Climate change confers a potential advantage to fleshy Antarctic crustose macroalgae over calcified species

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ABSTRACT

Climate change impacts were investigated in two species of crustose Antarctic macroalgae **that may be natural competitors in their habitat**. The seawater parameters oceanic pH and temperature were modified to near future projections for the western Antarctic Peninsula in microcosm experiments. Experiments included two crustose algae, the calcified coralline alga *Clathromorphum obtectulum* and the fleshy encrusting rhodophyte *Hildenbrandia* sp., and were run for six weeks. Treatments reflected near future ocean conditions under climate change predictions: increased temperature (3.5°C x pH 8.1), increased $p\text{CO}_2$ (1.5°C x pH 7.8), combined factors (3.5 °C x pH 7.8), and ambient conditions (1.5°C x pH 8.1). The physiological responses of the algae were evaluated through photosynthetic parameters (slope to saturation of photo centers (α), saturating irradiance (E_k), maximum electron transport rate (ETR_{max}), maximum quantum yield of photosystem II (F_v/F_m)), growth, chlorophyll *a* concentration, and for *C. obtectulum* calcium carbonate content and Mg/Ca ratio. No negative impacts of elevated temperature or increased $p\text{CO}_2$ were observed in either species. The fleshy alga decreased in size in low pH and high temperature treatments alone, but increased growth significantly when these factors were combined. Photosynthetic parameters were depressed by increased temperature in the calcified species and pH in the fleshy species but no significant differences were observed in other parameters in either species. This indicates that *Hildenbrandia* sp. may have a competitive advantage for space in the subtidal environment in near future oceanic conditions. However because benthic ecology in this geographic region is not well understood it is uncertain how these results will ultimately impact the community.

Keywords: Coralline algae, Antarctica, ocean acidification, warming, *Hildenbrandia* sp., climate change

Abbreviations: UVR: ultraviolet radiation, CO₂: carbon dioxide, CH₄: methane, N₂O: nitrous oxide, NOAA: National Oceanic and Atmospheric Agency, IPCC: International Panel on Climate Change, pH: logarithmic scale of hydrogen ions, *p*CO₂: partial pressure of carbon dioxide, C: Celsius, Mg: magnesium ion, Ca: calcium ion, PAR: photosynthetically active radiation, SEM: scanning electron microscope.

1. Introduction

In an era where anthropogenic activities have started to shape global climate, it has become pertinent to investigate the response of natural communities to these conditions. Greenhouse gases (CO₂, CH₄ and N₂O) are at their highest recorded levels in our atmosphere's recent history (IPCC, 2013; Tans and Keeling, 2013) and drive a multitude of shifts in the environment. For marine communities these include (but are not limited to) increases in temperature, ocean acidification, changes in sea ice duration and extent, and increased UVR (IPCC, 2013). This past decade has been the warmest on record (NOAA, 2014) and the oceans worldwide have increased by an average of 0.11 ± 0.2 °C every decade between 1971 and 2010 (IPCC, 2013). Ocean temperatures are projected to continue increasing by approximately 0.6-2.0 °C by the end of the century with warming of Southern Ocean deep water (> 1000m) predicted to be the most pronounced. This trend is alarming because ocean warming can severely impact species diversity in many ecosystems (Hillebrand et al., 2012), and may have more impact when coupled with other stressors.

Among greenhouse gases, CO₂ is of specific concern because it contributes not only to global warming but also to increases in ocean acidity. Increased concentration of CO₂ (*p*CO₂) translates

to an increased draw down of CO₂ into the oceans, which are the largest carbon sink on the planet (Sabine et al., 2004). This results in decreased ocean pH (Caldeira and Wickett, 2005; Doney et al., 2009; Guinotte and Fabry, 2008; Sabine et al., 2004; Takahashi et al., 1997), which is predicted to decline from a present day global average of 8.1 to 7.7 by the year 2100 (IPCC RCP8.5, 2013). In turn the increased concentration of hydrogen ions will shift saturation states of carbonate ions in seawater (Fabry et al., 2008; Feely et al., 2004; Orr et al., 2005) which are significant to organisms that utilize calcium carbonate (CaCO₃) polymorphs (aragonite, calcite, and **high** magnesium calcite) to strengthen their shells and cell walls. Aragonite and magnesium calcite (MgCO₃) are **over** 50% more soluble **than calcite** in seawater (Feely et al., 2004), and the **saturation state of these ions directly relates to the calcification rates of organisms (Gattuso et al., 1999). Shifts in environmental variables have a direct effect on the molar percentage of MgCO₃ in skeletons (Chave, 1954), and this will be significant in polar seas that have low saturation states of mg-calcite and aragonite already (Andersson et al., 2008).**

