). These endophytes are mostly green filamentous algae (observation), although three brown endophyte genotypes, one being unique among host algae, grew in culture when removed from mesograzer pressure (Amsler et al., 2009). Pathogenicity is not visually apparent in *I. cordata* (i.e. galls or deterioration of host) as it can be in other host species (Gauna et al., 2009, Thomas et al., 2009), and endophyte presence does not weaken thallus toughness or change palatability to sympatric mesograzers (Schoenrock et al., unpublished), although endophytes increase coverage of the host as *I. cordata* ages (Schoenrock et al., 2013). In some species endophyte infection can be considered an infectious disease and be extremely destructive (Fujita et al., 1972, Ishikawa and Saga, 1989, Potin, 2012), removing the host from populations (Goff and Cole, 1976, Buschmann et al., 1997) as well as the pathogen (Toft and Karter, 1990). This would ultimately decrease the fitness of a host (perhaps as well as a pathogen) but few studies translate the effect of endophyte infections to fitness of the individuals.

Endophyte infection has varied effects on different life history stages of the related marine rhodophyte, *Chondrus crispus* Stackhouse (Gigartinaceae). The endophyte *Achrochaete operculata* J.A. Correa & R. Nielsen infects the sporophytes of *C. crispus* profusely, causing
bacterial infection and deterioration of the thallus, but does not penetrate the cortical layer of gametophytes (Correa et al., 1988, Correa and McLachlan, 1991, 1992, 1994). These stages differ in sulfation patterns of their extracellular matrix carrageenans (Bouarab et al., 1999); λ-carrageenan oligosaccharides in sporophytes elicit a H$_2$O$_2$ response from the endophyte A. operculata, which triggers a molecular cascade resulting in increased pathogenicity of specific polypeptides of A. operculata (Bouarab et al., 1999). κ-carrageenans in gametophytes hinder carrageenolytic responses in A. operculata and enhance pathogen recognition by the host, which responds to the endophyte with a H$_2$O$_2$ response 10-15 times greater than that of the sporophyte (Bouarab et al., 1999). Other studies have shown that endophyte presence, especially in the reproductive structures of an alga, can decrease fitness of specific life history stages in their host species (Muller, 1996, Faugeron et al., 2000).

The goal of the present study is to elucidate endophyte impact on the fitness of I. cordata by evaluating endophyte coverage and fertility in all life history stages. In order to define the impact of variation in endophyte infection between stages in I. cordata it is necessary to ascertain the species demography in the study area (Thornber et al., 2006). Isogamous life history stages thrive in stable conditions like those in Antarctica (John, 1994, Wiencke et al., 2007), but the haploid to diploid ratio within populations can impact the life history cycle of a species. Many algal populations are sporophyte dominated (De Wreede and Klinger, 1988) including rhodophytes in Gracilariaceae and Ceramiaceae, but Gigartinaceae populations are often gametophyte dominated (Fierst et al., 2005).

Haploid:diploid ratios shift when spore recruitment, coalescence, fecundity, fertilization success, survival, and disease differentially impact life history stages within a species (Carrington et al., 2001, Thornber et al., 2006, Krueger-Hadfield et al., 2013). Densities of
asexually reproducing organisms are known to increase towards margins of populations, which our study site may be, in geographic parthenogenesis (Craigie and Pringle, 1978, De Wreede and Klinger, 1988). Populations under adverse conditions such as the cold temperatures or high disturbance levels characterizing the western Antarctic Peninsula are hypothesized to be sporophyte dominated (Hansen and Doyle, 1976) because diploid individuals have the ability to mask mutations (increased genetic variability; Sosa and Garcia-Reina, 1992) and adapt quickly to environmental variation (Bell, 1982). Still, there is no pattern describing every species population (De Wreede and Green, 1990). By coupling population demography with differential endophyte presence and impacts on fertility in the life histories of *I. cordata* we will confirm whether the observed pathogenicity of these endophytes (Schoenrock et al., 2013, unpublished) also affects the fitness of populations along the western Antarctic Peninsula.

**METHODS**

The present study was conducted within the archipelago surrounding Palmer Station (USAP) located toward the south of Anvers Island along the western Antarctic Peninsula (Figure 2). Individuals were collected from six locations where *I. cordata* is relatively abundant using SCUBA; the Bahia Paraiso shipwreck (S 64° 46.829’ W 64° 05.749’) at 6 m depth, Bonaparte point (S 64° 46.679’ W 64° 04.013’) at 4 m depth, Kristie Cove at 6 m depth, the southeast cove of Shortcut Island (S 64° 46.991’ W 64° 02.379’) at 4 m depth, Stepping Stones Island (S 64° 47.111’ W 64° 59.691’) at 4 m depth, and the northern cove at Laggard Island (S 64° 98.374’ W 64° 00.720’) at 5 m depth. A 1 m² quadrat was dropped sporadically over dense crops of *I. cordata* from a height where reproductive status could not be determined and all *I. cordata*
individuals within the quadrat were collected. Sampling was done twice in 2011; March and late May which represents autumn and early winter in the Antarctic Peninsular region.

Individuals were immediately transported to Palmer Station, 50 individuals from each site were haphazardly chosen to be photographed on a fluorescent light table for use in image analysis, and life history stages were distinguished. The image analysis program CPCe (Kohler and Gill, 2006) was used to determine sporangial density and percent cover of endophyte presence in individuals. Sporangial density (sporangia/cm²) was calculated by averaging the number of carpogonia or tetrasporangia within five 1cm² quadrats along a transect from the stipe to distal end of an individual. Endophyte presence was determined as a percentage of the host covered (cm² endophyte/ cm² blade).

*Life history stages*

Life history stages were easily identified and grouped as carposporophyte, tetrasporophyte, or ‘sterile’ gametophyte. Sterile individuals were identified as gametophytes by characterizing carrageenan content. 10 individuals of each apparent life history stage were subsampled from collections, frozen at −20 °C and shipped back to the University of Alabama at Birmingham (UAB). At UAB, each blade was thawed, patted dry with lab towels, and a 1.2 cm diameter disk was excised and placed in a 10 mL test-tube. These disks were treated with resorcinol-acetal test reagent and incubated for 1 min at 80 °C following the methods of Garbary and DeWreede (1988). Using this reaction, the haploid (κ-carrageenan) and diploid (λ-carrageenan) individuals were distinguishable. All sterile gametophytes and carposporophytes contained κ-carrageenan and were grouped as haploid individuals.
Since individuals were haphazardly sampled from each site during the two sampling periods, they are assumed to be representative of each population. A Goodness of Fit test was used to determine whether populations deviated from the expected ratio of $\sqrt{2}:1$ (haploid:diploid) within each site for each season, assuming they have life history stages that have equal fitness (Destombe et al., 1989, Thornber and Gaines, 2004). Ratios at all sites were compared between seasons using a paired t-test (SPSS) to evaluate whether population demography shifts with season (De Wreede and Green, 1990). To determine whether populations at each site and time could be grouped in further analyses, the percent cover of endophyte presence and fertility data of each sex were square root transformed and used to calculate a Bray-Curtis (BC) similarity matrix with the factors site and collection season (factor). A CLUSTER analysis with a SIMPROF test was run using the BC matrix to show similarities between sites and sampling times using PRIMER (v6.3) (Clarke and Gorley, 2006).

*Endophyte coverage and fertility*

Because all the collection sites are similar in terms of wave exposure and other physical features (Figure 2), and there was no significant structure found in site data using the CLUSTER analysis (SIMPROF; P= 0.67), the populations were grouped. Endophyte coverage and fertility data were square root transformed to fit the assumptions of normality for the following tests. To determine differences in endophyte presence between life history stages (gametophyte, carposporophyte, and tetrasporophyte), all sites and seasons were grouped for each collection time, and a 1-way ANOVA was used to compare percent cover of endophyte (dependent variable) and life history stage (independent variable). To determine the effect of endophyte cover on fertility of the host, all sites and seasons were grouped and correlations run to determine
if sporangial density (dependent variable) varies with percent cover endophytic algae (independent variable) in both tetrasporic and carposporic life-history stages.

RESULTS

Life history stages

The Goodness of Fit test showed that most sites in all seasons had significantly more haploid individuals than expected, with the exception of Shortcut Island (autumn and winter) and the Bahia Paraiso (early winter) which had even ratios (Table 1). Only the winter population of Shortcut Island exhibited the expected haploid:diploid ratio. Overall the population surrounding Palmer Station had a haploid to diploid ratio of ~ 3:1 (the populations are 78% haploid). Sex ratios did not change significantly between autumn and early winter, but population trends included a decrease in total number of individuals, as well as fraction of carposporophytes, and an increase in the fraction of tetrasporophytes at many sites (Figure 3). A Bray-Curtis similarity matrix and CLUSTER analysis with SIMPROF test showed that all sites exhibited extreme likeness in fertility and endophyte presence through both seasons.

Endophyte coverage and fertility

The 1-way ANOVA showed significant differences in endophyte cover between life history stages ($F = 11.484, p < 0.0005$, Figure 4). A Tukeys HSD test showed significantly more endophyte coverage in the tetrasporophytes and gametophytes than endophyte coverage in carposporophytes. Correlations in both tetrasporophytes and carposporophytes showed no relationship between endophyte coverage of a host and fertility (Pearson correlation = 0.082 and -0.037 respectively, $p = 0.226$ and 0.626 respectively).
DISCUSSION

Variation in the ratio of haploid to diploid morphs may represent a variety of adaptations at each ploidy level: 1) greater fitness in either morph, 2) a specific advantageous attribute of a heteromorph in the environment, or 3) varied success of reproductive strategies by either ploidy level (i.e. apomeiosis) (De Wreede and Klinger, 1988). The understanding of a species population is fundamental to examining how it contributes to ecosystem processes, in this case when investigating isomorphic life history stage response to stressors. In *I. cordata*, endophytes are known to be pathogenic (Schoenrock et al., 2013, unpublished) making any differential presence of endophytes and impacts on fitness important in this species ecology. Populations around Palmer Station are haploid dominated; fertilized gametophytes with carposporangia and sterile gametophytes (presumably both male and female) are more abundant than tetrasporophytes. Though variation in endophyte presence did not impact fertility in any stage, the lower incidence of endophytes in the female gametophytes with carposporophytes is ecologically relevant because they amplify the products of sexual recombination (Searles, 1980) and are the source of genetic variation within these populations.

Lower incidence of endophyte presence in the cystocarpic female gametophytes can be ecologically important in two ways: fertilization of the female gametophyte may be more successful in individuals with lower endophyte coverage or fertilization may cause a shift in individual defenses. Defenses in the gametophytes of *C. crispus* protect them from expansive endophyte coverage (Bouarab et al., 1999), and this may be occurring in the Antarctic *I. cordata* as well. We did not measure either fertilization success or specific defensive chemistry of *I. cordata* life history stages. There are no known defenses against endophyte settlement in *I. cordata*, but carrageenan content of life history stages is consistent in the Gigartinaceae.
(Shaughnessy and De Wreede, 1991 and citations within) which indicates known sulfation of these compounds (Foltran et al., 1996) may trigger a defense response as seen in C. crispus (Bouarab et al., 1999). This species can produce reactive oxygen species (ROS) in response to wounding (McDowell et al., 2014), though it is uncertain if one life history stage would produce more ROS in the presence of a different stimuli such as endophyte presence.

In this study, fertility was not impacted by endophyte presence in any life history stage of I. cordata. Germination potential or fecundity of the reproductive structures was not examined (Santelices and Martinez, 1997, Faugeron et al., 2000), but endophyte structures were never seen proliferating throughout tetrasporangia or carpogonia indicating there is no mechanical damage to reproductive structures. Indeed, endophyte filaments rarely penetrate the cortical layer of these life history stages and both tetrasporangia and carpogonia develop in the medulla, just below the cortical layer (Wiencke and Clayton, 2002; Figure 1). Few studies show energy trade-offs between reproduction and growth or defense in algae (Dworjanyn et al., 2006) which can dictate reproductive potential in individuals (Faugeron et al. 2000) as well as survival (Thornber 2006). Costly defense of reproductive tissues, as outlined by the optimal defense theory, is often identified (Amsler and Fairhead, 2006, Pansch et al., 2008), but further studies are needed to understand if the decrease in growth of highly infected individuals seen in Schoenrock et al. (2013) is due to resource tradeoffs in I. cordata. Also, one would not expect carposporophyte fertility to decrease if a chemical defense were used to prevent endophyte infection. Therefore, further investigation of reproductive potential and defenses is warranted to understand this relationship between I. cordata and its endophytes.

Annual shifts in dominance are not uncommon in algal populations (De Wreede and Klinger, 1988, De Wreede and Green, 1990). We saw no shift in most haploid/diploid ratios over
our sampling periods but when the grouped ratio was compared with individual sites across seasons there is a difference at two sites. Both time periods at Stepping Stones and the winter sample from the Bahia Paraiso had close to a 1:1 haploid : diploid ratio, while other sites in the two seasons had ratios > 2:1. These populations are not at the margins of the Palmer Archipelago (Figure 2), but sites are separated by open-ocean and may be isolated through current systems, which although unlikely could potentially account for the observed differences. On average, populations in this area during the Austral fall-winter are 78% haploid and 22% diploid or 24% tetrasporophyte, 51% sterile gametophytes, and 25% carposporophytes. Haploid dominance may drive this fertilization success: ~30% of females (males are rare in late summer-early winter; Wiencke and Clayton, 2002). Because the deleterious effects of endophyte infection are less likely to occur in carposporophytes, the fitness of this life history stage is probably amplified which may or may not result in diploid dominance the following season.

An alternation of isomorphic generations is thought to be advantageous under constant conditions, where seasonality does not select for ecologically distinct phases (John, 1994). Still, subtle ecological differences probably stabilize isomorphy and there are advantages to both haploid and diploid stages (Thornber, 2006). Members of the Gigartinae often have haploid/gametophyte dominated populations (Fierst et al., 2005), which is advantageous because haploid individuals increase genetic differentiation in the population and are the substrate for sexual recombination (Thornber, 2006, and citations within). Diploid individuals have the ability to preserve genetic variability in a population by protecting potentially deleterious mutations and allowing more rapid adaptation to environmental variation. Both sporophyte and gametophyte dominance have been documented for the entity previously called *I. cordata* in the western USA, now *Mazzaella splendens* (Setchell & N.L. Gardner) Fredericq, (Hansen, 1977, May, 1986).
While conditions in the Antarctic marine environment are extreme, they are consistent, and both isomorphy and haploid dominance can be a response to the environment.

In this study we found that *I. cordata* populations along the western Antarctic Peninsula are haploid dominated and the female gametophytes with carposporophytes have significantly less endophyte infection. This difference between life history stages of the Antarctic *I. cordata* could potentially have ecologically significant influences on population demography.

ACKNOWLEDGEMENTS

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TABLE 1. Haploid to diploid ratios and results from the Goodness of Fit test at all sites in both seasons.