Polar environments including the Antarctic Peninsula are experiencing the fastest rates of climate change (Clarke et al., 2007; Ducklow et al., 2013; Fabry et al., 2009; Meredith and King, 2005; Schloss et al., 2012). These changes are specifically threatening to organisms inhabiting the Southern Ocean because they experience consistent environmental conditions (including the consistency of large disturbance events) and many organisms are stenothermal (Peck, 2005; Wiencke et al., 2007). Ocean temperatures range between -1.8 to 2.5 °C on the western Antarctic Peninsula (Drew and Hastings, 1992; Schram et al., in press) and pH can range from 8.0 to 8.62 units throughout the year in the Palmer archipelago off the mid-western Peninsula (Schram et al., in press), getting as low as 7.932 units in western Antarctic surface waters (Bjork et al., 2013). In

the last half century the oceans along the western Antarctic Peninsula have increased an average of 1 °C (Meredith and King, 2005; Schloss et al., 2012), while air temperature has increased ~ 0.25 °C every decade since the 1950s (Steig et al., 2009; Vaughan et al., 2003).

It is well understood that marine organisms have varied responses to climate change (Kroeker et al., 2011; Ries et al., 2009), including many marine algae (Harley et al., 2012; Koch et al., 2013; Roleda and Hurd, 2012). Climate change will likely obscure a range of cellular to behavioral processes in many marine organisms (Dixon et al., 2010; Elettra et al., 2014; Fabry et al., 2008; Hu et al., 2014; Kelley and Hofmann, 2013). There is a significant difference between the response of calcifiers like coralline algae and non-calcifiers like ‘fleshy’ macroalgae, the former being most prone to negative effects of ocean acidification (Harley et al., 2012). However the ability of species to persevere is relative to the resources in a habitat (Falkenberg et al., 2013), and the rate and severity of environmental change (Connell et al., 2013; Kamenos et al., 2013). Intuitively, marine autotrophs should benefit from these conditions (increased $p\text{CO}_2$; Koch et al., 2013) and fleshy macroalgae often do, especially species which do not use carbon concentrating mechanisms (CCMs) to acquire dissolved inorganic carbon (DIC; Hepburn et al., 2011), though there are exceptions (*Ulva* spp.; Rautenberger et al. 2015a).

Prominent brown macroalgal species are the non-calcified species most often studied in the context of climate change, while studies focused on non-calcifying reds and greens are fewer. Along the western Antarctic Peninsula two canopy forming species, *Desmarestia anceps* Montagne and *Desmarestia menziesii* J. Agardh, were shown to have mild responses to increases pH and temperature (Schoenrock et al., 2015a). Only saturating irradiance (E_k) and phlorotannin

content in *D. menziesii* increased significantly in combined and low pH treatments respectively (Schoenrock, et al., 2015a). In another study on Antarctic macroalgae, Rautenberger et al. (2015b) found that seven Antarctic species exposed to short term UVR and temperature stress were tolerant to these stressors if they occur simultaneously (damage repair of the photosynthetic apparatus was stimulated at 7 °C not 2 °C, though phlorotannin production had no consistent response). A neutral or generally positive response to climate change is seen in most fleshy species (Harley et al., 2012; Koch et al., 2013), even those that are moderately calcified. For instance, *Padina* sp. decreased calcification but increased growth in low pH conditions which allowed individuals to escape herbivory in the same treatment (Gil-Diaz et al., 2014; Johnson et al., 2012).

In the non-calcified red macroalga *Neosiphonia harveyi* (Bailey) M.-S. Kim, H.-G. Choi, Guiry & G.W. Saunders, lowered temperature reduces photosynthetic effort but this depression is alleviated by increased $p\text{CO}_2$ (Olischläger and Wiencke, 2013). Sub-canopy rhodophytes from the East Pacific Ocean respond and acclimate to variations in light level but overall photosynthetic effort is enhanced under high $p\text{CO}_2$ conditions (Pesce, 2012). Also, future climate conditions are predicted to increase biomass of a commercially important crop, *Gracilariopsis lemaneiformis* (Bory de Saint-Vincent) E.Y. Dawson, Acelto & Foldvik (Zou and Gao, 2014). The primary concern is the positive response of fleshy species will confer a competitive advantage to these species over calcified species. In field and lab experiments calcifying macroalgae often exhibit negative responses, becoming less abundant than fleshy macroalgae at low pH (Baggini et al., 2014; Hofmann et al., 2012), which thrive well in these conditions (turf algae; Falkenberg et al. 2013, Bender et al., 2014; Short et al., 2014).

Coralline algae **often** respond to high $p\text{CO}_2$ environments with decreased calcification or a net dissolution of thalli, decrease in structural integrity, and even mortality (Roleda et al., 2012; Hofmann and Bischof, 2014; McCoy and Kamenos, 2015). At the community level high $p\text{CO}_2$ 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). When high $p\text{CO}_2$ is coupled with other climate change factors the **species** response **may** change, and species do have varied responses (McCoy and Pfister, 2014; Ordonez et al., 2014). **Though we emphasize that negative responses are often measured in corallines, they are not ubiquitous.** Calcification of **corallines can** increase when conditions combine increased temperature and $p\text{CO}_2$ (Koch et al., 2013; Martin and Gattuso, 2009), though temperature and light have independent control on the growth of coralline algae (McCoy and Kamenos, 2015 and citations within). **Considering the large research effort in this area, few studies focus on high latitude corallines which are important ecosystem engineers in the Arctic (Adey et al., 2013; Teichert and Freiwald, 2014) and susceptible to range limitations in future ocean conditions (Brodie et al., 2014).**

The western Antarctic Peninsula is home to large macroalgal forests, composed largely of *Desmarestia* spp. and *Himantothallus grandifolius* (A. Gepp & E.S. Gepp) Zinova, which dominate the benthos in many areas (Wiencke and Amsler, 2012). Crustose algae are also abundant in this subtidal habitat and can reach 77% cover in habitats above 30 m depth (Amsler et al., 1995). Additionally, there are two taxa of fleshy crustose algae (Rhodophyta) that co-occur with corallines in lower abundance, *Hildenbrandia* sp. Hariot and *Gainia mollis* R.L. Moe

(Hommersand et al., 2011). Because there is very little known about Antarctic Peninsular benthic communities (but see Dayton et al. 1974 for the Ross Sea) in comparison to tropical, temperate and even Arctic reefs, it is hard to formulate predictions on how a species response to climate change will impact the community at large. 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). The primary aim of the present study was to evaluate the response of two common crustose macroalgae species from the western Antarctic Peninsula to increased temperature and decreased pH. Physiological responses to the climate variables were measured in the coralline algae *Clathromorphum obtectulum* (Foslie) Adey and the fleshy crustose algae *Hildenbrandia* sp. Responses of the algae were measured through CaCO_3 content and composition (Mg/Ca), growth, chlorophyll *a* content (chl *a*), and the photosynthetic characteristics of photosystem II (PSII): saturating irradiance (E_k), maximum quantum yield (F_v/F_m , MQY), slope to saturation of PSII (α), and maximum electron transport rate between PSII and photosystem I (PSI; ETR_{max}). The results of the present study will provide baseline information for future investigations into the complexity of community interactions involving crustose Antarctic rhodophytes.

2. Methods

2.1 Collections

This research was conducted at the United States Antarctic Program's Palmer Station, on Anvers Island on the western Antarctic Peninsula (for map see Schoenrock et al., 2015b). Both target species were collected in **March 2012, near the end of the Austral summer**, using SCUBA within a 3.5 km radius of Palmer Station. *Hildenbrandia* sp. were collected from ~15m at Hermit Island

(S 64° 45.620' W 64° 05.883) on comparable small granite rocks that could be used as anchors in the experiment. *Clathromorphum obtectulum* were collected from ~5 m at Kristie Cove (S 64° 46.697 W 64° 02.954') on comparable small granite rocks. Irradiance and other characteristics of these habitats are comparable (light intensity ~ 16-27 $\mu\text{mol photon m}^{-2} \text{ s}^{-1}$).

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 (Universidad de Granada, Spain) using scanning electron microscopy (SEM) and relevant taxonomic literature (Hommersand et al., 2011; Wiencke and Clayton, 2002). The morphotype used in this experiment was the gametophyte of *C. obtectulum*.

The collected individuals were on small rocks no more than 5 cm³. Additional fouling organisms were cleaned off of each rock using a wire brush and soldering iron before the experiment began and time 0 measurements were taken. All individuals were kept in flow through aquaria under irradiance levels of 20 to 30 $\mu\text{mol photon m}^{-2} \text{ s}^{-1}$ on a 12 : 12 h day night cycle which these individuals experience during the Austral summer-autumn. Algae were kept in these conditions approximately 2 weeks until the experiment began.

2.2 Microcosm 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, *Nacella concinna* and *Margarella antarctica*, which are described in another study (Schram, et al., 2014). 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 in the microcosm that reflect the natural environment (~ 16 to $22 \mu\text{mol photon m}^{-2} \text{s}^{-1}$). Treatments reflected current conditions and near-term predictions for 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°C), high temperature (pH 8.0 and 3.5°C), and low pH and high temperature (pH 7.8 and 3.5°C).