<table>
<thead>
<tr>
<th>site</th>
<th>season</th>
<th>haploid</th>
<th>diploid</th>
<th>ratio</th>
<th>G</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bonaparte point</td>
<td>Fall</td>
<td>81</td>
<td>31</td>
<td>2.62 : 1</td>
<td>9.147</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>Winter</td>
<td>66</td>
<td>14</td>
<td>4.72 : 1</td>
<td>21.076</td>
<td>4.41 × 10^-6</td>
</tr>
<tr>
<td>Kristie Cove</td>
<td>Fall</td>
<td>59</td>
<td>8</td>
<td>7.38 : 1</td>
<td>28.2</td>
<td>1.09 × 10^-9</td>
</tr>
<tr>
<td></td>
<td>Winter</td>
<td>67</td>
<td>11</td>
<td>6.09 : 1</td>
<td>27.589</td>
<td>1.5 × 10^-7</td>
</tr>
<tr>
<td>Bahia Paraiso</td>
<td>Fall</td>
<td>82</td>
<td>36</td>
<td>3.42 : 1</td>
<td>6</td>
<td>0.014</td>
</tr>
<tr>
<td></td>
<td>Winter</td>
<td>21</td>
<td>29</td>
<td>1 : 1.38</td>
<td>5.552</td>
<td>0.018</td>
</tr>
<tr>
<td>Shortcut Is.</td>
<td>Fall</td>
<td>138</td>
<td>44</td>
<td>1 : 1.04</td>
<td>23.838</td>
<td>1.05 × 10^-6</td>
</tr>
<tr>
<td></td>
<td>Winter</td>
<td>108</td>
<td>90</td>
<td>1.2 : 1</td>
<td>1.316</td>
<td>0.251</td>
</tr>
<tr>
<td>Laggart Is.</td>
<td>Fall</td>
<td>61</td>
<td>15</td>
<td>4.07 : 1</td>
<td>16.183</td>
<td>5.7 × 10^-4</td>
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<tr>
<td></td>
<td>Winter</td>
<td>109</td>
<td>8</td>
<td>13.63 : 1</td>
<td>72.325</td>
<td>1.85 × 10^-17</td>
</tr>
<tr>
<td>Stepping Stones</td>
<td>Fall</td>
<td>94</td>
<td>3</td>
<td>31.3 : 1</td>
<td>79.068</td>
<td>6 × 10^-19</td>
</tr>
<tr>
<td></td>
<td>Winter</td>
<td>57</td>
<td>25</td>
<td>2.28 : 1</td>
<td>4.186</td>
<td>0.041</td>
</tr>
</tbody>
</table>

1 indicates haploid: diploid ratio that is almost even

2 indicates haploid: diploid ratio that is expected (Thornber and Gaines 2004)
FIGURES

Figure 1. *Iridaea cordata* a) tetrasporophyte, b) endophytic filamentous algae between cortical cells, c) female gametophyte with carposporophytes, and d) sterile gametophyte. All arrows indicate endophyte thalli (scale bar indicate 1 cm in a, c, and d, and 0.1 cm in b).
**Figure 2.** Map of collections sites in the archipelago surrounding Palmer Station, Antarctica. Site names: a) the Bahia Paraiso shipwreck, b) Kristie Cove, c) Bonaparte Point, d) southern cove on Shortcut Island, e) Stepping Stones, and f) northern cove on Laggard Island. Arrow and continent indicate the position of Anvers Island along the western Antarctic Peninsula.
Figure 3. Total number of individuals at each site and each time are represented by shading for number of individuals in each life history stage and A= Autumn and W= Winter indicate sampling seasons.
Figure 4. A comparison of endophyte coverage between life history stages in *I. cordata*. Letters indicate significantly different groupings.
CLIMATE CHANGE IMPACTS ON OVER-STORY *DESMARESTIA* SPP. FROM THE WESTERN ANTARCTIC PENINSULA

by

KATHRYN M. SCHOENROCK, JULIE B. SCHRAM, CHARLES D. AMSLER, JAMES B. MCCLINTOCK, AND ROBERT A. ANGUS

Submitted to Marine Biology

Format adapted for dissertation
ABSTRACT

This study examines climate change impacts (increased temperature and $pCO_2$) on canopy forming *Desmarestia aniceps* and *D. menziesii* from the western Antarctic Peninsula during the Austral summer-winter 2013. These are ecologically important species that play a role functionally equivalent to kelp forests. Two way factorial experiments were run for 2 months in microcosms reflecting near future ocean conditions under climate change predictions: increased temperature alone (3.5 °C * pH 8.0), reduced pH alone (1.5 °C * pH 7.6), and both factors combined (3.5 °C * pH 7.6). Phlorotannin content, chlorophyll $a$ content, growth, and photosynthetic parameters ($\alpha$, $E_k$, $ETR_{max}$, and MQY) were used to assess the physiological responses of the individuals to the different climate change treatments. Few significant impacts were observed: in *D. menziesii* $E_k$ at the midpoint and phlorotannin content at the endpoint were significantly higher in the 3.5 °C * pH 7.6 treatment than others. All individuals in the experiment grew quickly through the midpoint, but growth declined thereafter. There was also a adjustment in photophysiological parameters between initial, midpoint, and endpoint measurements. Results indicate that *D. menziesii* is the more sensitive of the two species and that climate change factors have a synergistic effect on this species, increasing saturating irradiance and phlorotannin content. However, neither species appears to be especially sensitive to climate change factors predicted for near future ocean conditions, though the observed shifts in phlorotannin concentration and photosynthetic characteristics may have an unforeseen impact on the community dynamics in this geographic area.
INTRODUCTION

The western Antarctic Peninsula has been described as an area highly vulnerable to the effects of climate change (Clarke et al. 2007; IPCC 2007; Ducklow et al. 2013; Turley 2013). An important reason for this is many organisms in the Southern Ocean experience and may be adapted to relatively static environmental conditions (Peck 2005; Wiencke et al. 2007). This area is experiencing one of the fastest rates of climate change in the world, particularly warming (Meredith and King 2005; Clarke et al. 2007). A result of this is decreased annual sea ice cover along the Peninsula which affects a range of ice-dependant organisms from penguins to phytoplankton (Ducklow et al. 2013). The decline in annual sea ice cover also causes an increase in vertical mixing of the water column along the Peninsula (Ducklow et al. 2013). Other prominent environmental factors resulting from climate change include increased average ocean temperature, decreased pH, and increased UV radiation. All of these have been linked to an anthropogenic increase in the greenhouse gas CO₂ and will likely impact present day community structures.

Ocean temperature has a narrow range in this region, currently between -1.8 and 2.5 °C (Drew and Hastings 1992; Schram et al. unpublished), and pH in shallow coastal areas can range from 8.0 to 8.62 depending on time of year (Schram et al. unpublished). In the last half century ocean temperatures have increased by 1 °C along the western Antarctic Peninsula (Ducklow et al. 2013), and air temperatures have increased 0.25 °C per decade since the 1950s (Vaughan et al. 2003; Steig et al. 2009), and are projected to continue increasing. Ambient ocean pH is also projected to decline approximately 0.4 units globally by the year 2100 (IPCC 2007). Signs of change have already been documented in communities along the Peninsula (Ducklow et al. 2013), including a decrease in net primary productivity (NPP) of the ocean (Behrenfeld et al.
2006), specifically a 12% decrease in summer months, with a southward shift in NPP (Montes-Hugo et al. 2009). This decline certainly impacts light levels in the shallow subtidal, particularly because NPP reflects phytoplankton biomass. A reduction in phytoplankton may result in an increase in subtidal irradiance including detrimental ultraviolet radiation (UVR).

Organisms differ in their response to aspects of climate change depending on ecological characteristics such as trophic level or more specific physiological traits such as calcification or temperature tolerance (Ries et al. 2009; Kroeker et al. 2011; Harley et al. 2012; Roleda and Hurd 2012). Calcifying organisms like corals and coralline algae are predicted to be detrimentally impacted because the increased $pCO_2$ in sea water lowers the saturation states of carbonate species (Feely et al. 2004; Orr et al. 2005; Fabry et al. 2008), specifically those used as substrate for calcification (polymorphs aragonite and magnesium calcite) which are 50% more soluble in seawater than calcite (Feely et al. 2004). Climate change will likely affect metabolic processes in non-calcifying marine organisms as well since many cellular processes are driven by $H^+$ gradients across membranes (Fabry et al. 2008).

The western Antarctic Peninsula has an open coastline with periodic sea-ice cover which allows for large NPP (Ducklow et al. 2013) including the distinctive algal forests in the shallow subtidal (Wiencke and Amsler 2012). These forests are dominated by brown algae in the order Desmarestiales, including Desmarestia aniceps which dominates deeper photic zones, $D$. menziesii which dominates the shallower zone below ice scour, and Himantothallus grandifolius which dominates shallow slopes or habitats deeper than $D$. aniceps (Wienecke et al. in press). Canopies frequently include $D$. antarctica which rarely dominates an area, Ascoseira mirabilis which can be abundant at shallower depths, and Cystosphaera jacquinotti which occurs intermittently at deeper depths (Wienecke et al. in press). These brown algae perform the same
functional role as kelps do on temperate coastlines (Clayton 1994), providing 3-D structure for
the community. Many Antarctic species are uniquely adapted to the extreme characteristics of
their environment including the consistently low temperature and extreme flux in light levels.
Species are characterized by high photosynthetic efficiency at low irradiance (α), low saturating
irradiances (E_\text{k}), efficient P_{\text{max}} at low temperatures and low upper survival temperatures (USTs)
ranging between 10-11 °C in some Desmarestia spp. (Gomez et al. 2009).

Though they are functionally similar to kelps, these canopy-forming algae are largely
unpalatable to sympatric herbivores (Amsler et al. 2005a). Secondary metabolites in Antarctic
brown algae can act as anti-microbial or anti-feedant compounds (Ankisetty et al. 2004; Iken et
al. 2009). Compounds include quinones and chromenols (Lebar et al. 2007), and phlorotannins
which account for 0.5 - 9% DW of the thallus (Fairhead et al. 2005a; Iken et al. 2007).
Phlorotannins play more than one role in the ecology and physiology of brown algae. In addition
to anti-microbial and anti-feedant activity, phlorotannins are structural compounds, which aid in
wound healing, are metal ion chelators, and may be UV protectants (Amsler and Fairhead 2006).

Brown algae are known to structure the benthic community along the western Antarctic
Peninsula through these chemical defenses. The large mesograzer cohort along the Peninsula
uses the algal canopy as refuge from visual predators and subsequently grazes down epiphytic
filamentous algae, forcing a high prevalence of endophyte communities within the macroalgae
(Amsler et al. 2014). Because of their ecological importance to the subtidal community, and their
specific ecophysiological adaptations to their environment it is particularly important to
investigate the physiological response of the canopy algae D. menziesii and D. anceps to
anticipated impending changes in climate conditions. Insights into the response of non-calcified
macroalgae to these future conditions are few but show species-specific responses in fleshy macroalgae.

Temperature increases will presumably have a negative impact on Antarctic macroalgae because of their adaptation to consistently low temperatures. However, photosynthesis reaches optimal function at higher temperature than current ambient (Gomez et al. 2009). This may combine with decreased pH (increased $pCO_2$) to boost photosynthetic effort under future conditions. Previous studies have shown that increased temperature alone causes bleaching in chemically defended algae (Campbell et al. 2011). Additionally, it can amplify the effects of UV on photochemical parameters, increase peroxidative damage to photosystems, and increases phlorotannin induction (which are subsequently impaired) in sub-Antarctic macroalgae (Cruces et al. 2013). Different species probably respond differently to increased temperature and/or increased $pCO_2$ when experienced individually than they do when exposed to both simultaneously (Brown 2013). In temperate kelp, *Macrocystis pyrifera*, the sporophyte stage responds negatively to an increase in temperature alone, but increases growth and photosynthetic activity in addition to down regulating carbonic anhydrase activity in sporophytes when exposed to both increased temperature and $pCO_2$ (Brown 2013).

Increased $pCO_2$ may benefit non-calcifying autotrophs giving them a competitive advantage, at least for space, over calcifiers in their benthic habitat (Birrell et al. 2008; Diaz-Pulido et al. 2011; Betancor et al. 2013). High $pCO_2$ conditions enhanced sporophyte growth, photosynthetic effort, and gametogenesis in the kelp *Laminaria hyperborea* (Olischlager et al. 2012), increased size in *M. pyrifera* gametophytes (Roleda et al. 2012), and delayed senescence (Swanson and Fox 2007), or had little effect on Arctic kelps (Falkenberg et al. 2013). An intertidal brown alga, *Fucus serratus*, increased growth in high $pCO_2$ environments (Saderne
2012), as did two tropical *Padina* spp., though these tropical species decreased calcification in the same treatment (Johnson et al. 2012). In another study on tropical macroalgae, *Lobophora variegata* and *Padina pavonica* reduced both antioxidant activity and number of photo-protective compounds in high $p$CO$_2$ conditions, making photosystems more vulnerable to harmful radiation (Betancor et al. 2013). Alternatively, the concentration of photo-protective phlorotannins increased in arctic kelp species *Saccharina latissima* and *Nereocystis leutkeania* (Swanson and Fox 2007). These previous studies highlight the variability in response to climate change conditions between species and among the traits measured.

Polar ecosystems are likely to be highly sensitive to decreased pH and increased temperature that will accompany climate change (Aronson et al. 2013). The goal of this study is to determine the effects of increased temperature and decreased pH, individually and combined, on the physiology of *D. aniceps* and *D. menziesii*. Based on previous research and optimal growth temperature ranges for these species we hypothesize that these organisms will respond positively to near-future climate conditions. A microcosm experiment included both species to analyze how the physiological performance of the alga changes in isolated conditions. Physiological responses of the algae to each treatment was measured through growth, chlorophyll *a* concentration (chl *a*), phlorotannin concentration, and photosynthetic characteristics.

**METHODS**

*Collections*

Individuals were collected from the archipelago surrounding the United States Antarctic Program’s Palmer Station, situated on the southwestern coast of Anvers Island. All *Desmarestia aniceps* and *D. antarctica* individuals used in microcosms were collected on SCUBA at the Bahia
Paraiso shipwreck (S 64° 46.829' W 64° 05.749') and Shortcut Island (S 64° 46.991' W 64° 02.379') respectively. The distal end of a lateral branch from each individual was trimmed to ~12 cm length and cleaned of epibiota using Kim Wipes. Each individual was labeled and weighted with a PVC nut to keep it vertically oriented within the experiment. Prior to the start of each experiment, individuals were kept in flow-through aquaria under irradiance levels of 20 to 30 μmol photons m⁻² s⁻¹ on a 12 : 12 h day night cycle.

**Microcosms**

Microcosm experiments were maintained on four shelving units equipped with light fixtures, water baths, and a CO₂-air gas delivery system. pH treatments were randomly assigned to water baths of the appropriate temperature to avoid placement bias within the water baths. Each microcosm (36 x 20 x 15 cm) was partitioned into four compartments with mesh screening; one for each Desmarestia spp. and two for macroalgal associated amphipods. This design created a balance in photosynthesis and respiration within the microcosm, but prevented trophic interaction between organisms. Microcosms were covered with a Plexiglas lid to limit gas exchange with the ambient environment, but allowed light energy typical of the natural environment into the microcosm (~16-22 μmol photons m⁻² s⁻¹) on a 12 : 12 h day night cycle. The light source consisted of two Sylviana Cool White fluorescent bulbs (F48T12/CW/HO, 60W) which emitted 20 to 26 μmol photons m⁻² s⁻¹ and extended the length of the water table. The lid also had entry points for the pH meter and gas delivery hoses. Temperature was maintained by circulating a 30% glycol mixture through a water bath and large Plexiglas water table (114.3 x 58.42 x 15.24 cm) which housed the microcosms. A Hobo™ logger and daily temperature measurements provided a temperature record for each water bath and microcosm
over the course of the experiment. Target pH was maintained using an AT control system (Aqua Medic, USA) which monitored real time pH in each microcosm and delivered the CO₂-air mixture to increase $pCO_2$ (decrease pH) when necessary.

Microcosm experiments utilized a 2 x 2 factorial design consisting of four treatments ($n = 12$ per treatment) reflecting current ambient seawater conditions (Schram et al. unpublished) and near-term predicted conditions for seawater pH and temperature (Kurihara 2008; Riebesell et al. 2010) as follows: current (control, pH 8.0 * 1.5 °C), low pH (pH 7.6 * 1.5 °C), high temperature (pH 8.0 * 3.5 °C), and low pH and high temperature (pH 7.6 * 3.5 °C). Experiments were maintained for 79 days.

**Seawater Chemistry**

Seawater pH was controlled by an AT control system (Aqua Medic, USA) and a gas proportioner (Omega) that mixed pure CO₂ with ambient air drawn from directly outside the aquarium. This system used feedback from pH probes to regulate distribution of the CO₂-air mixture from the proportioner to the mesocosms to maintain target pH. Control treatments had a constant stream of air bubbling into them to replicate low pH treatment water movement. Partial water changes were performed for each microcosm with water from pretreated reservoirs every other day to maintain water quality.

In addition to the AquaMedic system, pH and temperature were monitored daily using a Durafet and DirectLine DL421 Sensor module (Honeywell, USA) and Cole-Parmer Digi-Sense ThermoLogR Thermister (Table 1). Seawater chemistry records for spectrophotometric pH and total alkalinity (TA) were calculated weekly for each microcosm (Table 2) and daily in seawater reservoirs and ambient seawater. Samples were siphoned from their microcosms into 300 ml
borosilicate stoppered bottles and allowed to overflow one volume in order to limit sample contact with air (Dickson et al. 2007). Seawater removed for analysis was replaced with water from reservoirs and sampling bottles were stored in a cold, dark area until they could be processed.

Spectrophotometric pH was measured following SOP 6b (Dickson et al. 2007) using a UV/Vis Spectrometer LAMBA 40 (Perkin Elmer, USA) with jacketed cells plumbed to a water bath held at 10 °C. Alkalinity was determined by titration using a T50 Titrator (Mettler Toledo, Switzerland), a jacketed 500 ml beaker plumbed to a water bath at 10 °C, and DGi113-SC pH probe (Mettler Toledo, Switzerland) following SOP 3b (Dickson et al. 2007). Salinity was measured in each sample using a YSI Conductivity meter (Model 3200) and conductivity cell (YSI 3253 model B) or a refractometer. Certified reference materials (CRMs) provided by A. Dickson (Scripps, UCSD) were periodically used to check accuracy of TA measurements. Replicate analysis of seawater samples was used to check precision. User and equipment error for TA was 4.02 μmol/kg SW (mean SD, n = 17 comparisons of replicate samples) and for pH was 0.03 units (mean SD, n = 11 comparisons of replicate samples). Recorded TA, pH, temperature, and salinity were used to calculate $pCO_2$, temperature corrected pH, calcite saturation state ($\Omega_{\text{cal}}$) and aragonite saturation state ($\Omega_{\text{arg}}$) using CO2calc (Robbins et al. 2010) for each seawater sample analyzed. CO$_2$ constants from Roy et al. (1993), the KHSO$_4$ acidity constant from Dickson (1990), and air-sea flux constant from Wanninkhof (1992) were used. Average TA, pH, $pCO2$, $\Omega_{\text{cal}}$, $\Omega_{\text{arg}}$, salinity and temperatures are shown in Table 2.
Physiological Measurements

Prior to beginning the microcosm experiment, specific physiological variables of each individual were measured for comparison with the mid and end points of the experiment. Variables used to measure the response of each species include growth (at midpoint and endpoint of the experiment), chl \( a \) concentration, photosynthetic parameters (at midpoint and endpoint of the experiment) and phlorotannin concentration. Initial measurements of non-experimental individuals were made using individuals in situ \((n = 9 \text{ per species; photosynthetic parameters})\) and individuals from the collections, denoted as time 0 treatment \((n = 12 \text{ per species; chl } a \text{ concentration, phlorotannin concentration, and photosynthetic parameters})\).