Temperature was maintained in microcosms by holding them in water tables ($114.3 \times 58.42 \times 15.24$ cm) that were connected to a circulating water bath (VWR Scientific 1186D Refrigerating Circulator, able to maintain temperatures $\pm 0.01^\circ\text{C}$) that recirculated a 30% glycol mixture at either 1.5°C or 3.5°C . Four water baths were set up on shelving units with Sylviana Cool White fluorescent bulbs (F48T12/CW/HO, 60W) which provided 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. Algae were exposed to experimental conditions for 47 days.

2.3 Water Chemistry

Target pH was maintained in reservoirs using an AT control system (Aqua Medic, USA) which monitors real time pH in target reservoirs and delivered CO_2 gas as necessary to a given reservoir to increase $p\text{CO}_2$ (decrease pH). Control reservoirs had a constant stream of air bubbling into them to maintain water movement.

230 Seawater pH and temperature were monitored daily in microcosms using an Orion pH_{NBS} probe
231 and Cole-Parmer Digi-Sense ThermoLogR Thermister. Total alkalinity (TA) and
232 spectrophotometric pH (pH_T) were measured twice a week in each microcosm and once daily in
233 reservoirs and ambient seawater (Table 1). Seawater samples were siphoned from their
234 microcosms into 300 ml borosilicate bottles and allowed to overflow one full volume, then
235 sealed with greased ground glass stoppers in order to limit sample contact with air (Dickson et
236 al., 2007). After sampling, water was replaced with pretreated water from reservoirs and
237 sampling bottles were stored in a cold-dark area until they could be processed (within 4 h).
238
239 Spectrophotometric pH was measured following methods in SOP 6b (Dickson et al., 2007). A
240 UV/Vis Spectrometer LAMBA 40 (Perkin Elmer), equipped with jacketed cells plumbed to a
241 water bath held at 25 °C, was used for pH_T measurements. Seawater TA was titrated using a T50
242 Titrator (Mettler Toledo, Switzerland), a jacketed 500 ml beaker plumbed to a water bath at 25
243 °C, and DGi113-SC pH probe (Mettler Toledo) following methods in SOP 3a (Dickson, et al.,
244 2007). Salinity was measured in each sample using an YSI Conductivity Instrument (Model
245 3200) and conductivity cell (YSI 3253 model B). Certified reference materials (CRMs) provided
246 by A. Dickson (Scripps, UCSD) were used to calibrate pH and TA. User error or equipment error
247 margins were 4.02 µmol kg⁻¹ seawater for TA, and 0.03 pH units. All TA, pH, temperature, and
248 salinity measurements were used to calculate carbonate chemistry parameters including *p*CO₂,
249 temperature corrected pH, calcite saturation state (Ω_{cal}) and aragonite saturation state (Ω_{arg}) using
250 CO2calc (Robbins et al., 2010). The CO₂ constants from Roy et al. (1993), the KHSO₄ acidity
251 constant from Dickson (1990), and air-sea flux constant from Wanninkhof (1992) were used

within the CO2calc program to calculate values. Average TA, pH, $p\text{CO}_2$, Ω_{cal} , Ω_{arg} , salinity and temperatures are expressed in Table 1.

2.4 Measurements

Prior to beginning the experiment, physiological variables of each experimental individual were measured including a set of time 0 individuals ($n = 18$ for each treatment). Growth was measured through change in surface area (cm^2) by analyzing photographs of each individual taken at the beginning and end of the experiment (47 days exposure) with an image analysis program (CPCe, Kohler and Gill, 2006). Change in surface area was calculated as percentage of original surface area $((\text{SA}_t - \text{SA}_0) / \text{SA}_0)$.

Photosynthetic parameters were measured using a Diving PAM fluorometer (WALZ, Germany). *In situ* measurements were taken from *C. obtectulum* and *Hildenbrandia* sp. in Kristie Cove. The photo-parameters measured were maximum quantum yield of PSII (F_v/F_m , saturation pulse 1.0 s) after 30 min of dark adaptation. Following this the light clip was moved to a different part of the blade (light adapted) and nine saturation pulses (1.0 s) 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, and $199.1 \mu\text{mol photon m}^{-2} \text{s}^{-1}$) to measure effective quantum yield of PSII ($\Delta F/F_m'$). From these measurements electron transport rates (ETRs) were calculated using the measured absorbance of the two species (*C. obtectulum*: 60% irradiance, *Hildenbrandia* sp.: 60% irradiance) and these were fitted to the Platt equation (Jassby and Platt, 1976). Absorbance was measured by removing thalli from their substrate and placing them between a PAM light diode and Li-COR sensor (see Schoenrock et al. 2015c). From these light curves α , ETR_{max} , and E_k were calculated (see

Schoenrock et al., 2015a for all equations) and photosynthetic parameters from the end of the experiment (47 days exposure) were translated into change from the initial parameter in order to measure the response of the algae to each treatment.