Growth rate was measured through change in wet weight (WW, mg), which was measured after individuals were blotted dry and epibiota were removed. This was done three times during the experiment because individuals grew rapidly through the beginning of the experiment. We express growth rate as a fraction of change in wet weight for the two growth periods (eq. 1, \( M_0 \) is start wet weight, \( M_t \) is wet weight at time).

\[
\mu = \frac{(M_t - M_0)}{M_0}
\]

Photosynthetic parameters were measured using a Diving PAM fluorometer (Walz, Germany). Like growth rate, photosynthetic parameters were measured in experimental individuals at the start, the mid and end points of the experiment in microcosms. In situ measurements were made in individuals from Palmer Station pier \((64° 46.473' \ W 64° 03.284'\), Janus Island \((64° 47.074' \ W 64° 05.987'\)), east Norsel Point \((64° 45.677' \ W 64° 04.660'\)), and DeLaca Island \((64° 46.753' \ W 64° 05.935'\)). The photo-parameters measured were maximum quantum yield \((MQY; Fv/Fm, \text{ saturation pulse 1.0 s})\) after 30 min of dark adaptation. Following this, nine saturation pulses were delivered every 2 min after exposure to increasing
irradiance levels (0, 2.9, 11.3, 36.7, 76.8, 112.1, 192.1, and 273.3 µmol photons m\(^{-2}\) s\(^{-1}\)). From these measurements electron transport rates (ETRs) were calculated (eq. 2) using measured absorbance (abs) of the two species (D. anceps: 55.04% irradiance, D. menziesii: 56.62% irradiance) and these were fitted to the Platt equation (Jassby and Platt 1976). From these light curves slope to saturation of photocenters (\(\alpha\)), maximum electron transport rate (ETR\(_{\text{max}}\)), and saturating irradiance were calculated (E\(_k\), eq. 3, 4, 5).

\[
\text{ETR} = \text{abs} \cdot 0.5 \cdot \frac{F_v}{F_m} \cdot \text{PAR} \quad \text{(2)}
\]

\[
\alpha = X \cdot (k_2 - k_1) \quad \text{(3)}
\]

\[
E_k = \ln(k_2) - \ln(k_1) / (k_2 / k_1) \quad \text{(4)}
\]

\[
\text{ETR}_{\text{max}} = X \cdot (e^{-k_1E_k} - e^{-k_2E_k}) \quad \text{(5)}
\]

Photosynthetic parameters were measured as a change from the initial measurement in order to measure the response of the algae to each treatment.

Chl \(a\) was extracted from individuals in microcosms and time 0 individuals using a small percentage of thallus material (0.4 to 5.8 mg tissue). Tissue was lyophilized, ground with a mortar and pestle, and extracted in 20 ml 9:1 acetone: H\(_2\)O solution at -20 °C for 24 hours. A TD-700 fluorometer (Turner Designs) was used to calculate µg/L chl \(a\) in solution and this was standardized to weight of sample material (µg/g algae).

Approximately 1 g of thallus material from each microcosm individual was blotted dry and frozen at -20 °C at the end of the experiment for phlorotannin analysis. Thallus material was transported to the University of Alabama at Birmingham for phlorotannin extraction. Tissue was extracted in 20 ml 70% acetone for 24 at 4 °C on a shaker table (Koivikko et al. 2005). Samples were dried off and resuspended in 10 ml H\(_2\)O. The Folin-Denis assay (Stern et al. 1996) was used to determine phlorotannin concentration in samples and absorption was measured at 725
nm, using phloroglucinol (anhydrous, Sigma) to create a standard curve. Each sample was then acidified with 20 µl of acetic acid (pH 4.5), treated with 3 additions of polyvinylpolypyrrolidone (PVPP) to precipitate phlorotannins (every 5 hours under same incubation conditions), and absorbance was measured again at 725 nm to correct for non-phenol compounds in the extraction (Stern et al. 1996). Phlorotannin content was standardized to a % dry weight (% DW) using WW : DW from Fairhead et al. (2005a).

**Statistics**

All data were added to a constant and square root transformed for analysis. The four photosynthetic parameters were compared between time 0, *in situ*, and control treatments using a 1-way ANOVA to determine whether there was a lab effect. Mean phlorotannin and chl *a* concentration were compared between time 0 and control treatments using a Students t-test. A MANOVA was used to look for significant treatment effects in all measured parameters at the midpoint and endpoint measurement periods. When a significant effect was found in the MANOVA, a 2-way ANOVA was run with the specific variable to ensure significant differences between means (main effects or interactive effects) seen in the MANOVA were not due to violation of the requirement for sphericity. Change in mass (as a metric for growth) and photosynthetic parameters were compared between initial, midpoint, and endpoint measurements using a repeated measures 2-way ANOVA. This was done to look at changes in these parameters throughout the experiment. All statistics were run using SPSS (IBM Statistics) and *p* ≤ 0.05 was used to determine significant differences.
RESULTS

Microcosms

Growth was measured in individuals twice during the microcosm experiment; at the midpoint and the endpoint. Midpoint measurements show that *Desmarestia menziesii* individuals gained 4 to 50% and *D. anceps* individuals gained 15 to 28% of their original mass, however this declined to -2 to 15% and -6 to 1.7% respectively by the endpoint (Figure 1). There is a significant difference in growth between mid and endpoints in both species (Table 3) but there were no significant differences in growth between any treatments at both times (Table 4). Though there is a trend in greater growth in high temperature treatments at the midpoint, it disappears at the endpoint.

In *D. menziesii* the mean $\alpha$ was significantly different between time 0, *in situ*, and control treatments (ANOVA, $F_{(2, 32)}=84.433$, $P < 0.0005$). $E_k$ and MQY means were not significantly different between *in situ* and time 0 treatments, but both means were significantly different from the control treatment ($E_k$ increased in the control, but MQY decreased; ANOVA, $F_{(2, 32)}= 40.187$, $P < 0.0005$ and $F_{(2, 32)}= 100.61$, $P < 0.0005$ respectively), but $ETR_{max}$ was significantly higher in time 0 treatments than control and *in situ* treatments (ANOVA, $F_{(2, 32)}= 100.571$, $P < 0.0005$). In *D. anceps*, $ETR_{max}$ were not significantly different between *in situ*, time 0, and control treatments, but the parameters $\alpha$ and MQY had significantly higher means in time 0 treatments than control and *in situ* (ANOVA, $F_{(2, 32)}= 11.192$, $P < 0.0005$ and $F_{(2, 32)}= 7.069$, $P = 0.003$ respectively), and mean $E_k$ was significantly higher in the control treatment than *in situ* and time 0 treatments (ANOVA, $F_{(2, 32)}=7.168$, $P = 0.003$). Microcosm conditions impacted these parameters while few time 0 treatments were altered by the aquarium conditions.
All photosynthetic parameters, like growth, were measured during both time periods (Table 5). At the mid-point in \textit{D. anceps} no treatment significantly impacted the change in mean $\alpha$, MQY, $E_k$ and $ETR_{\text{max}}$ (Table 4, 5). Mid-point measurements in \textit{D. menziesii} showed no significant differences in mean $\alpha$, MQY, and $ETR_{\text{max}}$ between treatments, but the interaction between the high temperature and low pH significantly altered $E_k$ in microcosms (Table 4, 5). No parameters were significantly different from each other at end point measurements (Table 4, 5). However, the repeated measures ANOVA showed all parameters changed significantly, specifically due to the factor time (start, mid, and endpoint) except in \textit{D. menziesii} where MQY stayed constant and $ETR_{\text{max}}$ fluxed due to treatment as well (Table 3, Figure 2). Trends in the change of the parameters include an increased $E_k$, lower or more efficient $\alpha$, and lowered MQY and $ETR_{\text{max}}$ (Figure 2).

The mean concentration of chl $a$ was significantly greater in time 0 treatments than control treatments in both \textit{D. menziesii} and \textit{D. anceps} (Figure 3; Students t-test, $t_{(22)}$= 19.302, $P< 0.0005$, and $t_{(22)}$= 6.658, $P < 0.0005$ respectively). In both species mean chl $a$ concentrations did not differ significantly between any of the microcosm treatments (Table 4, Figure 3).

In \textit{D. anceps}, no significant differences were found in mean phlorotannin content between treatments or between treatments and time 0 individuals (Table 4, Figure 4). There was significantly higher mean phlorotannin content in \textit{D. menziesii} in microcosm treatments vs. time 0 (Figure 4; Students t-test $t_{(22)}$= 28.393, $P = 0.032$) and significantly higher mean phlorotannin content in low pH conditions across all microcosms (Table 4, Figure 4).
DISCUSSION

The multiple end products of the global increase in greenhouse gases (increased temperature and $pCO_2$ of the ocean) threaten the western Antarctic Peninsula in a variety of ways (Ducklow et al. 2013). Overall our results indicate that increased ocean temperature and $pCO_2$ actually have few significant effects on the canopy-forming algae from the subtidal western Antarctic Peninsula. In near future ocean conditions the two Desmarestia spp. do not appear to be physiologically stressed, although the changes we do see in their physiology may have the potential to be ecologically consequential. In D. menziesii, the mean $E_k$ increased significantly in the high temperature * low pH treatment after short term exposure, indicating that individuals need more light to saturate their photocenters in these conditions. Microcosm containers did have a steady light source, which is unusual for Antarctic species which have very low light requirements and may have been responsible for observed changes (Gomez et al. 2009). Both species showed oscillating trends in growth over the two time periods; increased growth to the midpoint but negative growth to the endpoint. Also, individuals in low pH treatments had significantly higher mean phlorotannin content than other treatments, and all experimental treatments had higher phlorotannin levels than time 0 individuals in D. menziesii. The overall plasticity in these physiological parameters has been reported before (Amsler and Fairhead 2006; Rautenberger et al. 2013), but the few significant changes in D. menziesii should be noted.

This experiment was relatively short in duration and the changes in growth and photo parameters over two sampling periods highlight the need to look at the effects of climate change parameters over short and long term exposure periods. Species may be acclimated or adapted to constant or high-fluctuating environments (Kelley and Hofmann 2012). The environment in the archipelago surrounding Palmer Station is characterized by moderate daily and annual variations
in water chemistry parameters (Schram et al. unpublished). Low pH levels and elevated temperature may be experienced in this area, but they are not sustained at these levels for long periods of time. Our results describe two temporal responses to climate change conditions that highlight physiological change over the two periods; short and moderate duration. There are also significant differences between experimental treatments and \textit{in situ} and time 0 measurements. Because there were mostly no significant differences between treatments, though there was high variation around the means, the more static controlled environment alone may have impacted many parameters of algal physiology. Prime examples are decrease in chl \textit{a} in both species in experiments and increased phlorotannin concentration in \textit{D. menziesii}.

The shift in photosynthetic parameters throughout the experiment may show an initial acclimation to the light source in experiments at the midpoint, which is supported by the reduction in chl \textit{a} concentration in these algae. Measurements at the endpoint likely reflect adaptation to pH and temperature treatments, or the longer exposure period, and these are not consistently different from midpoint measurements. The significant increase in \( E_k \) in \textit{D. menziesii} at the mid-point in the low pH * high temperature treatment indicates that these factors synergistically impact these individuals in that their photosystems require more irradiance to saturate PSII. Overall the individuals in the experiments reduced their \( \alpha \) values significantly through the midpoint, becoming less efficient, and increased slightly through the endpoint. Individuals also decreased MQY initially, with a continued decrease through the endpoint. \( E_{\text{TR}_{\text{max}}} \) initially decreased at the midpoint, though it increased to a higher level than time 0 individuals at the endpoint. Finally, \( E_k \) increased through both sampling periods. The combination of all these shifts indicates a depression in the light curve generated using PAM fluorometry, though this is not specific to any climate change conditions.
Initial growth in both species across treatments may be due to the increased $p\text{CO}_2$ and constant light source. CO$_2$ diffuses 4 orders of magnitude less rapidly in water than it does in air (Gomez and Huovinen 2012). Therefore increases in partial pressure will likely amplify the diffusive mode of carbon acquisition in larger seaweeds over other carbon concentrating mechanisms (i.e. utilizing HCO$_3^-$ through carbonic anhydrase, CCMs). A prediction for this experiment was that Desmarestia spp. would positively respond to an increase in $p\text{CO}_2$ (as a substrate for photosynthesis) and constant light source, which they did initially. However, throughout the last half of the experiment individuals lost mass and in some treatments growth (% change in mass) was negative (D. anceps; Figure 1). These perennial species grow primarily during the Antarctic spring and summer (August-December; Wiencke 1990), which may account for the change in growth except the growth cycle in these species is largely controlled by light levels which were constant in our experiment. It is unlikely that the algae override light cues to follow an annual cycle and temperatures were not outside optimal growth parameters for these species (sporophytes optimal growth temperature: 0 to 5 ºC; Wienck and Dieck 1990). Therefore the reason for this decline in mass in all treatments is as of yet unidentified but may be due to imbalance in photosynthesis and respiration or another factor that we could not monitor (e.g. CCMs).

Environmental variation does impact phlorotannin levels in brown algae (Targett and Arnold 1999; Amsler and Fairhead 2006; Jormalainen and Honkanen 2008), making it a plastic characteristic of brown algae. Therefore, significant differences that we see in concentrations between time 0 and control treatments are not unexpected. Phlorotannin concentrations increased only in D. menziesii, the species which commonly inhabits shallower zones. Because we used the distal portion of lateral branches for the assays and all individuals of each species were from the
same site, we know that phlorotannin content should have initially been consistent across treatments (Fairhead et al. 2005a; Fairhead et al. 2005b; Iken et al. 2007). As before, exposure to 12 h of constant light probably affected this aspect of the algal physiology, but treatments responded unexpectedly. In previous studies, *D. anceps* had higher phlorotannin concentration than *D. menziesii* (Fairhead et al. 2005b), which was true for our initial measurements (2.81% DW and 1.07% DW respectively). However in these experiments, *D. menziesii* values increased to 4.38 - 8.7% (significantly more in low pH treatments) whereas *D. anceps* values increased to only 3.36 - 5.59%. The former species is presumably exposed to higher levels of natural irradiance and wave action which both impact phlorotannin concentration (Amsler and Fairhead 2006). Therefore this aspect of the *D. menziesii* phenotype may be easier to up or down regulate than it is in *D. anceps*.

Aside from a persistent debate over the specific functions of phlorotannins, the general consensus is that they are important to the functional and chemical ecology of algae (Amsler and Fairhead 2006). Phlorotannins are structural compounds found in physodes which are next to or embedded in cell walls of brown algae. These compounds can also increase in response to herbivory and UV exposure (Amsler and Fairhead 2006). Both species are strongly chemically defended against herbivory (Amsler et al., 2005b), primarily through lipophyllic compounds including menzoquinone in *D. menziesii* and hydrophillic compounds in *D. anceps* (Ankisetty et al., 2004, Amsler et al., 2005a), and anti-herbivory compounds include phlorotannins (Fairhead et al., 2006). Phlorotannin content in *D. menziesii* may have increased as a response to basic experiment conditions and in low pH treatments, increased as a response to the increased carbon resource or perhaps as a response to the acidity of the environment. Regardless, when low pH is
uncoupled from increased temperature it may impact uptake of algal carbon in detrital systems and distribution or zonation of the algae in situ.

Along with specific impacts on individual species, there is a potential for effects to cascade within communities, shifting ecological interactions (Diaz-Pulido et al. 2011; Hofmann et al. 2012) and complexity (Kroeker et al. 2011; Kroeker et al. 2013), which could amplify the effects of ocean acidification (Fabry et al. 2008; Guinotte and Fabry 2008; Hall-Spencer et al. 2008; Widdicombe and Spicer 2008). This increases the importance of doing multiple species or even ecosystem wide experiments when examining the biological impacts of climate change (Riebesell et al. 2008) and modeling treatments around environmental complexity (Denman et al. 2011; Dupont and Portner 2013). This experiment looked at the response of species in individual treatments but it would be relevant to investigate the effects the minor shifts in algal physiology on the palatability of these dominant species, their toughness, and other ecologically relevant traits. While these results indicate benign impacts of low pH and high temperature on canopy forming species, future experiments should focus on broad scale community responses on to confirm null effects of physiological shifts that were not evaluated.

ACKNOWLEDGEMENTS

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Table 1. pH and temperature records for experiment treatments and ambient water samples using the Durafet and digital temperature sensor (means ± SD).

<table>
<thead>
<tr>
<th>Treatments</th>
<th>pH 8 * 1.5 °C</th>
<th>pH 7.6 * 1.5 °C</th>
<th>pH 8.0 * 3.5 °C</th>
<th>pH 7.6 * 3.5 °C</th>
<th>ambient</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>8.07 ± 0.09</td>
<td>7.6 ± 0.15</td>
<td>8.04 ± 0.06</td>
<td>7.58 ± 0.19</td>
<td>8.02 ± 0.04</td>
</tr>
<tr>
<td>Temperature</td>
<td>1.51 ± 0.25</td>
<td>1.49 ± 0.23</td>
<td>3.52 ± 0.35</td>
<td>3.51 ± 0.29</td>
<td>-0.69 ± 0.49</td>
</tr>
</tbody>
</table>
Table 2. Water Chemistry parameters for microcosms calculated using AT and spectrophotometric pH data in CO2calc (means ± SD).

<table>
<thead>
<tr>
<th></th>
<th>in situ seawater</th>
<th>1.5°C pH 7.6</th>
<th>1.5°C pH 8.0</th>
<th>3.5°C pH 7.6</th>
<th>3.5°C pH 8.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>8.10 ± 0.03</td>
<td>7.55 ± 0.18</td>
<td>8.05 ± 0.134</td>
<td>7.55 ± 0.171</td>
<td>8.05 ± 0.095</td>
</tr>
<tr>
<td>TA (μmol/kg SW)</td>
<td>2284 ± 36</td>
<td>2292 ± 55.44</td>
<td>2269 ± 60.75</td>
<td>2294 ± 35.61</td>
<td>2271 ± 55.98</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>0.91 ± 0.72</td>
<td>1.46 ± 0.566</td>
<td>1.45 ± 0.296</td>
<td>3.32 ± 0.976</td>
<td>3.19 ± 0.782</td>
</tr>
<tr>
<td>Salinity (ppt)</td>
<td>35.6 ± 0.603</td>
<td>35.74 ± 0.632</td>
<td>35.83 ± 0.784</td>
<td>35.67 ± 0.561</td>
<td>35.81 ± 0.633</td>
</tr>
<tr>
<td>pCO2 (μatm)</td>
<td>327 ± 28</td>
<td>1407 ± 583.4</td>
<td>410 ± 290.7</td>
<td>1433 ± 530.8</td>
<td>393 ± 127.4</td>
</tr>
<tr>
<td>DIC (μmol/kg SW)</td>
<td>2132 ± 44</td>
<td>2316 ± 82.05</td>
<td>2134 ± 70.98</td>
<td>2311 ± 66.78</td>
<td>2125 ± 53.6</td>
</tr>
<tr>
<td>Ω_{arig}</td>
<td>1.67 ± 0.12</td>
<td>0.57 ± 0.25</td>
<td>1.55 ± 0.32</td>
<td>0.61 ± 0.257</td>
<td>1.66 ± 0.295</td>
</tr>
<tr>
<td>Ω_{cal}</td>
<td>2.66 ± 0.19</td>
<td>0.91 ± 0.397</td>
<td>2.46 ± 0.508</td>
<td>0.96 ± 0.408</td>
<td>2.63 ± 0.467</td>
</tr>
</tbody>
</table>
Table 3. Results from repeated measure 2-way ANOVAs using the parameters growth, $E_k$, $\text{ETR}_{\text{max}}$, MQY and $\alpha$.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>$D. \text{ anceps}$</th>
<th></th>
<th>$D. \text{ menziesii}$</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F</td>
<td>df</td>
<td>P</td>
<td>F</td>
</tr>
<tr>
<td>growth</td>
<td>50.295</td>
<td>2</td>
<td>&lt; 0.0005</td>
<td>32.656</td>
</tr>
<tr>
<td>time</td>
<td>61.083</td>
<td>2</td>
<td>&lt; 0.0005</td>
<td>49.304</td>
</tr>
<tr>
<td>treatment</td>
<td>0.771</td>
<td>3</td>
<td>0.513</td>
<td>0.518</td>
</tr>
<tr>
<td>$\alpha$</td>
<td>0.221</td>
<td>3</td>
<td>0.881</td>
<td>1.019</td>
</tr>
<tr>
<td>time</td>
<td>30.7</td>
<td>2</td>
<td>&lt; 0.0005</td>
<td>19.323</td>
</tr>
<tr>
<td>treatment</td>
<td>2.007</td>
<td>3</td>
<td>0.132</td>
<td>2.837</td>
</tr>
<tr>
<td>$E_k$</td>
<td>211.863</td>
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<td>&lt; 0.0005</td>
<td>1.802</td>
</tr>
<tr>
<td>time</td>
<td>2.443</td>
<td>3</td>
<td>0.082</td>
<td>0.676</td>
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<tr>
<td>treatment</td>
<td>26.072</td>
<td>2</td>
<td>&lt; 0.0005</td>
<td>9.983</td>
</tr>
<tr>
<td>MQY</td>
<td>1.299</td>
<td>3</td>
<td>0.291</td>
<td>3.341</td>
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</tbody>
</table>
Table 4. MANOVA tables for both *D. anceps* and *D. menziesii*.

<table>
<thead>
<tr>
<th>species</th>
<th>time</th>
<th>parameter</th>
<th>df</th>
<th>F-value</th>
<th>P-value</th>
<th>power</th>
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</thead>
<tbody>
<tr>
<td><em>D. anceps</em></td>
<td>1</td>
<td>growth</td>
<td>3</td>
<td>0.877</td>
<td>0.46</td>
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<td></td>
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<tr>
<td></td>
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<td>chl α</td>
<td>3</td>
<td>2.503</td>
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<td>0.581</td>
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<tr>
<td></td>
<td>2</td>
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<td>3</td>
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<td>0.657</td>
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<tr>
<td></td>
<td>1</td>
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<tr>
<td></td>
<td>1</td>
<td>EFq</td>
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<tr>
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<tr>
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<td>ETR_{max}</td>
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<tr>
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<td>3</td>
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<td>0.137</td>
<td>0.467</td>
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<tr>
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<td>growth</td>
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<td>0.211</td>
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<tr>
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<td>0.309</td>
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<tr>
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<tr>
<td></td>
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<td>1.71</td>
<td>0.179</td>
<td>0.416</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>EFq</td>
<td>3</td>
<td>5.45</td>
<td>0.003</td>
<td>0.916</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>MQY</td>
<td>3</td>
<td>0.192</td>
<td>0.901</td>
<td>0.083</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>ETR_{max}</td>
<td>3</td>
<td>2.017</td>
<td>0.125</td>
<td>0.483</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>α</td>
<td>3</td>
<td>2.189</td>
<td>0.103</td>
<td>0.519</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>EFq</td>
<td>3</td>
<td>0.723</td>
<td>0.544</td>
<td>0.191</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>MQY</td>
<td>3</td>
<td>1.566</td>
<td>0.211</td>
<td>0.384</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>ETR_{max}</td>
<td>3</td>
<td>0.657</td>
<td>0.583</td>
<td>0.177</td>
</tr>
</tbody>
</table>

1= mid-point measurements, 2= end-point measurements. F-values are equivalent to F-test values.
Table 5. Photosynthetic parameters of *D. anceps* and *D. menziesii* in microcosms at the mid-point and end-point of the experiment. Values are reported as means ± SE (n = 12).

<table>
<thead>
<tr>
<th>Microcosm</th>
<th>Species</th>
<th>MQV</th>
<th>( J_i )</th>
<th>( \alpha )</th>
<th>( \text{FTR}_{\text{max}} )</th>
<th>MQV</th>
<th>( J_i )</th>
<th>( \alpha )</th>
<th>( \text{FTR}_{\text{max}} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH 8.0 * 1.5°C</td>
<td><em>D. menziesii</em></td>
<td>0.737 ± 0.011</td>
<td>171.89 ± 16.99</td>
<td>0.1204 ± 0.013</td>
<td>8.37 ± 1.66</td>
<td>0.794 ± 0.029</td>
<td>201.21 ± 19.93</td>
<td>0.1445 ± 0.0155</td>
<td>11.83 ± 1.33</td>
</tr>
<tr>
<td></td>
<td><em>D. anceps</em></td>
<td>0.714 ± 0.023</td>
<td>195.99 ± 9.41</td>
<td>0.084 ± 0.005</td>
<td>6.04 ± 0.51</td>
<td>0.564 ± 0.068</td>
<td>211.35 ± 47.81</td>
<td>0.078 ± 0.012</td>
<td>6.11 ± 1.06</td>
</tr>
<tr>
<td>pH 7.6 * 1.5°C</td>
<td><em>D. menziesii</em></td>
<td>0.734 ± 0.012</td>
<td>170.78 ± 4.34</td>
<td>0.1207 ± 0.0066</td>
<td>7.81 ± 0.39</td>
<td>0.682 ± 0.038</td>
<td>219.26 ± 9.17</td>
<td>0.1475 ± 0.0067</td>
<td>11.79 ± 0.51</td>
</tr>
<tr>
<td></td>
<td><em>D. anceps</em></td>
<td>0.693 ± 0.020</td>
<td>195.99 ± 10.32</td>
<td>0.082 ± 0.008</td>
<td>6.84 ± 0.98</td>
<td>0.669 ± 0.036</td>
<td>191.91 ± 16.66</td>
<td>0.092 ± 0.010</td>
<td>6.32 ± 0.73</td>
</tr>
<tr>
<td>pH 8.0 * 3.5°C</td>
<td><em>D. menziesii</em></td>
<td>0.744 ± 0.016</td>
<td>192.74 ± 5.61</td>
<td>0.114 ± 0.0089</td>
<td>8.08 ± 0.54</td>
<td>0.628 ± 0.063</td>
<td>175.64 ± 18.11</td>
<td>0.142 ± 0.0221</td>
<td>9.79 ± 1.47</td>
</tr>
<tr>
<td></td>
<td><em>D. anceps</em></td>
<td>0.575 ± 0.085</td>
<td>185.73 ± 4.41</td>
<td>0.076 ± 0.006</td>
<td>5.08 ± 0.39</td>
<td>0.631 ± 0.064</td>
<td>138.82 ± 19.47</td>
<td>0.087 ± 0.014</td>
<td>5.35 ± 0.85</td>
</tr>
<tr>
<td>pH 7.6 * 3.5°C</td>
<td><em>D. menziesii</em></td>
<td>0.722 ± 0.027</td>
<td>228.67 ± 9.15</td>
<td>0.134 ± 0.0073</td>
<td>10.64 ± 0.55</td>
<td>0.711 ± 0.034</td>
<td>222.22 ± 10.03</td>
<td>0.1577 ± 0.0013</td>
<td>12.98 ± 1.11</td>
</tr>
<tr>
<td></td>
<td><em>D. anceps</em></td>
<td>0.704 ± 0.015</td>
<td>185.73 ± 4.73</td>
<td>0.083 ± 0.004</td>
<td>5.67 ± 0.34</td>
<td>0.596 ± 0.067</td>
<td>180.71 ± 24.97</td>
<td>0.100 ± 0.014</td>
<td>6.17 ± 0.78</td>
</tr>
<tr>
<td>Time 0</td>
<td><em>D. menziesii</em></td>
<td>0.690 ± 0.016</td>
<td>159.18 ± 10.49</td>
<td>0.1994 ± 0.0053</td>
<td>11.54 ± 0.68</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td><em>D. anceps</em></td>
<td>1.055 ± 0.012</td>
<td>195.99 ± 15.34</td>
<td>0.175 ± 0.012</td>
<td>7.28 ± 0.71</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>In situ</td>
<td><em>D. menziesii</em></td>
<td>0.738 ± 0.009</td>
<td>166.99 ± 9.92</td>
<td>0.134 ± 0.0077</td>
<td>8.28 ± 0.67</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td><em>D. anceps</em></td>
<td>0.741 ± 0.044</td>
<td>141.09 ± 10.52</td>
<td>0.088 ± 0.012</td>
<td>5.01 ± 1.04</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

\( a, b, c \) indicate significant differences in a 1-way ANOVA (\( p < 0.05 \)) between control, time 0, and *in situ* treatments.

\( * \) indicate significantly different treatments in a 2-way ANOVA (\( p < 0.05 \))
FIGURES

Figure 1. Growth (% original thallus weight) in both *D. anceps* and *D. menziesii* at both mid (black) and end points (grey) of the experiment. Values represent means + SE.
Figure 2. Changes in photosynthetic parameters over the experimental time period in both *D. anceps* and *D. menziesii* (repeated measures 2-way ANOVA with raw data; means ± SE).
Figure 3. Chlorophyll $a$ concentration in *D. aniceps* and *D. menziesii* from microcosm experiment and time 0 individuals (means + SE). Time 0 individuals to the right of the figure exemplify a shift in the photosynthetic apparatus in microcosm treatments overall.
Figure 4. Phlorotannin concentration of *D. aniceps* and *D. menziesii* in microcosm treatments (means + SE). Asterisks denote a significant difference in a treatment.
CLIMATE CHANGE FACTORS INCREASED SEAWATER TEMPERATURE AND PCO2 INCREASE GROWTH IN FLESHY CRUSTOSE ANTARCTIC MACROALGAE

by

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ABSTRACT

Climate change impacts were investigated in two species of crustose Antarctic macroalgae. The seawater parameters oceanic pH ($p$CO$_2$) and temperature were modified to near future projections for the western Antarctic Peninsula in microcosm experiments. Experiments included two crustose algae, the calcified species *Clathromorphum obtectulum* and the fleshy species *Hildenbrandia* sp., and were run for six weeks in treatments reflecting near future ocean conditions under climate change predictions: increased temperature (3.5°C and pH 8.1), increased pCO$_2$ (1.5°C and pH 7.8), and both factors (3.5 °C and pH 7.8). The physiological responses of the algae were evaluated through photosynthetic parameters (MQY, $E_k$, $ETR_{\text{max}}$, and $\alpha$), growth, chlorophyll *a* concentration, and calcium carbonate content. No negative impacts were observed in either species, though some trends in data indicate photosynthetic parameters are depressed by increased temperature in the calcified species and pH in the fleshy species. The fleshy algae decreased in size in low pH and high temperature treatments alone, but increased size significantly when these factors were combined. No other parameters changed significantly in either species. These results indicate that *Hildenbrandia* sp. may have a competitive advantage for space in the subtidal environment in near future oceanic conditions; however it is uncertain how this may broadly impact the community.
INTRODUCTION

As greenhouse gases (CO$_2$, CH$_4$ and N$_2$O) are increasing to highest recorded levels in our atmosphere’s recent history (IPCC 2013; Tans and Keeling 2013), in an era where anthropogenic activities have started to shape global climate, it has become pertinent to investigate how natural communities will respond to these conditions. Products of climate change coupled with other human induced stressors to marine communities include (but are not limited to) sea surface temperature increases, ocean acidification, change in sea ice duration and extent, eutrophication, and increased UVR (IPCC 2007; 2013). Since the 1950s the average global increase in ocean temperature was 0.11 ± 0.2 °C per decade between 1971 and 2010 (IPCC 2013), and this past decade is the warmest on record. Ocean temperatures are projected to increase by 0.6 to 2.0 °C by the end of the century, and deep water warming (> 1000m) will be most pronounced in the Southern Ocean. Warming has been shown to severely impact species diversity in many ecosystems (Hillebrand et al., 2012), and when coupled with other stressors may have a greater impact.

Of specific concern is CO$_2$ because it contributes not only to global warming but also acidifies our oceans. Increased CO$_2$ concentrations translate to increased draw down of CO$_2$ into the oceans, which are the largest carbon sink on the planet (Sabine et al., 2004), resulting in decreased ocean pH (Caldeira and Wickett 2005; Doney et al., 2009; Guinotte and Fabry 2008; Sabine et al., 2004; Takahashi et al., 1997) through the following reaction mechanism (eq. 1-3):

\[
\begin{align*}
\text{CO}_2 + \text{H}_2\text{O} & \rightarrow \text{H}_2\text{CO}_3 \\
\text{H}_2\text{CO}_3 & \leftrightarrow \text{H}^+ + \text{HCO}_3^- \\
\text{HCO}_3^- & \leftrightarrow \text{H}^+ + \text{CO}_3^{2-}
\end{align*}
\]

Ambient ocean pH is projected to decline from a present day global average of 8.1 to 7.7 by the year 2100 (IPCC 2007; 2013), and this increased concentration of hydrogen ions will shift
saturation states of carbonate (Fabry et al., 2008; Feely et al., 2004; Orr et al., 2005). The calcium carbonate (CaCO$_3$) polymorphs aragonite and magnesium calcite (MgCO$_3$) are 50% more soluble in seawater than calcite (Feely et al., 2004), which will make it difficult for organisms that use these ions to strengthen their shells or thalli.

The Antarctic Peninsula is experiencing one of the fastest rates of global climate change today (Clarke et al. 2007; Ducklow et al. 2013; Meredith and King 2005). These changes could be detrimental to organisms inhabiting the Southern Ocean because they experience somewhat consistent environmental conditions (aside from disturbance and light) and many organisms are stenothermal (Peck 2005; Wiencke et al., 2007). Ocean temperatures range between -1.8 to 2.5 ºC on the western Antarctic Peninsula (Drew and Hastings 1992; Schram et al., unpublished) and pH can range from 8.0 to 8.62 units throughout the year in our study area (Schram et al., unpublished) and pH can get as low as 7.932 units in the western Antarctic surface waters (Bjork et al., 2013). In the last half century the oceans around the western Antarctic Peninsula have increased 1 ºC in average temperature (Ducklow et al., 2013), while air temperature has increased ~ 0.25 ºC every decade since the 1950s (Steig et al., 2009; Vaughan et al., 2003). In addition, because of the consistently cold seawater temperatures the polar oceans may become under-saturated in carbonate species before other oceans (Orr et al., 2005), making it difficult for the already thin-shelled organisms to maintain calcification.