Chl *a* extractions from time 0 individuals and individuals at the end of the experiment were done with a small percentage of thallus material (0.25 to 1.0 cm²; 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 : H₂O solution at -20 °C for 24 hours. A TD-700 fluorometer (Turner Designs, USA) 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 (CaCO₃ including MgCO₃) content was measured in *C. obtectulum* by drying a percentage of the thallus material (0.5 to 1.0 cm²; 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 CaCO₃, 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 CaCO₃ content (not differentiating between calcite species) was then standardized to the surface area of the sample (µg cm⁻²).

To determine Mg/Ca ratio of skeletal material approximately 10mg of sample material was ground by hand to a fine, homogeneous powder in an agate mortar and pestle and suspended in ~10 drops 70% ethanol. This was transferred to a glass slide to air dry flat and used to generate a powder X-ray diffraction (XRD) pattern in an X'Pert diffractometer (Phillips, USA). The grazing angle thin film diffraction geometry was employed where incidence angle was set to 5° and 2θ angle was scanned to record the diffraction pattern. Mg/Ca ratios were calculated from the 2θ-value of the calcite peak following methods from Ries (2011).

2.5 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 due to ambient environment. Chl *a* content and Mg/Ca were 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 ≤ 0.05 was used to determine significance in comparisons of means.

3. Results

Growth in both species was slow; 4.2-15.5% (*Hildenbrandia* sp.) and 3.1-16.7% (*C. obtectulum*) of original thallus area which amounted to <1 mm at any edge. There was significantly greater growth in *Hildenbrandia* sp. in combined pH 7.8 and 3.5 °C (Figure 1; 2-way ANOVA, $F_{(3, 72)}=$

2.809, $p = 0.046$) but there were no significant differences in growth across treatments in *C. obtectulum*.

Mean values for photosynthetic parameters did not significantly change between *in situ*, time 0, and control treatment 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 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 α 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_{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 treatments with low pH *Hildenbrandia* sp. have decreased mean photosynthetic parameters overall (Table 2), while in *C. obtectulum*, mean ETR_{max} and E_k are generally depressed in treatments with high temperature (Table 2).

Mean chl *a* content of individuals in time 0 and control treatments was not significant different in either *Hildenbrandia* sp. or *C. obtectulum* (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 and in comparison to *C. obtectulum* (Figure 3).

Mean CaCO_3 content recorded in *C. obtectulum* ranged between 0.095 and 0.211 $\mu\text{g cm}^{-2}$.

Individuals in high temperature or low pH treatments had the least CaCO_3 while individuals in the low pH and high temperature treatment had highest mean CaCO_3 content, although these differences were not significant (Figure 2, Table 3). Although aragonite never became undersaturated in experimental treatments (Table 1), MgCO_3 is a more soluble species and dissolution of thallus material could be possible in low pH treatments. Mg/Ca was not significantly different across treatments (Table 3), and ranged between 0.111-0.116 indicating that these are relatively low Mg-calcite species (Ries, 2006).

4. Discussion

In the present study, the climate change parameters of reduced oceanic pH and increased ocean temperature had few effects on crustose algae from the western Antarctic Peninsula. Notably, the fleshy *Hildenbrandia* sp. grew significantly more in microcosm treatments that reflect near future conditions (pH 7.8 and 3.5 °C); 15.5% the original size whereas low pH and low temperature had negative growth and control treatments had 8% growth (Figure 1). This species also exhibited trends in decreased chl *a*, and photosynthetic parameters, in treatments with low pH (not statistically significant), indicating they may modify their photosynthetic machinery in environments containing higher $p\text{CO}_2$. The coralline *Clathromorphum obtectulum* did not respond significantly to any climate change treatments, but higher temperatures slightly depressed photosynthetic parameters in this species and there was large variation across individuals in the treatments. *C. obtectulum* grew at a rate between 3.1 % and 16.7% of its original thallus size which is somewhat comparable to growth of *Hildenbrandia* sp. Overall our results indicate no negative effects from short term exposure to climate change conditions (47

days), but a positive effect on growth in the in *Hildenbrandia* sp. which may confer a competitive advantage over calcified species in the future.

Unfortunately, our understanding of competitive interactions and the functional role of Antarctic crustose algae in benthic ecology is far behind Arctic analogs (Adey et al., 2013; Chenelot et al., 2011). In the Arctic corallines can provide important habitats for invertebrates within their skeletal matrix both in crustose morphologies and in rhodolith assemblages (Tiechert et al., 2014; Chenelot et al., 2011). The physiology and competitive interactions between corallines and sympatric species are also better understood in Arctic corallines (Adey et al. 2013). While Antarctic corallines are prevalent in the subtidal, persisting below 40m depth and at the highest southern latitudes with open ocean (Amsler et al., 1995; Miller and Pearse, 1991), current research provides distribution (Wiencke et al., 2014), taxonomy (Hommersand et al., 2011; Wiencke and Clayton, 2002), photosynthetic rates in the Ross Sea (Schwarz et al., 2003), and response to canopy loss (Irving, et al., 2005). Even less is known about *Hildenbrandia* sp. which is among two species of fleshy crustose in the Antarctic subtidal. In both calcified and non-calcified algae growth rates are slow, but *Hildenbrandia* sp. grows 15.5% while *C. obtectulum* grew 3.3% on average in near future conditions; this signifies an increase in growth for *Hildenbrandia* sp. but no significant change in growth for *C. obtectulum* (Figure 1). We assume that these species are in direct competition for space because of their close spatial relationship (Figure 4), therefore the difference in growth will alter community composition in the near future. But direct evaluation of competitive interactions and life history traits are needed to fully support this conclusion.

Photosystems in Antarctic algae are characteristically flexible due to the highly variable light environment (Wiencke and tom Dieck, 1990) and species are acclimated or adapted to low light conditions (Gomez et al., 2009). Perhaps surprisingly, photosystems in both species showed no significant changes across treatments despite the significant differences in growth of *Hildenbrandia* sp. Trends include decreases in ETR_{max} and E_k in high temperature treatments for *C. obtectulum* and decrease in all parameters in treatments including low pH in *Hildenbrandia* sp. (Table 3). Photosynthetic parameters in *C. obtectulum* significantly differed between time 0 measurements, *in situ* measurements, and the control treatment highlighting an effect experimental conditions might have had on these photosystems, 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 after exposure to a constant light environment as it has with canopy species from this region (Schoenrock et al., 2015a). Additionally the exposure to a consistent light source did not cause visible bleaching in this experiment, which can occur in Antarctic corallines when their macroalgal canopy is removed (Irving et al., 2005) or in excessive levels of PAR (Gao et al., 2012). But light levels are ecologically relevant in this habitat and future experiments should include variation in UVR. Annual daylight patterns should also be included in future experiments and according to predictions for the future western Antarctic Peninsula (early break up of sea ice; Thompson et al., 2011) would aid in near-future predictions for algal species. UVR has sometimes been shown to exert stronger influence than other environmental parameters on the physiology of macroalgae (Rautenberger et al., 2015a; Russell et al., 2011).

Photosynthesis is coupled with calcification in algae (Lobban and Harrison, 1997) and it can liberate CO₂ for photosynthesis while protecting photosystems from UVR (Borowitzka, 1982; Gao and Zheng, 2010). However, a direct relationship between the two metabolic processes has not yet been demonstrated. Many studies indicate that low pH should result in net dissolution of calcified thalli especially in low light environments (Budenbender et al., 2011), and dissolution may be more sensitive to ocean acidification than calcification itself (Roleda et al. 2012; Silbiger and Donahue, 2015). This response is not consistent across coralline species (McCoy and Pfister, 2014; McCoy and Kamenos, 2015; Ragazzola et al., 2012) but would be of high concern in cold water species who experience low saturation states of Mg calcite. The calcification and dissolution of *C. obtectulum* were not specifically measured in this study so we were unable to discern whether either processes were affected. On Arctic reefs CaCO₃ composes 80-90% of thallus mass; in our samples CaCO₃ accounted for 62.5-86.2% of thallus mass suggesting this Antarctic species may invest more in organic content of their thallus. XRD analyses have been used previously to investigate potential shifts Mg/Ca in response to temperature (Kamenos et al., 2009). This ratio is used as a paleoclimate indicator in Arctic corallines (summarized in McCoy and Kamenos, 2015) and XRD gives accurate Mg content for ions associated specifically with the calcite matrix (Nash et al., 2013; Ries, 2011). We saw no differences across treatments, but this study does provide the first record of Mg/Ca for an Antarctic coralline; between 0.111-0.116. We were unable to determine if these values compare with the Mg/Ca in the seawater of the experimental treatments, but they appear to be low for crustose coralline algae (Ries, 2006). All carbonate analyses would be more robust if they could be compared to field specimens in the future.