Organisms differentially respond to aspects climate change (Harley et al., 2012; Kroeker et al., 2011; Ries 2009; Roleda and Hurd 2012), and calcifying invertebrates like corals and coralline algae are most prone to negative effects of ocean acidification specifically, though climate change will likely obscure metabolic processes in many marine organisms (Fabry et al., 2008; Kelley and Hofmann 2012). The physiological response of coralline algae to high $p$CO$_2$
environments is largely decreased calcification (Anthony et al., 2008; Budenbender et al., 2011; Bulach 2012; Johnson and Carpenter 2012; Kuffner et al., 2008; Yildiz et al., 2013) or a net dissolution of thalli, decrease in structural integrity, or mortality (Diaz-Pulido et al., 2012; Ragazzola et al., 2013; Roleda et al., 2012). Calcification of thalli can increase when high $p$CO$_2$ is coupled with increased temperature in some species (Koch et al., 2013; Martin and Gattuso 2009). Although high $p$CO$_2$ also reduces external carbonic anhydrase activity in some species (Hofmann et al., 2012) and decreases productivity and growth (Anthony et al., 2008; James et al., 2014; Jokiel et al., 2008), when high $p$CO$_2$ is coupled with an increase in ultraviolet radiation (UVR) growth rates increase (Yildiz et al., 2013). At the community level high $p$CO$_2$ can lower recruitment rates of coralline algae to reef communities (Kroeker et al., 2013; Kuffner et al., 2008; Porzio et al., 2013) and increase susceptibility to grazing (Johnson and Carpenter 2012). Overall, coralline algae commonly show negative responses to factors of climate change, particularly high $p$CO$_2$.

Fleshy macroalgae have a variety of responses to climate change factors. Patterns of decreased calcification are seen in fleshy brown algae, specifically Padina spp. which decrease calcification but grow more in those conditions (Johnson et al., 2012). The ability of species to persevere is relative to the resources in a habitat, and the rate and severity of climate changes (Connell et al., 2013; Kamenos et al., 2013). For the most part, marine autotrophs should benefit from these conditions (Koch et al., 2013) and fleshy macroalgae do, especially species which do not use carbon concentrating mechanisms (CCMs) to acquire inorganic carbon (DIC; Hepburn et al. 2011). Many marine communities have natural diurnal variations in pH that macroalgae buffer (Frieder et al., 2012; Hendriks et al., 2013) and calcifying species benefit from association with these macroalgae (Saderne 2012; Semesi et al., 2009). These predominantly positive
responses of fleshy algae confer a competitive advantage over the negatively impacted sympatric calcified species.

Species specific responses have the potential to broadly affect whole communities causing a shift in ecological interactions (Diaz-Pulido et al., 2011; Hofmann et al., 2012) and community complexity (Kroeker et al., 2013; Kroeker et al., 2011), which could amplify the effects of ocean acidification already observed (Fabry et al., 2008; Guinotte and Fabry 2008; Hall-Spencer et al., 2008; Widdicombe and Spicer 2008). The goal of this experiment is to determine species specific response to increased temperature, decreased pH, and both factors on the physiology of two common crustose macroalgae species from the western Antarctic Peninsula.

The western Antarctic Peninsula is home to large macroalgal forests, composed largely of Desmarestia spp. and Himantothallus grandifolius, which dominate the benthos in some areas (Wiencke and Amsler, 2012). Crustose algae are also abundant in this subtidal habitat; coralline algae can reach 77% cover in some areas above 30 m depth (Amsler et al., 1995) and there are two taxa of fleshy crustose algae (Rhodophyta) that co-occur with corallines, Hildenbrandia sp. and Gaina mollis, but these are less abundant. The response of common crustose species from the western Antarctic Peninsula to near-future climate change conditions was evaluated using the coralline algae Clathromorphum obtectulum and the fleshy crustose algae Hildenbrandia sp. Physiological response of the algae to each treatment will be measured through calcification (coralline only), growth, chlorophyll a content, and photosynthetic characteristics.
METHODS

Collections

This research was conducted at the United States Antarctic Program’s Palmer Station, on Anvers Island along the western Antarctic Peninsula (for map see Amsler et al. 2009). Both target species were collected using SCUBA within a 3.5 km radius of Palmer Station. *Hildenbrandia* sp. were collected from ~15m at North Hermit (S 64° 45.620’ W 64° 05.883) on small granite rocks that could be used as anchors in the experiment. *C. obtectulum* were collected from ~5 m at Kristie Cove (S 64° 46.697 W 64° 02.954’) on small granite rocks. Crustose coralline algae were differentiated using morphological characteristics detectable under a dissecting scope. Subsequent morphological groupings were identified to species through collaboration with Dr. Juan C. Braga at the Universidad de Granada in Spain. Scanning electron microscopy (SEM) was used to key out groupings (Wiencke and Clayton 2002; Hommersand et al., 2009, 2011). The morphotype used in this experiment was the gametophytic stage of *C. obtectulum*.

Experimental individuals were on small rocks no more than 5 cm$^3$ in dimension. Additional fouling organisms were cleaned off of each rock using a wire brush and soldering iron prior to the start of the experiment and time 0 measurements. All individuals were kept in flow through aquaria under irradiance levels of 20 to 30 µmol photon m$^{-2}$ s$^{-1}$ on a 12 : 12 h day night cycle until the experiment began.

Experimental set-up

Microcosms (36 x 20 x 15 cm) were quartered using plastic screening so organisms would not come in contact; each microcosm held one *Hildenbrandia* sp. and *C. obtectulum*.
thallus and two benthic gastropods. This design was used to balance photosynthesis and respiration in the experiment without allowing the organisms to interact. Microcosms were covered with a Plexiglas lid limiting gas exchange with the ambient environment, but allowing irradiance levels which mimicked those in their natural environment (~16 to 22 µmol photon m\(^{-2}\) s\(^{-1}\)). Treatments reflected current conditions and near-term predictions of pH and temperature (Kurihara 2008, Riebesell et al. 2010): ambient conditions (control, pH 8.0 and 1.5°C), low pH (pH 7.8 and 1.5 ºC), high temperature (pH 8.0 and 3.5 ºC), and low pH and high temperature (pH 7.8 and 3.5 ºC).

Temperature in microcosms was maintained by circulating a 30% glycol mixture through a circulating water bath and large Plexiglas water table (114.3 x 58.42 x 15.24 cm) which held microcosms. Four water baths were set up on shelving units with Sylviana Cool White fluorescent bulbs (F48T12/CW/HO, 60W) providing irradiance on a 12 : 12 h day night cycle. The pH was maintained by exchanging 50% of microcosm water volume daily with pre-treated reservoir water. Treatments were exposed to experimental conditions for 47 days.

**Water Chemistry**

Target pH was maintained in reservoirs using an AT control system (Aqua Medic, USA) which monitors real time pH in each microcosm, delivering CO\(_2\) gas to the tanks to increase \(p\)CO\(_2\) (decrease pH) when necessary. Control reservoirs had a constant stream of air bubbling into them to maintain water movement.

Seawater pH and temperature were monitored daily in microcosms using an Orion \(p\)H\(_{NBS}\) probe and Cole-Parmer Digi-Sense ThermoLogR Thermister. Total alkalinity (TA) and spectrophotometric pH were measured twice a week in each microcosm and once daily in
reservoirs and ambient seawater (Table 1). Seawater samples were siphoned from their microcosms into 300 ml borosilicate stoppered bottles and allowed to overflow one full volume in order to limit sample contact with air (Dickson et al., 2007). After sampling, water was replaced with pretreated water from reservoirs and sampling bottles were stored in a cold-dark area until they could be processed.

Spectrophotometric pH was measured following methods in SOP 6b (Dickson et al., 2007) using a UV/Vis Spectrometer LAMBA 40 (Perkin Elmer) with jacketed cells plumbed to a water bath held at 25 ºC. Seawater TA was measured using a T50 Titrator (Mettler Toledo, Switzerland), a jacketed 500 ml beaker plumbed to a water bath at 25 ºC, and DGi113-SC pH probe (Mettler Toledo) following methods in SOP 3a (Dickson, et al., 2007). Salinity was measured in each sample using a YSI Conductivity Instrument (Model 3200) and conductivity cell (YSI 3253 model B). Certified reference materials (CRMs) provided by A. Dickson (Scripps, UCSD) were periodically used to calibrate pH and TA. User error or equipment error margins were minor: 4.15 μmol/kg seawater for TA, and 0.03 pH units. All TA, pH, temperature, and salinity measurements were used to calculate $pCO_2$, temperature corrected pH, calcite saturation state ($\Omega_{\text{cal}}$) and aragonite saturation state ($\Omega_{\text{arg}}$) using CO2calc (Robbins et al., 2010). We used $CO_2$ constants from Roy, et al. (1993), the KHSO$_4$ acidity constant from Dickson (1990), and air-sea flux constant from Wanninkhof (1992) were used to calculate temperature corrected pH and carbonate parameters. Average TA, pH, $pCO_2$, $\Omega_{\text{cal}}$, $\Omega_{\text{arg}}$, salinity and temperatures are expressed in Table 1.
**Measurements**

Prior to beginning the experiment, physiological variables of each experimental individual were measured, as were a set of time 0 individuals \( n = 18 \) for each treatment. Growth was measured through change in surface area \( (\text{cm}^2) \) by analyzing photographs of each individual taken at the beginning and end of the experiment with an image analysis program (CPCe, Kohler and Gill 2006). Change in surface area was calculated as fraction of original surface area \( \left( \frac{\text{SA}_t - \text{SA}_0}{\text{SA}_0} \right) \).

Photosynthetic parameters were measured using a pulse amplitude modulated (PAM) fluorometer (WALZ, Germany). Each individual was dark adapted for 30 min to empty photocenters to get a realistic estimation of the capacity of PSII. A saturating light pulse (sat pulse) was then delivered to calculate maximum quantum yield of photosystems \( (\text{MQY}, \frac{F_v}{F_m}, \text{sat pulse } 1.0 \text{ s}) \). Light curves were generated after this; nine sat pulses were delivered every 2 min after exposure to increasing irradiance levels \( (0, 0.48, 3.25, 11.82, 31.41, 55.1, 84.51, 121.3, \text{ and } 199.1 \mu\text{mol photon m}^{-2} \text{s}^{-1}) \) to calculate effective quantum yield \( (\frac{F_q}{F_m}) \). From the yield measurements electron transport rates (ETRs) were calculated (eq. 1) using measured absorbance \( (\text{abs}) \) of the two species \( (C. \text{ obectulum}: 60\% \text{ irradiance}, \ Hildenbrandia \text{ sp.}: 60\% \text{ irradiance}) \) and these were fitted to the Platt equation (Jassby and Platt 1976). From these light curves the parameters slope to saturation of photocenters \( (\alpha, \text{ eq. 2}) \), maximum electron transport rate \( (\text{ETR}_{\text{max}}, \text{ eq. 3}) \), and saturating irradiance were calculated \( (E_k, \text{ eq. 4}) \).

\[
\text{ETR} = \text{abs} \cdot 0.5 \cdot \frac{F_v}{F_m} \cdot \text{PAR} \\
\alpha = X \cdot (k_2-k_1) \\
E_k = \frac{((\ln]\cdot k_2) - (\ln]\cdot k_1))}{(k_2 / k_1)} \\
\text{ETR}_{\text{max}} = X \cdot (e^{-k_1 E_k} - e^{-k_2 E_k})
\]
Photosynthetic parameters were calculated as a change from the initial parameter in order to measure the response of the algae to each treatment.

Chlorophyll \( a \) was extracted from experimental and time 0 individuals using a small percentage of thallus material (0.25 to 1.0 cm\(^2\); quantified using CPCe, Kohler and Gill 2006). The tissue was lyophilized, ground using a mortar and pestle, and extracted in 20 ml 9 : 1 acetone : H\(_2\)O solution at -20 °C for 24 hours. A TD-700 fluorometer (Turner Designs) was used to calculate µg/L chl \( a \) in solution, and this was standardized to surface area of sample material (µg cm\(^{-2}\) algae).

Calcium carbonate (CaCO\(_3\)) content was measured in \( C. \) obtectulum by drying a percentage of the thallus material (0.5 to 1.0 cm\(^2\); quantified with CPCe; Kohler and Gill 2006) at 65 °C for 24 h. Samples were stored in air-tight bags and transported back to the United States for further processing. Individuals were weighed initially, combusted in a muffle furnace (Thermoline, Thermo Scientific, USA) for 4 h at 500 °C, and reweighed to determine ash weight. Samples were then dissolved in 12M HCl to eliminate CaCO\(_3\), then diluted with deionized water and dried in a vacuum oven at 65 °C. This step was repeated until all salts were removed and the weight of the inorganic material was subtracted from ash weight. This provided CaCO\(_3\) content (not differentiating between calcite species) was then standardized to the surface area of the sample (µg cm\(^{-2}\)).

Statistics

All data were added to a constant and square root transformed prior to statistical analysis in order to meet the assumptions of normality. Photosynthetic parameters of \( in \) \( situ \), time 0, and control individuals were compared using a 1-way ANOVA (SPSS) to test for physiological
changes do to ambient environment. Chl $a$ content was compared between time 0 and microcosm control treatments using a Students t-test (SPSS). Parameters from microcosm treatments were compared using a MANOVA, and significant effects were further investigated using a 2-way ANOVA (SPSS). A $p$ value of $\leq 0.05$ was used to determine significance in comparisons of means.

RESULTS

Growth in both species was slow; 4.2 to 15.5% in *Hildenbrandia* sp. and 3.1 to 16.7% in *C. obtectulum* of original thallus area which amounted to <1 mm at any edge. There were no significant differences in growth across treatments in *C. obtectulum*, however in the low pH * high temperature treatment *Hildenbrandia* sp. there was a significantly more growth (Figure 1; 2-way ANOVA, $F_{(3, 72)}= 2.809, p = 0.046$).

Mean values for photosynthetic parameters did not significantly change between *in situ*, time 0, and control treatment individuals in *Hildenbrandia* sp. but they did in *C. obtectulum* (Table 2). Mean $E_k$ was not significantly different between *in situ*, time 0, and control treatments, but mean values for other photosynthetic parameters were significantly different. Mean MQY of *in situ* individuals was significantly lower than time 0 and control treatment MQY (ANOVA, $F_{(2, 38)}=9.668, P< 0.0005$), and mean $\alpha$ of time 0 individuals was significantly lower than those of control and *in situ* treatments (ANOVA, $F_{(2, 38)}= 10.701, P<0.0005$). Mean $ETR_{\text{max}}$ was significantly lower in time 0 individuals than those *in situ* and in control treatments (ANOVA, $F_{(2, 38)}= 6.416, P=0.004$). There were no significant differences in mean values across experimental treatments for either species (Table 3), but there are a few trends. In *C. obtectulum*, mean $ETR_{\text{max}}$ and $E_k$ are generally depressed in treatments with high temperature (Table 2),
while in treatments with low pH *Hildenbrandia* sp. have decreased mean photosynthetic parameters overall (Table 2).

Mean CaCO$_3$ content recorded in *C. obtectulum* ranged between (0.095 to 0.211 μg cm$^{-2}$). Individuals in high temperature or low pH treatments had the least CaCO$_3$ while individuals in the low pH * high temperature treatment had highest mean CaCO$_3$ content, although these differences were not significant (Figure 2, Table 3). Mean chl a content of individuals in time 0 and control treatments was not significant different in either *C. obtectulum* or *Hildenbrandia* sp. (Students t-test, $t_{(2, 18)} = 0.445$, $P = 0.659$ and $t_{(2, 18)} = 0.133$, $P = 0.895$ respectively). The same was true for the mean chl a content in experimental treatments for both species (Figure 3, Table 3). The only trend in these data is lower levels of mean chl a content in *Hildenbrandia* sp. in low pH treatments (Figure 3).

**DISCUSSION**

In the present study, the climate change parameters increased $p$CO$_2$ and ocean temperature had few effects on crustose species from the western Antarctic Peninsula. *Hildenbrandia* sp. grew significantly more in microcosm treatments with low pH and high temperature. This species also exhibited trends of decreased chl a and depressed photosynthetic parameters in treatments with low pH. *Clathromorphum obtectulum* did not respond significantly to climate change treatments but there were trends of a depression in photosynthetic parameters in treatments with high temperature. In both species, growth is a slow process (personal observation) and the short duration of the experiment did not allow for large changes in thallus size. Overall our results indicate no negative effects from these climate change treatments, but a
positive effect on growth in the fleshy species, potentially conferring a competitive advantage for space in this community.

Seawater chemistry manipulations were designed to act as a sublethal stress and carbonate species such as \( \Omega_{\text{calc}} \) never became under saturated in our treatments (Table 1; \( pCO2 \) 922 to 944 μatm). The two experimental species in the present study are only found in the Southern Ocean and surrounding sub Antarctic habitats (Hommersand et al., 2011) and may or may not be stenothermal. They also experience periodic fluctuations in pH in their habitat throughout the year down to pH 7.938 (Bjork et al., 2013), indicating that exposure to the static pH of 7.8 of the near future treatments in the present study may not be extreme for these algae. Macroalgae are able to buffer pH in their overlying boundary layers (Cornwall et al., 2013), and the extra source of carbon may be beneficial for growth and other physiological processes in both species (Porzio et al., 2011). Many studies indicate that low pH should result in net dissolution of calcified thalli especially in low light environments (Budenbender et al., 2011), but based on the results in the present study this may not be the case in \emph{C. obtectulum}.

Though aragonite is the most common carbonate species in the ocean, coralline algae impregnate their cell walls with calcite (an orthogonal conversion probably occurs inside the alga; Lobban and Harrison 1994), 13 to 25% of which is MgCO\(_3\) (Budenbender et al., 2011). Although aragonite never became under-saturated in experimental treatments (Table 1), MgCO\(_3\) is a more soluble species. Trends in calcification indicate that high temperature and low pH independently decrease CaCO\(_3\) but together they increase CaCO\(_3\) above control treatment levels. On arctic reefs CaCO\(_3\) composes 80 to 90% of thallus mass; in our samples CaCO\(_3\) content ranged between 62.5 and 86.2% of thallus mass. Variation in this content showed no trends across treatments, and it would be relevant to look into \textit{in situ} CaCO\(_3\) content in the future.
Calcification is an important defense against herbivores and epibiota, and a more refined look at the ultrastructure of experimental individuals including cellular organization, thickness of the calcified thalli, and composition of the cell wall mineralization could show finer scale responses (Diaz-Pulido et al., 2014; Ries 2006; Ries 2010). This experiment was short in duration, and a longer experiment might have shown more differences across treatments.