The reasons for null effects in the present experiments could be many, and other studies have observed similar responses depending on exposure period and level of pCO₂ (Kamenos, 2015; Ragazzola et al., 2012). The experimental seawater chemistry and temperature manipulations were designed as sub-lethal stressors: a consistent pH of 7.8 and temperature of 3.5 °C. It is likely that both crustose species in this experiment utilize CCMs (Giordano et al., 2005), which indicates the physiological measurements used may capture a response to increased carbon resources alone. Many metabolic processes in Antarctic macroalgae operate better at higher than observed ambient temperatures (Gomez et al., 2009) and this may explain why no significantly negative effects were observed in high temperature treatments. Coastal environments along the western Antarctic Peninsula also experience periodic fluctuations in pH throughout the year from pH 7.938 to 8.62 (Bjork et al., 2013; Schram et al., in press) in tune with biological productivity (Tortell et al., 2014). This may mean that they experienced the low pH used in these treatments. Also, the short duration of this experiment (47 days) might have fallen short of producing different responses within the species across treatments (because of slow growth rate, for example). Schoenrock et al. (2015a) found temporal shifts in photosynthetic parameters and growth rate of Antarctic species over exposure periods (39 and 79 days). Specifically, a shift from positive to negative growth and depression in photosynthetic parameters (E_k , ETR_{max} , α , and MQY) show that the initial response period (~one month) can be misleading as to the final outcome these conditions would elicit (Martin and Gattuso, 2009). Other studies have shown that algae should be acclimated to their experimental environment for at least a month specifically to get accurate measurements of photosynthetic characteristics (Burdett et al., 2014).

The results of this work and our inability to draw conclusions about trickle down effects on the benthic community highlight the importance of performing multiple species/ ecosystem wide experiments when examining the biological impacts of climate change (Riebesell et al., 2008) and modeling experimental treatments around environmental complexity (Denman et al., 2011; Dupont and Portner, 2013; Hurd et al., 2009). Many experiments now use fluctuation around a decreased mean pH to mimic the ambient sea water conditions, highlighting stronger responses from organisms (variation in calcification; Johnson et al., 2014). Corallines comprise a much larger portion of the benthic crustose algal community than fleshy crustose species (Amsler et al. 1995), but their role in the subtidal along the Antarctic Peninsula is still unknown (Schoenrock, 2014). Calcification is an important defense against herbivores and epibiota in coralline algae, and a more refined look at the ultrastructure of this species could show finer scale responses (Diaz-Pulido et al., 2014; Ragazzola et al., 2012; Ries, 2006; Ries, 2010). Still, it would be unfounded to infer that antagonism like herbivory or fouling would increase on *C. obtectulum* since they have no known grazers. Overall, to make implications about these results it is important to consider the response of other benthic organisms in similar conditions (e.g. Schram et al., 2014). In light of these human induced oceanic conditions the primary concern is ecosystem response (Gaylord et al., 2015) and the potential for extinction, species range shift, and successful invasive species is high in this remote environment (Ducklow et al., 2013; Olabarria et al., 2012; Quartino et al., 2013).

5. Conclusion

This study provides initial data on the response of these two Antarctic organisms to climate change conditions. The fleshy crustose alga *Hildenbrandia* sp. increased growth in environments

478 with high $p\text{CO}_2$ and seawater temperatures while *C. obtectulum* had no significant response. This
479 is a unique response for a calcified alga and indicates that *Hildenbrandia* sp. could become a
480 better competitor for space in the benthic subtidal in future conditions.

481

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Table 1. Water chemistry parameters for microcosms sampled twice weekly over the 47 day experiment. Values were calculated using AT and spectrophotometric pH data in CO2calc (means $\pm$ SD).

	<i>in situ</i> seawater	1.5°C		3.5°C	
		pH 7.8	pH 8.0	pH 7.8	pH 8.0
pH	8.06 $\pm$ 0.01	7.81 $\pm$ 0.02	8.11 $\pm$ 0.01	7.79 $\pm$ 0.02	8.03 $\pm$ 0.01
TA (μ mol/kg SW)	2342 $\pm$ 28	2379 $\pm$ 6	2358 $\pm$ 5	2358 $\pm$ 6	2339 $\pm$ 14
Temperature (°C)	0.83 $\pm$ 0.11	1.34 $\pm$ 0.02	1.36 $\pm$ 0.02	3.41 $\pm$ 0.03	3.42 $\pm$ 0.04
Salinity (ppt)	34.0 $\pm$ 0.06	34.2 $\pm$ 0.03	34.2 $\pm$ 0.02	34.2 $\pm$ 0.02	34.2 $\pm$ 0.03
pCO ₂ (μ atm)	383 $\pm$ 11	944 $\pm$ 70	371 $\pm$ 13	922 $\pm$ 64	455 $\pm$ 16
Ω_{arg}	1.51 $\pm$ 0.02	1.10 $\pm$ 0.06	1.82 $\pm$ 0.06	1.10 $\pm$ 0.05	1.67 $\pm$ 0.05
Ω_{cal}	2.41 $\pm$ 0.03	1.76 $\pm$ 0.10	2.90 $\pm$ 0.09	1.74 $\pm$ 0.07	2.66 $\pm$ 0.08

Table 2. Photosynthetic parameters of *C. obtectulum* and *Hildenbrandia* sp. in microcosms.