Photosynthesis is coupled with calcification in algae (Lobban and Harrison 1994) and liberates CO$_2$ for photosynthesis while protecting it from UVR (Borowitzka 1982; Gao and Zheng 2010), although a direct relationship between the two processes is still unknown. Photosystems in Antarctic algae are characteristically flexible due to the highly variable light environment (Wienck and tom Dieck 1990) and acclimated or adapted to low light conditions (Gomez et al., 2009). Neither species showed significant changes across treatments, but trends include decreases in ETR$_{\text{max}}$ and $E_k$ in high temperature treatments for *C. obtectulum* and decrease in all parameters in low pH treatments in *Hildenbrandia* sp. (Table 3). In the coralline species, photosynthetic parameters changed between time 0 measurements and *in situ* measurements from the field and the end of experimental treatments, but this did not occur in the fleshy species. Interestingly, the calcified species had markedly more chl *a* than the fleshy species (Figure 3), but chl *a* concentration did not exhibit drastic changes in crustose species from exposure to a constant light environment as it did in canopy species from this region (Schoenrock et al., unpublished-b). Also, exposure to a consistent light source did not cause visible bleaching in this experiment, which occurs in Antarctic corallines when their macroalgal canopy is removed (Irving et al., 2005) or in excessive levels of PAR (Gao et al., 2012). This experiment did not include variation in UVR, which might impact the western Antarctic Peninsula in near future climate conditions (Thompson et al., 2011).
Corallines comprise a much larger portion of the benthic crustose algal community than fleshy crustose species (personal observation), but their role in the subtidal is still unknown (Schoenrock et al., unpublished-a). It would be hard to characterize what the effect on community dynamics would be if *Hildenbrandia* sp. increased its presence in the subtidal. But *Hildenbrandia* sp. did opportunistically respond to increased $pCO_2$ only in high temperature conditions, while *C. obtectulum* had the opposite response (not significant). In many communities coralline algae are dominant and constant through seasons in the subtidal (Hepburn et al., 2011) and primary reef builders found from the intertidal to 268 m (Littler et al., 1985). Both species likely have CCMs (Giordano et al., 2005) and may not respond readily to increased carbon resources alone, though we did not evaluate CCM activity in this experiment. Many metabolic processes in Antarctic macroalgae operate better at higher than ambient temperatures (Gomez et al., 2009) and this may explain why we do not see significantly negative effects (even a positive trend in only elevated temperature in *C. obtectulum*).

This study was relatively short in duration. Some studies have shown that species have parabolic responses to climate change treatments (Martin and Gattuso, 2009). The initial response period (one month) can be misleading as to the final outcome these conditions would elicit (Martin and Gattuso, 2009). This potentially increases the importance of performing multiple species or even ecosystem wide experiments when examining the biological impacts of climate change (Riebesell et al., 2008) and modeling treatments around environmental complexity (Denman et al., 2011; Dupont and Portner, 2013; Hurd et al., 2009). This study provides initial data on the response of these two organisms to climate change conditions, but it is important to understand that many physiological parameters that were not characterized, as well as community interactions, and those may nullify or amplify our findings. Overall,
*Hildenbrandia* sp. increased growth in environments with high pCO2 and water seawater temperatures while *C. obtectulum* had no significant response. This may confer a competitive advantage to the fleshy crustose species, but it is also important to understand the role of these species within the benthic community before broadly implying negative impacts to community structure on this polar reef.

**ACKNOWLEDGEMENTS**

This work was supported by National Science Foundation awards ANT-1041022 (CDA, RAA, JBM). Field support was provided by the Raytheon Polar Services staff of Palmer Station, especially Neal Scheibe, Steve Sweet, Paul Queior and Lily Glass, and the University of Alabama at Birmingham field team including Maggie Amsler and Nell Herman. We would like to thank Jackie (von Salm) Fries and Ryan Young for editorial review and comments.
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Table 1. Water Chemistry parameters for microcosms calculated using AT and spectrophotometric pH data in CO2calc (means ± SD).

<table>
<thead>
<tr>
<th></th>
<th>in situ seawater</th>
<th>1.5°C</th>
<th>3.5°C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pH 7.8</td>
<td>pH 8.0</td>
<td>pH 7.8</td>
</tr>
<tr>
<td>pH</td>
<td>8.06 ± 0.01</td>
<td>7.81 ± 0.02</td>
<td>8.11 ± 0.01</td>
</tr>
<tr>
<td>TA (μmol/kg SW)</td>
<td>2342 ± 28</td>
<td>2379 ± 6</td>
<td>2358 ± 5</td>
</tr>
<tr>
<td>Temperature (ºC)</td>
<td>0.83 ± 0.11</td>
<td>1.34 ± 0.02</td>
<td>1.36 ± 0.02</td>
</tr>
<tr>
<td>Salinity (ppt)</td>
<td>34.0 ± 0.06</td>
<td>34.2 ± 0.03</td>
<td>34.2 ± 0.02</td>
</tr>
<tr>
<td>pCO2 (μatm)</td>
<td>383 ± 11</td>
<td>944 ± 70</td>
<td>371 ± 13</td>
</tr>
<tr>
<td>Ω&lt;sub&gt;arg&lt;/sub&gt;</td>
<td>1.51 ± 0.02</td>
<td>1.10 ± 0.06</td>
<td>1.82 ± 0.06</td>
</tr>
<tr>
<td>Ω&lt;sub&gt;cal&lt;/sub&gt;</td>
<td>2.41 ± 0.03</td>
<td>1.76 ± 0.10</td>
<td>2.90 ± 0.09</td>
</tr>
</tbody>
</table>
Table 2. Photosynthetic parameters of *C. obtectulum* and *Hildenbrandia* sp. in microcosms.

Values are reported as means ± SE (n = 18).

<table>
<thead>
<tr>
<th>microcosm</th>
<th>species</th>
<th>MQY</th>
<th>E&lt;sub&gt;k&lt;/sub&gt;</th>
<th>α</th>
<th>ETR&lt;sub&gt;max&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH 8.0 * 1.5 ºC</td>
<td><em>C. obtectulum</em></td>
<td>0.677 ± 0.024</td>
<td>114.62 ± 14.595</td>
<td>0.136 ± 0.006</td>
<td>8.323 ± 2.555</td>
</tr>
<tr>
<td></td>
<td><em>Hildenbrandia</em> sp.</td>
<td>0.595 ± 0.062</td>
<td>80.348 ± 10.910</td>
<td>0.102 ± 0.017</td>
<td>3.719 ± 1.228</td>
</tr>
<tr>
<td>pH 7.8 * 1.5 ºC</td>
<td><em>C. obtectulum</em></td>
<td>0.589 ± 0.023</td>
<td>116.324 ± 9.633</td>
<td>0.133 ± 0.009</td>
<td>5.669 ± 0.636</td>
</tr>
<tr>
<td></td>
<td><em>Hildenbrandia</em> sp.</td>
<td>0.636 ± 0.053</td>
<td>63.879 ± 6.780</td>
<td>0.086 ± 0.011</td>
<td>2.328 ± 0.419</td>
</tr>
<tr>
<td>pH 8.0 * 3.5 ºC</td>
<td><em>C. obtectulum</em></td>
<td>0.656 ± 0.016</td>
<td>87.735 ± 5.637</td>
<td>0.133 ± 0.012</td>
<td>4.264 ± 0.401</td>
</tr>
<tr>
<td></td>
<td><em>Hildenbrandia</em> sp.</td>
<td>0.511 ± 0.08</td>
<td>65.912 ± 13.095</td>
<td>0.099 ± 0.030</td>
<td>2.508 ± 0.491</td>
</tr>
<tr>
<td>pH 7.8 * 3.5 ºC</td>
<td><em>C. obtectulum</em></td>
<td>0.658 ± 0.022</td>
<td>86.376 ± 5.814</td>
<td>0.129 ± 0.007</td>
<td>3.944 ± 0.296</td>
</tr>
<tr>
<td></td>
<td><em>Hildenbrandia</em> sp.</td>
<td>0.539 ± 0.084</td>
<td>57.990 ± 10.879</td>
<td>0.076 ± 0.014</td>
<td>2.397 ± 0.453</td>
</tr>
<tr>
<td>time 0</td>
<td><em>C. obtectulum</em></td>
<td>0.735 ± 0.024</td>
<td>104.224 ± 7.832</td>
<td>0.075 ± 0.010</td>
<td>2.681 ± 0.301</td>
</tr>
<tr>
<td></td>
<td><em>Hildenbrandia</em> sp.</td>
<td>0.691 ± 0.024</td>
<td>66.244 ± 14.262</td>
<td>0.104 ± 0.017</td>
<td>2.482 ± 0.391</td>
</tr>
<tr>
<td>in situ</td>
<td><em>C. obtectulum</em></td>
<td>0.52 ± 0.058</td>
<td>93.350 ± 13.620</td>
<td>0.127 ± 0.027</td>
<td>5.743 ± 0.696</td>
</tr>
<tr>
<td></td>
<td><em>Hildenbrandia</em> sp.</td>
<td>0.534 ± 0.04</td>
<td>93.429 ± 13.612</td>
<td>0.138 ± 0.020</td>
<td>4.387 ± 0.181</td>
</tr>
</tbody>
</table>
Table 3. MANOVA table for *C. obtectulum* and *Hildenbrandia* sp.

<table>
<thead>
<tr>
<th>species</th>
<th>parameter</th>
<th>df</th>
<th>$F$ -value</th>
<th>$P$ -value</th>
<th>power</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. obtectulum</em></td>
<td>growth</td>
<td>3</td>
<td>0.518</td>
<td>0.641</td>
<td>0.16</td>
</tr>
<tr>
<td></td>
<td>chl $a$</td>
<td>3</td>
<td>0.476</td>
<td>0.7</td>
<td>0.141</td>
</tr>
<tr>
<td></td>
<td>CaCO$_3$</td>
<td>3</td>
<td>0.664</td>
<td>0.577</td>
<td>0.183</td>
</tr>
<tr>
<td></td>
<td>$\alpha$</td>
<td>3</td>
<td>0.177</td>
<td>0.911</td>
<td>0.081</td>
</tr>
<tr>
<td></td>
<td>$E_k$</td>
<td>3</td>
<td>1.055</td>
<td>0.374</td>
<td>0.274</td>
</tr>
<tr>
<td></td>
<td>MQY</td>
<td>3</td>
<td>0.493</td>
<td>0.688</td>
<td>0.145</td>
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<tr>
<td></td>
<td>ETR$_{\text{max}}$</td>
<td>3</td>
<td>2.001</td>
<td>0.122</td>
<td>0.493</td>
</tr>
<tr>
<td><em>Hildenbrandia</em> sp.</td>
<td>growth</td>
<td>3</td>
<td>2.809</td>
<td>0.046*</td>
<td>0.651</td>
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<tr>
<td></td>
<td>chl $a$</td>
<td>3</td>
<td>0.949</td>
<td>0.422</td>
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<tr>
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<td>$\alpha$</td>
<td>3</td>
<td>0.921</td>
<td>0.436</td>
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<td></td>
<td>$E_k$</td>
<td>3</td>
<td>1.082</td>
<td>0.363</td>
<td>0.28</td>
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<tr>
<td></td>
<td>MQY</td>
<td>3</td>
<td>0.408</td>
<td>0.748</td>
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<tr>
<td></td>
<td>ETR$_{\text{max}}$</td>
<td>3</td>
<td>0.113</td>
<td>0.952</td>
<td>0.069</td>
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</table>

* indicate significant differences ($p \leq 0.05$)

F-values are equivalent to F-test values.
Figure 1. Growth rate data from both *C. obtectulum* and *Hildenbrandia* sp. (means + SE). * indicate significant differences.
Figure 2. CaCO₃ concentration in *C. obtectulum* across microcosm treatments (means + SE).
Figure 3. Chlorophyll $a$ concentration in *C. obtectulum* and *Hildenbrandia* sp. from microcosm experiment and time 0 individuals (means + SE).
DEPTH-RELATED SETTLEMENT PATTERNS OF SUBTIDAL INVERTEBRATE LARVAE
ALONG THE WESTERN ANTARCTIC PENINSULA

by

KATHRYN M. SCHOENROCK, CHARLES D. AMSLER, JAMES B. MCCLINTOCK, AND
BILL J. BAKER

Prepared as a note for Antarctic Science
Format adapted for dissertation
INTRODUCTION

The structure of benthic marine communities relies largely on the demographics, dispersal, recruitment, and further settlement of organism propagules (Mileikovsky 1971, Caley et al. 1996). The near shore environment of the subtidal western Antarctic Peninsula is composed of many diverse benthic communities, typically dominated by macroalgae in the shallow habitats (< 20 to 35 m depth, Wiencke & Amsler 2012) and in deeper waters by dense invertebrate assemblages (Dayton et al. 1994, Arntz et al. 1997). Crustose coralline algae (CCA) are abundant in these habitats from shallow to deep water along the peninsula covering 70-85% of the hard substrate at some times (Amsler et al. 1995, Wiencke & Amsler 2012). On tropical and temperate reefs, CCA are important contributors to reef structure and community dynamics from the intertidal down to 268 m (Littler et al. 1986) and can contribute to the recruitment and subsequent settlement (or morphogenesis) of marine organisms to a reef (Morse & Morse 1984, Heyward & Negri 1999). Many abiotic and biotic factors in a habitat play into this stage in the life histories of marine organisms (Hadfield 1986, Fletcher & Callow 1992, Steinberg et al. 2002). In this study we investigate the influence of potential chemical cues from CCA and depth on the distribution of dominant invertebrate taxa along the western Antarctic Peninsula.

Chemical cues associated with CCA play a large role in establishment of invertebrate assemblages (Morse & Morse 1984, Hadfield 1986, Morse et al. 1994, Morse et al. 1996, Heyward & Negri 1999, Negri et al. 2001, Harrington et al. 2004). It is possible that the responsible molecules are conserved within many genera of CCA or their associated bacteria (Heyward & Negri 1999), including Antarctic CCA species. Both cell wall components of CCA and microbial biofilms on CCA contain morphogens that have been found to induce invertebrate larval settlement (Morse & Morse 1984, Morse et al. 1994, Zimmer-Faust & Tamburri 1994,
Heyward & Negri 1999, Harrington et al. 2004). Few structures of chemical cues are known, but these are often primary metabolites with a water soluble component that functions as the cue (Steinberg et al. 2002). One study identified the molecule likely to prompt morphogenesis in coral larvae; a sulfated lipoglycosaminoglycan with repeated β (1,4)- linked N-acetyl-lactosamine sulfate units and lipid like aliphatic side chains (Morse et al. 1994). An γ–aminobutyric acid (GABA) peptide associated molecule from CCA or associated bacteria targets the morphogenic pathway in red abalone larval causing them to settle (Morse 1990). Chemical extracts targeting these specific molecules and can be used to create larval “flypaper” following the methods of Morse et al. (1994) in order to determine settlement preference of invertebrate larvae to these potential cues. Antarctic invertebrate larvae may have retained or developed a response to these cues akin to those on other reef systems.

The goal of this experiment was to investigate the influence of cues from common CCA species on the settlement of invertebrate larvae in the shallow subtidal along the western Antarctic Peninsula. If a preferential settlement is observed in invertebrate larvae, it would warrant further investigation regarding the nature of this interaction, the role it plays in facilitating recruitment across polar systems, as well as the importance of pre-settlement processes in shaping benthic community assemblages along the western Antarctic Peninsula. Also, the Antarctic Peninsula is experiencing one of the fastest rates of global climate change in the world (Clarke et al. 2007, IPCC 2013). CCA dissolution is amplified in these conditions (McClintock et al. 2009, Diaz-Pulido et al. 2012). Classically, the morphogenesis inducer from CCA is solubilized using the chelators EGTA or EDTA, indicating that it is within calcified cell walls (Morse et al. 1994, Morse & Morse 1996, Heyward & Negri 1999). Likely, interference with CCA physiology or dissolution of the calcified cell wall will alter any observed morphogen-
larval interactions. If a preference in settlement is seen in invertebrate larvae, climate change processes may have cascading effects on pelagic and benthic marine community dynamics.

**METHODS**

*Collections*

Three morphologies of CCA were collected on SCUBA (Schoenrock *et al.* 2012) from the subtidal habitat surrounding Palmer Stations, Antarctica (Figure 1). Thalli were categorized by morphology and individuals were then chipped from their substrate and frozen at -20°C for transport to University of South Florida (USF). Morphologies were identified to species through collaboration with Dr. Braga (Universidad de Granada, Spain) and include *Clathromorphum obtectulum* (Foslie) Adey, both tetrasporophyte and gametophyte, and *Hydrolithon subantarcticum* (Foslie) M.L. Mendoza & Cabioch.