Values are reported as means $\pm$ SE (n = 18).

microcosm	species	MQY	E_k	α	ETR _{max}
time 0	<i>C. obtectulum</i>	0.735 $\pm$ 0.024	104.224 $\pm$ 7.832	0.075 $\pm$ 0.010 *	2.681 $\pm$ 0.301 *
	<i>Hildenbrandia</i> sp.	0.691 $\pm$ 0.024	66.244 $\pm$ 14.262	0.104 $\pm$ 0.017	2.482 $\pm$ 0.391
in situ	<i>C. obtectulum</i>	0.52 $\pm$ 0.058 *	93.350 $\pm$ 13.620	0.127 $\pm$ 0.027	5.743 $\pm$ 0.696
	<i>Hildenbrandia</i> sp.	0.534 $\pm$ 0.04	93.429 $\pm$ 13.612	0.138 $\pm$ 0.020	4.387 $\pm$ 0.181
pH 8.0 * 1.5 °C	<i>C. obtectulum</i>	0.677 $\pm$ 0.024	114.62 $\pm$ 14.595	0.136 $\pm$ 0.006	8.323 $\pm$ 2.555
	<i>Hildenbrandia</i> sp.	0.595 $\pm$ 0.062	80.348 $\pm$ 10.910	0.102 $\pm$ 0.017	3.719 $\pm$ 1.228
pH 7.8 * 1.5 °C	<i>C. obtectulum</i>	0.589 $\pm$ 0.023	116.324 $\pm$ 9.633	0.133 $\pm$ 0.009	5.669 $\pm$ 0.636
	<i>Hildenbrandia</i> sp.	0.636 $\pm$ 0.053	63.879 $\pm$ 6.780	0.086 $\pm$ 0.011	2.328 $\pm$ 0.419
pH 8.0 * 3.5 °C	<i>C. obtectulum</i>	0.656 $\pm$ 0.016	87.735 $\pm$ 5.637	0.133 $\pm$ 0.012	4.264 $\pm$ 0.401
	<i>Hildenbrandia</i> sp.	0.511 $\pm$ 0.08	65.912 $\pm$ 13.095	0.099 $\pm$ 0.030	2.508 $\pm$ 0.491
pH 7.8 * 3.5 °C	<i>C. obtectulum</i>	0.658 $\pm$ 0.022	86.376 $\pm$ 5.814	0.129 $\pm$ 0.007	3.944 $\pm$ 0.296
	<i>Hildenbrandia</i> sp.	0.539 $\pm$ 0.084	57.990 $\pm$ 10.879	0.076 $\pm$ 0.014	2.397 $\pm$ 0.453

* indicate that values are significantly different from other treatments (p < 0.05)

946 **Table 3.** MANOVA table for *C. obtectulum* and *Hildenbrandia* sp.

species	parameter	Df	<i>F</i> - value	<i>P</i> - value	power
<i>Clathromorphum obtectulum</i>	growth	3	0.518	0.641	0.16
	chl <i>a</i>	3	0.476	0.7	0.141
	CaCO ₃	3	0.664	0.577	0.183
	Mg/Ca	3	1.445	0.276	0.406
	α	3	0.177	0.911	0.81
	E _k	3	1.055	0.374	0.274
	MQY	3	0.493	0.688	0.145
	ETR _{max}	3	2.001	0.122	0.493
<i>Hildenbrandia</i> sp.	growth	3	2.809	0.046*	0.651
	chl <i>a</i>	3	0.949	0.422	0.249
	α	3	0.921	0.436	0.242
	E _k	3	1.082	0.363	0.28
	MQY	3	0.408	0.748	0.127
	ETR _{max}	3	0.113	0.952	0.69

947

948 F-values are equivalent to F-test values.

949

950 **Figure 1.** Growth rate data from both *C. obtectulum* and *Hildenbrandia* sp. (means + SE).

951 * indicate significant differences.

952

953

954 **Figure 2.** CaCO₃ concentration in *C. obtectulum* across microcosm treatments (means + SE).

955

956 **Figure 3.** Chlorophyll *a* concentration in *C. obtectulum* and *Hildenbrandia* sp. from microcosm
957 experiment and time 0 individuals (means + SE).

958

959 **Figure 4.** Two images reflect the benthic community underneath the algal canopy at 15 m depth.
960 a. depicts the normal crustose community where coralline algae (C) dominate substrate. b. is a
961 rare example of *Hildenbrandia* sp. (H) dominance over coralline algae.

Figure 1

Figure 2

Figure 3

Figure 4