*Extraction methods*

Cell wall molecules were extracted from the CCA species using modified methods from Morse *et al.* (1994). 4 g of each species were ground using mortar and pestle until it is a smooth paste. 10 ml of filtered seawater (FSW; 2µm, Millepore GFC) was mixed with each g of algae paste and then diluted with 40 ml rifampicin treated FSW (RFSW, 2µg/ml) and centrifuged for 5 min (10,000 x g). The resulting supernatant was discarded and this process was repeated twice. The remaining pellet was added to 50 mM EDTA 1L/g algae (pH 8.2, adjusted with NaOH) and stirred for 22 to 24 h to allow decalcification of cell walls. The solution was then readjusted to pH 8.2 (NaOH) when necessary and clarified with glass fiber filters (Millipore GFC, 47mm). This crude extract was adjusted to 2 M NaCl to facilitate adsorption of the morphogen to t-butyl
HIC resin beads (50 µm particle size, BioRad Laboratories Inc.). 2 g of DI water rinsed HIC resin was added to the crude extract and sat on a shaker table for 12 h at 18 °C. Target molecules couple with the inert acrylic beads, which are a dense network of pores and channels designed to maximize internal surface area for adsorption and maximizing chromatographic efficiency. The extract and resin were filtered (47mm GFC filter) and refiltered with filtrate thrice. The resin was rinsed twice with 10 ml 2 M NaCl to rid the beads of chelator, and then blotted dry with Kim Wipes. Beads were then resuspended in TrisCl buffer (1mM, pH 8.2 at 2 ºC adjusted with NaOH, 3ml/L of original clarified solution). This solution was poured into a chromatography column (0.9 cm in diameter) and the column eluted with the same chilled buffer using a low pressure chromatography pump. The first four aliquots from each extraction were frozen at -20 °C for transport back to Palmer Station.

At Palmer Station, resin based larval ‘flypaper’ was reconstituted and painted onto acrylic settlement plates (7 cm x 7 cm) (Morse et al. 1994). The resin beads were resuspended in aliquots of each species, adjusted to 2 M NaCl, and mixed on a shaker table at 1 ºC for 24 h prior to attachment to plates. A multi-purpose sealant (Dow Corning, RTV sealant) was used to attach the rim of a Petri dish (5.7 cm in diameter) to the top of the plate, and t-butyl HIC resin beads were secured inside this enclosure and allowed to set in solution for ~12 h at 2 ºC. During this period resin was rinsed repeatedly with FSW after setting. Only a fraction of the purified molecules should be present on the surface of the plate, however the molecule was likely retained on these plates for some time due to the high salinity naturally found in seawater (35-37 ppt in the Palmer vicinity).
Preliminary settlement plates (without extracts) were deployed at three depths (10 m, 20 m, and 30 m) at Norsel Point, Amsler Island (S 64° 45’37.38” W 64° 05’47.4”) in spring of 2011 and left in situ for one year. This was done to determine whether we could quantify invertebrate settlement in the Palmer area and how long it would take to get significant numbers of different taxa on the plates. A variety of invertebrate and algal taxa were found on these plates, both up- and down facing sides, at 30 m and 20 m depth, but not 10 m.

Two sites were chosen for this experiment; one in the northwestern portion of the study area, east Norsel Point (S 64° 45.677’ W 64° 04.660’), and one in the southern part of the study area, southeast Bonaparte Point (S 64° 46.765’ W 64° 02.554’) (Figure 1). Both sites have dense algal canopies above 30 meters and robust invertebrate assemblages below. Experiment plates (with extracts) were attached to seine-nets using 4” cable ties and nets were weighed to the seafloor with lead dive weights. Each net contained 5 plates from the 3 treatments (extracts from 3 species) and one control group (t-butyl HIC resin beads and FSW). Plates were randomly assigned locations in a 5 x 4 grid on each net to prevent bias in settlement data due to algal cover or sedimentation of portions of the experiment. Because Antarctic CCA are primarily upward facing substrata, settlement plates were only upward facing in this experiment.

Extract plates were left in situ for ~ 1 yr; the 20 m experiment at east Norsel Point for 360 d, the 30 m experiment at east Norsel Point for 368 d, the 20 m experiment at southeast Bonaparte Point for 346 d, and the 30 m experiment at southeast Bonaparte Point for 396 d. The experiments were brought up from depth in a collection bag, and each plate was removed from the seine net and isolated in 16 oz. Qorpack bottle with FSW for transport. Plates were stored at 1 °C under minimum irradiance until they could be processed. The number of invertebrate and
algal recruits (successfully settled individuals) were counted using a dissecting scope with upwelling light. Taxon groupings include encrusting bryozoan, fleshy bryozoan, hydroid, foraminifera, bivalve, coral, spirorbid worms, other calcified worms, mobile invertebrates, anemones, sponge, tunicate, calcified algae, red algal crust, red algal blade, and branching red algae. Algae were included in these count data because they comprised a large portion of settled organisms at both depths. Photos were taken of each phylogenetic grouping and unknown taxa for later identification and plates were dried in an oven at 65 ºC and stored at the University of Alabama at Birmingham (UAB).

Statistics

Invertebrate and algae count data were standardized within a sample to get relative abundance of each taxonomic unit. A bray-curtis dissimilarity matrix was created using Euclidean distance with these data, and an analysis of variance using distance matrices (adonis test) was run to determine which factors (depth, site or CCA) impacted species diversity and density the most (R ver 3.1.0). A SIMPER analysis was run using factors depth and site in PRIMER (Clarke K.R. & Gorley, 2006) to determine which taxa were driving dissimilarity between samples. A significance value ≤ 0.05 was used to determine differences in communities.

RESULTS

Results of this settlement study indicate that dissimilarity between treatments plates after one year was driven by depth, site, and the interaction between the two factors rather than CCA species used in extracts (Table 1). The SIMPER analysis showed that at both deep and shallow sites fleshy bryozoans, encrusting bryozoans, red algae (including CCA, red crusts, red blades
and branching red algae) and spirorbid worms drove most of the variance (average squared distance Deep = 1885.15, Shallow = 2263.78; Table 2). The same trend was seen in the SIMPER analysis run with the site factors (average squared distance North = 1929.79, South = 2436.22; Table 3).

Trends in the colonizing taxa show that red algal recruits were the most dominant on settlement plates at all sites. Patterns between depths include a higher abundance of red algal recruits, sponges, and fleshy bryozoans in deeper sites, and a higher abundance of spirorbid and serpulid polychaetes, mobile invertebrates at shallower depths (Table 2). Percent contribution of each of these taxa to the whole community changed between depths as well; despite differences in abundance red algae have a higher percent contribution to the community at shallow depths. Across sites variation included higher abundance of fleshy bryozoans and spirorbid polychaetes at the northern site, east Norsel Point, and higher abundance of red algae at the southern site, southeast Bonaparte (Table 3). Spirorbid and serpulid polychaetes have higher percent contribution at the northern site while red algae and encrusting bryozoans have higher percent contribution at the southern site (Table 3).

DISCUSSION

The settlement dynamics of Antarctic invertebrate larvae have been studied in the Ross Sea (Dayton 1989), East Antarctica (Stark 2008), Signy Island and Admiralty Bay on the northern Antarctic Peninsula (Stanwell-Smith & Barnes 1997, Freire et al. 2005), and south of the Antarctic circle on the western Antarctic Peninsula (Bowden 2005b, Bowden et al. 2006, Bowden et al. 2009). Recruitment and settlement is slow on these reefs in comparison to temperate and tropical processes (Pearse et al. 1991, Bowden et al. 2006, Koner et al. 2007) and
though the meroplankton assemblage is as diverse in Antarctica as it is on temperate reefs, larvae
are much less abundant (Stanwell-Smith & Barnes 1997, Sewell 2006).

Marine benthic community composition naturally varies with depth along the western
Antarctic Peninsula; algal forests can dominate habitats above 30 m depth (Amsler et al. 1995)
while invertebrate assemblages proliferate below that depth (Dayton et al. 1994, Arntz et al.
1997). Many sessile invertebrates in Antarctica brood their young rather than broadcasting them
(Picken 1980), though the planktotrophic mode of development is found in many ecologically
dominant species (Poulin et al. 2002). Settlement in Antarctic benthic species is seasonal; mobile
invertebrates settle in the summer months (September-December) and sessile invertebrates can
settle throughout the year with peak recruitment and morphogenesis in early winter months
(Freire et al. 2005, Bowden et al. 2009). These patterns are tied to phytoplankton productivity
(e.g. molluscs in late summer-early winter; Frier et al. 2005), and seasonal disturbance in the
subtidal which impact both mobile and sessile life histories (Bowden 2005a, Mincks & Smith
2007). Though the primary influence on abundance and diversity of settling invertebrate larvae
in Antarctica is season (Stanwell-Smith & Barnes 1997), depth and site characteristics also carry
influence on this process (Bowden et al. 2006, Bowden et al. 2009).

Biological elicitors of recruitment and settlement in Antarctic invertebrate larvae have
not been investigated but in the marine environment no surface is free of at least microbial taxa
that larger benthic organisms will interact with. Though colonization rates of substrata along the
western Antarctic Peninsula are one order of magnitude lower than those on neighboring
continents (Stanwell-Smith & Barnes 1997), coverage can reach 6 to 100% on downfacing and
~10% on upfacing surfaces of settlement plates over extended time periods (Bowden et al.
2006), which reflects mean epifaunal coverage of upfacing substrata at 25 m depth (~ 6.5%,
Barnes et al. (1996). Aside from surface cues, factors like grazing, environmental disturbance, light, and physical cues also impact settlement patterns on both upfacing and downfacing substrate orientations. Organisms may preferentially settle in either orientation and factors regulating the assemblages in these orientations vary. Post settlement mortality occurs through interactions with abiotic factors such as ice-scour or biotic factors such as grazing on the upfacing side of substrata, while downfacing surfaces may have little regulation (Bowden et al. 2009). The aim of this study was to determine whether settlement cues from CCA species contribute to invertebrate settlement on upfacing surfaces in the marine benthic community found along the western Antarctic Peninsula.

Because variation in disturbance levels across depths and sites, as well as grazer populations, affect both abundance and diversity of the settling organisms (Bowden et al. 2006, Smale 2008, Stark 2008) multiple sites and depths were used to characterize invertebrate larval affinity to different species of CCA. This design excludes site or depth bias in settlement preference towards one CCA species and SIMPER analyses confirm high values of dissimilarity across depth and site (Table 2, 3). Our results indicate that red algal recruits outnumbered other settlers at all sites and depths, but the dominant invertebrate groups were spirorbid worms and bryozoans. These organisms drove differences across sites and depths, but there was no difference across CCA extract treatments.

On tropical reefs it is known that extracts maintain potency from hours after release to more than 30 days (Morse & Morse 1991). The CCA extracts used in this study may remain on settlement plates longer due to the cold temperatures of the study site, however latency of these extracts was not tested. Post-settlement mortality can obscure recruitment and settlement rates (Hunt & Scheibling 1997), which would be the primary result of a chemical cue from CCA
extracts. Because field experiments using SCUBA are logistically challenging in the Antarctic (Schoenrock et al. 2012) and seasonality of invertebrate recruitment means most successful settlement occurs late summer through winter months (Bowden 2005a, Bowden et al. 2009) settlement plates could not be extracted or photographed throughout the experiment. This means we were unable to monitor growth, seasonality of recruitment and settlement, post-settlement mortality of taxa, and we may have missed an initial settlement preference for CCA extracts early on in the experiment. Also, invertebrate larvae are known to settle in habitats suitable for adult lifestyles (Coon & Bonar 1985) or recruit to cues from adult invertebrates (Green et al. 2002) and this may be a more dominant influence than a cue associated with CCA.

Though no relationship is found between benthic macroalgae and invertebrate larvae, this is the first study of subtidal recruitment dynamics on open substrata in this area of the western Antarctic Peninsula, and as such our results provide a valuable addition to our knowledge of settlement patterns in this region. The dominant invertebrate taxa reported are among the most common colonizers in other studies from the western Antarctic Peninsula, north and south of our study site (Stanwell-Smith & Barnes 1997, Bowden et al. 2006).

ACKNOWLEDGEMENTS
This study was supported by the National Science Foundation award ANT- 0838773 (CDA, JBM) and ANT-0828776 (BJB) from the Antarctic Organisms and Ecosystems program and the Grant in Aid of Research from the Phycological Society of America. This work benefitted from the assistance of Jason Cuce, Julie Schram, Maggie Amsler, and the Raytheon Polar Services/Antarctic Service Contract staff of Palmer Station. We thank Kenan Matterson and Cole Easson for review of text and statistics in this manuscript.
REFERENCES


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Table 1. Results of the adonis test using a Bray-Curtis distance matrix to determine dissimilarity between settlement plates and which factors (depth, site, or CCA) drove observed community differences.

<table>
<thead>
<tr>
<th>Factor</th>
<th>df</th>
<th>Sum of squares</th>
<th>Means squared</th>
<th>F</th>
<th>R²</th>
<th>sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>depth</td>
<td>1</td>
<td>42356</td>
<td>42356</td>
<td>33.781</td>
<td>0.293</td>
<td>0.001</td>
</tr>
<tr>
<td>site</td>
<td>1</td>
<td>24313</td>
<td>24313</td>
<td>19.391</td>
<td>0.1316</td>
<td>0.001</td>
</tr>
<tr>
<td>CCA</td>
<td>4</td>
<td>7085</td>
<td>1771</td>
<td>1.413</td>
<td>0.0384</td>
<td>0.137</td>
</tr>
<tr>
<td>depth * site</td>
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<td>22186</td>
<td>22186</td>
<td>17.694</td>
<td>0.1201</td>
<td>0.001</td>
</tr>
<tr>
<td>depth * CCA</td>
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<td>839</td>
<td>0.669</td>
<td>0.0136</td>
<td>0.776</td>
</tr>
<tr>
<td>site * CCA</td>
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<td>1090</td>
<td>0.869</td>
<td>0.0177</td>
<td>0.587</td>
</tr>
<tr>
<td>depth * site * CCA</td>
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<td>5252</td>
<td>1751</td>
<td>1.396</td>
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<td>0.172</td>
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<tr>
<td>Residuals</td>
<td>62</td>
<td>77739</td>
<td>1254</td>
<td></td>
<td>0.4209</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>78</td>
<td>184720</td>
<td></td>
<td>1</td>
<td></td>
<td></td>
</tr>
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</table>
Table 2. Invertebrate and algal assemblages on recruitment plates left in situ for one year. Average abundance and percent contribution of each taxonomic grouping is represented for plate communities at the two depths (SIMPER analysis).

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Average Abundance</th>
<th>% Contribution</th>
<th>Average Abundance</th>
<th>% Contribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deep</td>
<td></td>
<td></td>
<td>Shallow</td>
<td></td>
</tr>
<tr>
<td>serpulid polchaetes</td>
<td>0</td>
<td>0</td>
<td>4.7</td>
<td>0.19</td>
</tr>
<tr>
<td>mobile invertebrates</td>
<td>0</td>
<td>0</td>
<td>0.00427</td>
<td>0</td>
</tr>
<tr>
<td>bivalve</td>
<td>0.0023</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>hydroid</td>
<td>0.114</td>
<td>0</td>
<td>0.00199</td>
<td>0</td>
</tr>
<tr>
<td>anthozoans</td>
<td>0.00783</td>
<td>0.01</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>cyclostome bryozoans</td>
<td>0.427</td>
<td>0.04</td>
<td>1.38</td>
<td>0.06</td>
</tr>
<tr>
<td>tunicate</td>
<td>0.337</td>
<td>0.08</td>
<td>0.614</td>
<td>0.02</td>
</tr>
<tr>
<td>sponge</td>
<td>1.01</td>
<td>0.21</td>
<td>0.443</td>
<td>0.08</td>
</tr>
<tr>
<td>spirobid polychaetes</td>
<td>5.75</td>
<td>1.66</td>
<td>15.3</td>
<td>11.85</td>
</tr>
<tr>
<td>encrusting bryozoan</td>
<td>9.54</td>
<td>15.82</td>
<td>10.9</td>
<td>13.51</td>
</tr>
<tr>
<td>fleshy bryozoan</td>
<td>26.6</td>
<td>39.14</td>
<td>20.8</td>
<td>24.36</td>
</tr>
<tr>
<td>red algae</td>
<td>56.0549</td>
<td>43.05</td>
<td>49.616</td>
<td>49.91</td>
</tr>
</tbody>
</table>
Table 3. Invertebrate and algal assemblages on recruitment plates left in situ for one year. Average abundance and percent contribution of each taxonomic grouping is represented for plate communities at the two sites (SIMPER analysis).

<table>
<thead>
<tr>
<th>Taxa</th>
<th>North Average Abundance</th>
<th>% Contribution</th>
<th>South Average Abundance</th>
<th>% Contribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>serpulid polchaetes</td>
<td>0.445</td>
<td>1.53</td>
<td>0.135</td>
<td>0.03</td>
</tr>
<tr>
<td>mobile invertebrates</td>
<td>0</td>
<td>0</td>
<td>0.00386</td>
<td>0</td>
</tr>
<tr>
<td>bivalve</td>
<td>0.00171</td>
<td>0</td>
<td>0.00205</td>
<td>0</td>
</tr>
<tr>
<td>hydroid</td>
<td>0.00355</td>
<td>0</td>
<td>0.103</td>
<td>0</td>
</tr>
<tr>
<td>anthozoans</td>
<td>0</td>
<td>0</td>
<td>0.00698</td>
<td>0</td>
</tr>
<tr>
<td>cyclostome bryozoans</td>
<td>0.385</td>
<td>0.03</td>
<td>0.419</td>
<td>0.03</td>
</tr>
<tr>
<td>tunicate</td>
<td>0.00831</td>
<td>0</td>
<td>0.32</td>
<td>0.05</td>
</tr>
<tr>
<td>sponge</td>
<td>0.744</td>
<td>0.16</td>
<td>0.578</td>
<td>0.06</td>
</tr>
<tr>
<td>spirorbid polychaetes</td>
<td>11.6</td>
<td>11.89</td>
<td>5.62</td>
<td>0.93</td>
</tr>
<tr>
<td>encrusting bryozoan</td>
<td>24.7</td>
<td>19.89</td>
<td>23.6</td>
<td>28.47</td>
</tr>
<tr>
<td>fleshy bryozoan</td>
<td>21.3</td>
<td>27.33</td>
<td>16</td>
<td>27.15</td>
</tr>
<tr>
<td>red algae</td>
<td>40.77</td>
<td>38.55</td>
<td>53.208</td>
<td>43.27</td>
</tr>
</tbody>
</table>
Figure 1. The archipelago surrounding Palmer Station, Antarctica, including North and South study sites. Inset shows location of Palmer Station on the Antarctic continent.
CONCLUSION

Antarctica is a characteristically harsh environment for the phycobenthos: low light levels, extreme physical conditions (Wiencke et al., 2007), and large mesograzer populations (Amsler et al., 2008) make it so. In chapters 1 and 2 I examined the response of Antarctic macroalgae to potential biotic stressors endophytes. In chapters 3 and 4 I examined the response of Antarctic macroalgae to potential environmental stressors produced by climate change. I also investigated the potential the role of calcified macroalgae in invertebrate settlement dynamics on this polar reef in chapter 5, which could have potentially been altered by findings in chapter 4. The findings from this dissertation should allow us to formulate broader hypotheses about ecological interactions, even in light of global climate change.

Endophyte presence

The presence of a large mesograzer cohort is thought to drive high levels of endophytic algae along the western Antarctic Peninsula (Peters, 2003, Amsler et al., 2014). From an evolutionary perspective the persistence of filamentous algal endophytes in Antarctic macroalgae may also be credited to their benign impacts on many algal hosts, shown in most species in this study. Overall, Antarctic endophytes should be classified as innocuous or harmful depending on their host alga. In chapter 1 endophytes decreased the toughness of Pachymenia sp., specifically decreased the force required to puncture the thallus area heavily infected with endophytes. In I. cordata, endophytes are known to be pathogenic (Schoenrock et al., 2013, unpublished) and I found that areas adjacent to endophyte infection on a hosts thallus have lowered photosynthetic efficiency. Therefore, the presence of endophytes is important to this species’ ecology.
In chapter 2 I found that populations of *I. cordata* around Palmer Station are haploid dominated; fertilized gametophytes with carposporangia and sterile gametophytes (presumably both male and female) are more abundant than tetrasporophytes. We found that variation in endophyte presence did not impact fertility in any stage, but there was a lower incidence of endophytes in the carposporophytes when compared with unfertilized gametophytes and tetrasporophytes. This could be ecologically relevant as they represent individuals who amplify the products of sexual recombination (Searles, 1980).

Future work should focus on nutritional aspects of the endophytes (e.g. carbon sequestration), high resolution information on biomechanical properties of the algae, and microscale characterization of photosynthetic properties and palatability of the algae. Weakness in host physiology caused by endophyte presence, or pathogenicity, could reduce growth and survival of ‘infected’ individuals in a community, effectively reducing the fitness of that species. Loss of these individuals would include any symbionts, potentially reducing fitness of the filamentous endophytes as well. Studies should also examine the effect of endophyte presence on reproductive potential and gamete dispersal in these algae. These two studies provide a basis for future investigations into the innocuous, sometimes pathogenic, relationships between many Antarctic macroalgae and their endophytes.

*Climate Change*

While the results of the two experiments indicate mostly benign and some positive impacts of low pH and high temperature on canopy forming and crustose species, future experiments should focus on broad scale community responses to confirm null effects of physiological shifts that were not evaluated. These experiments looked at the response of species
in individual treatments but it would be relevant to investigate the effects the minor shifts in algal physiology have on the palatability of these dominant species, their toughness, and other ecologically relevant traits. We found that *D. menziesii*, the shallower of the two canopy species, increased E_k and phlorotannin content in low pH and high temperature conditions, while *D. anceps* had no significant response. *Hildenbrandia* sp. increased growth in environments with high pCO2 and water seawater temperatures while *C. obtectulum* had no significant response. This may confer a competitive advantage to the fleshy crustose species, but it is also important to understand the role of these species within the benthic community before broadly implying negative impacts to community structure on this polar reef.

Of particular interest are the phlorotannins; phlorotannin content in *D. menziesii* may have increased as a response to basic experiment conditions and in low pH treatments. This increase could have been a response to the increased carbon resource or perhaps as a response to the acidity of the environment. Aside from a persistent debate over the specific functions of phlorotannins, the general consensus is that they may be important to the functional and chemical ecology of algae (Amsler & Fairhead, 2006). Both species are strongly chemically defended against herbivory (Amsler et al., 2005), primarily through lipophyllic compounds including menzoquinone in *D. menziesii* and hydrophyllic compounds in *D. anceps* (Ankisetty et al., 2004, Amsler et al., 2005), and anti-herbivory compounds include phlorotannins (Fairhead et al., 2006). Phlorotannins are a diverse group of compounds generally found in the 10-100 kDa range, but compounds from 126 Da to 650 kDa are known (Boettcher & Targett, 1993, McClintock & Baker, 2001). Further investigation of phlorotannin compound diversity would highlight how climate conditions affect the ecology of these species through specific phlorotannins produced in each treatment.
The two climate change studies were relatively short in duration. Some studies have shown that species have parabolic responses to climate change treatments (Martin & Gattuso, 2009). The initial response period (one month) can be misleading as to the final outcome these conditions would elicit (Martin & Gattuso, 2009). Along with specific impacts on individual species, there is a potential for effects to cascade within communities, shifting ecological interactions (Diaz-Pulido et al., 2011, Hofmann et al., 2012) and complexity (Kroeker et al., 2011, Kroeker et al., 2013), which could amplify the effects of ocean acidification. This increases the importance of doing multiple species or even ecosystem wide experiments when examining the biological impacts of climate change (Riebesell et al., 2008) and modeling treatments around environmental complexity (Hurd et al., 2009, Denman et al., 2011) as well as looking for indirect effects within communities (Alsterberg et al., 2013).

**Invertebrate settlement**

Invertebrate recruitment and settlement on artificial substrates laced with CCA extracts was assessed. The aim of this study was to determine whether CCA species attract invertebrate settlement in the subtidal along the western Antarctic Peninsula. Our results indicate red algal recruits are by large the most abundant settlers at two depths across sites, but spirorbid worms and bryozoans dominate the invertebrate taxa that settle on upward facing plates. The abundance of these organisms drives differences in settlement across sites and depths, but there was no difference across CCA extract treatments. A more direct approach of investigating the impact of CCA on invertebrate larvae would be to isolate the molecule commonly associated with CCA or their bacterial community to determine if it is indeed a vestigial characteristic. From that point, CCA extracts could be further tested with meroplankton from the Palmer area to examine
recruitment and morphogenesis in the presence of the cue. The results of this study indicate that
invertebrate larvae have no specific affinity for CCA species, but the restrictions of this
experiment may have obscured settling preferences over the year plates spent in the field.
Despite this, this is the first study of subtidal recruitment dynamics on open substrata in this area
of the western Antarctic Peninsula, and as such our results provide a valuable addition to our
knowledge of settlement patterns in this region.
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APPENDIX A

IDENTIFICATION OF ANTARCTIC CRUSTOSE CORALLINE ALGAE
Algae are abundant along the western Antarctic Peninsula, biomass sometimes reaching levels comparable to temperate kelp forests (Amsler et al. 2008). Today approximately 124 species have been recorded on the continent (Wiencke et al. 2014) however the majority of the continent is under sampled. Endemism is prominent among Antarctic algae; 44% of Phaeophyceae, 36% of Rhodophyceae and 18% of Chlorophyceae (Wiencke & Amsler 2012). New taxa are continually being identified (e.g. Hommersand et al. 2009, 2011) but many individuals are similar morphologically and hard to distinguish using common microscopy methods. Crustose coralline algae (CCA) are particularly hard to identify (Wiencke & Clayton 2002) in Antarctica. These algae can cover up to 77% of the benthos in some areas (Amsler et al. 1995), including the upper 5m where most algae are absent due to ice scour. In many marine habitats, CCA play important roles in shaping communities by inducing invertebrate recruitment to reefs (Heyward & Negri 1999, Morse et al. 1996, 1994) as well as providing habitat structure (Chenelot et al. 2011, Foster 2011, Steller et al. 2003). They also contribute geologically (Goreau 1963) by contributing largely to the inorganic carbon cycle in the ocean (Adey & Macintyre 1973). Therefore the role CCA can play in subtidal communities highlights the importance of identifying communities to species.

CCA species from the western Antarctic Peninsula have been described in Wiencke & Clayton (2002) and Hommersand et al. (2009, 2011), however vegetative and reproductive material need to be used in morphological identification as there are very few species which can be identified based on external morphology alone (Bailey & Chapman 1998). For this reason we employed scanning electron microscopy (SEM) is informative to the morphological structure of
these calcified species, but morphology may not definitive in defining CCA species (Gabrielson, personal communication). An alternative to this is to distinguishing the species is using molecular identification. Using both molecular identification and morphological identification with SEM we should be able to ground truth identification of these morphotypes.

The three morphologies of CCA examined here are common on small rocks located in the shallow subtidal throughout the archipelago surrounding Palmer Station. These rocks were collected on SCUBA and brought back to Palmer Station to be processed. Morphotypes were identified by using a dissecting microscope (Zeiss) and then the samples was preserved in two ways. Half of the thallus was chipped from the rocks using a hammer and chisel, then cleaned with Kimwipes, and placed in silica gel in an air tight bag. The other half of the thallus was dried in an oven at 65 °C for ~48 hours, and preserved in an air tight bag (on its original substrate). SEM samples were shipped to Spain for morphological identification in collaboration with Dr. Braga at the University of Granada, Spain. Dr Braga provided identification using thin sections and morphological key of Wiencke & Clayton (2002) and Hommersand et al. (2011) (Figure 1, Table 1). Morphology 1 (samples 1-11) and morphology 2 (samples 12-21) both keyed out as the tetrasporophyte and gametophyte/carposporophyte of Clathromorphum obtectulum. Morphology 4 keyed out as Hydrolithon subantarcticum.
Figure 1. SEM photos from coralline identification by Dr. Braga.
Table 1. Character checklist for species identification of coralline morphologies. In

*Clathromorphum obtectulum* tetrasporangia and gametangia are in separate thalli.

DNA extractions were done in the lab of Dr. Bailey at the University of North Carolina at Wilmington. Samples were described under a dissecting scope and then ground in a mortar and pestle and placed in 1.5 mL Eppendorf tube with 3x extraction buffer. Eight μL beta-mercaptoethanol was added to each tube and samples were incubated at 60 °C for one hour. DNA was then extracted twice using and equal volume of phenyl: chlorophorm: iso-amyl alcohol. One volume was added and inverted for 30 s, samples were spun for 3 min at 14k rpm, and the aqueous layer was removed and re-extracted. After second extraction, the aqueous layer was transferred to a new tube and 0.8 volumes of ice cold ethanol with 0.1 volumes 4M NaCl was added to the mix, then stored at -20 °C for one hour. Samples were spun down for one hour at 14k rpm and then dried. Following, samples were resuspended in 200 uL of DI water and then cleaned with GeneClean kit (MP Biomedical) following manufacturers protocols. ‘Clean’ DNA was then stored in 40 uL DI water for PCR.
PCR was run with primers G1, G7, G6, and G8 from Saunders and Kraft (1994) which target the 18s smRSU gene. Master mix included 1 μL of each primer, 20 μL 5x PCR buffer, 2 μL dNTPS, 0.5 μL taq polymerase, and 73.5 μL H2O and 2 μL DNA. The thermocycling profile included a 4 min denaturation step of 94 ºC followed by 35 cycles of 30 sec at 94 ºC, primer annealing for 30 sec at 50 ºC, and extension for 90 sec at 72 ºC and a final extension time of 7 min at 72 ºC. Amplification products were sequenced at the Heflin Center for Genomic Sciences at UAB and edited and in CodonCode Aligner (LiCor Inc.). Sequences were aligned with published sequences obtained from GenBank in Mega 6.06 and phylogenetic relationships among corallines were inferred using maximum likelihood criteria with a Kimura-2 parameter model with Gamma distributed rates among sites and 500 bootstrap replicates. Published sequences from NCBI GenBank with 95% similarity to our sample sequences were included in this analysis (Table 2).

The maximum likelihood tree showed that the Antarctic coralline morphologies grouped away from coralline algae sequences from neighboring continents, and sequences found through BLAST searched on GenBank. Some samples did not amplify as well as others and the *H. subantarcticum* morphology is underrepresented in our data set. It is however distinct from *C. obtectulum* sequences which is a good indication they are different species (Figure 2). There are no GenBank accession numbers for the species in question. In the future other genes including, psbA, rbcL and LSU will be used to reconstruct these phylogenies.
<table>
<thead>
<tr>
<th>Taxon</th>
<th>Source</th>
<th>Number of bases determined</th>
<th>Accession number</th>
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</table>

Table 2. GenBank Accession numbers for 18S rRNA, or SSU gene.
Figure 2. Maximum likelihood tree generated from sample sequences and published sequences from GenBank, rooted with *Rhodogorgon carriebowensis*. Morphological identification is labeled next to sample numbers.
APPENDIX B

IDENTITY OF GREEN FILAMENTOUS ALGAL ENDOPHYTES FROM THE WESTERN ANTARCTIC PENINSULA
Endophytes of four host macroalgal were targeted in particular; Gymnogongrus turquetii, Myriogramme manginii, Iridaea cordata, and Trematocarpus antarcticus. In experiments and in culture brown endophyte species grow erect filaments in the absence of herbivores (Amsler et al. 2009, Aumack et al. 2011). However, most green endophytes do not grow out of the host thallus, even though they account for a majority of the endophyte cover in many red algal hosts (Amsler et al. 2009). Two green endophyte morphologies have been identified and sequenced; both are branched and genetically unique, however comparison with known sequences was unsuccessful in determining species identification (Lopez-Bautista, personal communication). Recent attempts were successful in amplifying green endophyte DNA using primers for the rbcL gene of the Ulvophyceae (Lopez-Bautista, personal communication). Identifying species that settle and are successful at proliferating within rhodophyte hosts may help to formulate future hypotheses and describe pathogenic relationships (Schoenrock et al., 2013, unpublished).

Target species, particularly G. turquetii, M. manginii, T. antarcticus, and I. cordata, were collected from the subtidal environment around Palmer Station on SCUBA. Sections of thallus material with large amounts of endophyte/s were cut from the thalli and preserved in silica gel. Surfaces of sections from the same thalli were cleaned with Kimwipes and 1% betadine solution. These sections were then cultured in 1% bacterioagar-PES media (DIFCO bacterioagar) at 1 °C on a 12:12 h day:night cycle with ~ 20 μmol photon m\(^{-2}\) s\(^{-1}\) irradiance. Cultures were cleaned regularly to reduce presence of brown algal endophytes and fungal colonies. All samples were transported back to UAB where DNA extractions were done and cultures remain at 1 °C on a
12:12 h day:night cycle. Two morphologies came out in cultures; a flat branching crust (morphology 1) and a uniseriate morphology with whorled branching pattern (morphology 2).

DNA extractions: fresh thallus material and desiccated material was ground under liquid nitrogen in 1.5 ml Eppendorf tubes with a pestle and sterile sand. DNA was extracted from ground material using DNAeasy Plant Mini Kit (Quiagen, Crawley, UK) following the manufacturers protocol. DNA quality will be checked by running gel electrophoresis in agarose gel with ethidium bromide. PCR amplification of the green endophytes was done with tufAF and tufAR primers from Fama et al. (2002). Amplification products were verified using gel electrophoresis and then sequenced at the Heflin Center for Genomic Sciences at UAB. Sequences were edited in CodonCode Aligner (LiCor Inc.) and aligned with published sequences obtained from GenBank in MEGA 6.06 (Table 1). Few PCR products resulted in high quality sequences, therefore 3 samples were included in the following analyses; sample 2 (morphotype 2) and sample 8 and 13 (morphotype 1). Phylogenetic hypotheses of relatedness were inferred using maximum likelihood criteria, with Tamura (1992) model and Gamma distributed rate of base substitution, and 50 bootstrap replicates (Figure 1).

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Source</th>
<th>Number of bases determined</th>
<th>Accession number</th>
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Table 1. GenBank accession numbers for the tufA gene.
Figure 1. Maximum likelihood tree generated from sample sequences and published sequences from GenBank, rooted with *Ochlochaete hystrix*.

The tufA sequence obtained from sample 2 may be from a common contaminant, *Urospora* sp.. In the future, cleaner cultures should be used for DNA extractions in order to avoid possible misidentification. Sequences from samples 8 and 15 represent species closely related to the *Acrosiphonia* sp. which is in the order Ulotrichales. This is a different order from many green algal endophytes that were previously identified using the tufA marker (Neilsen et al. 2013) which were in the order Ulvales. Hopefully future attempts at extraction and morphological description will provide better resolution in endophyte identification.