Ellie Bergstrom

EFEITOS DO AUMENTO DA TEMPERATURA E ACIDIFICAÇÃO OCEÂNICA NA FISIOLOGIA DAS ALGAS CALCÁRIAS E GRAMAS MARINHAS

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“Efeitos do aumento da temperatura e acidificação oceânica na fisiologia das algas calcárias e gramas marinhas”

por

Ellie Regina Bergstrom

Dissertação julgada e aprovada em sua forma final pelos membros titulares da Banca Examinadora (Port. 03/PPGFAP/2016) do Programa de Pós-Graduação em Biologia de Fungos, Algas e Plantas - UFSC, composta pelos Professores Doutores:

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To my grandparents for always supporting, to my parents and brother
for always pushing and to my husband for always encouraging…

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RESUMO

O aumento de CO_2 e temperatura dos oceanos, decorrente das atividades antrópicas, causam e causarão enormes impactos para uma grande diversidade de organismos marinhos em todo o planeta. Os produtores primários costeiros são especialmente sensíveis devido ao seu importante papel na assimilação de carbono inorgânico nos ecossistemas marinhos costeiros. No entanto, ainda existem muitas lacunas no conhecimento sobre as respostas dos produtores primários calcários e não calcários às mudanças climáticas globais, bem como o efeito das interações metabólicas entre espécies nessas respostas. No presente trabalho, foram realizados experimentos que manipularam múltiplas variáveis (CO_2, temperatura e espécies presentes), usando uma instalação inovadora, o Mesocosmo Marinho, no Brasil, a fim de analisar os efeitos da acidificação e aumento da temperatura dos oceanos (OA & OW) na fisiologia das algas calcárias (*Halimeda cuneata, Lithophyllum sp.*, *Lithothamnion sp.* e *Mesophyllum sp.*) e a grama marinha (*Halodule wrightii*). Primeiramente, foi avaliado o impacto de OA e OW no rendimento fotossintético dos três táxons dominantes e morfologicamente distintos de algas calcárias não articuladas de um banco de rodolitos tropical. A análise de fluorescência revelou respostas específicas para cada espécie de rodolito. *Lithophyllum sp.* não mostrou nenhuma mudança de F_v/F_m, independente do tratamento, *Lithothamnion sp.* teve uma redução no F_v/F_m sob elevada temperatura e *Mesophyllum sp.* sofreu uma queda de F_v/F_m relacionado ao acoplamento de alto CO_2 e temperatura. Sugerimos que as diferentes taxas de rendimento fotossintético são indicadores de estratégias fisiológicas diferentes que podem ser enraizadas no nível de complexidade da morfologia de cada espécie de rodolito. Caso essa hipótese seja corroborada por estudos específicos, podemos prever uma redução da complexidade de bancos de rodolitos imposta pela pressão seletiva de valores extremos de CO_2 e de temperatura. Em seguida, testou-se as respostas de calcificação e produção primária líquida (NPP) da alga calcária verde, *H. cuneata*, e grama marinha, *H. wrightii*, bem como a interação metabólica entre as duas espécies. Sob elevado CO_2, *H. cuneata* teve uma resposta positiva de NPP e negativa de calcificação, enquanto *H. wrightii* não apresentou variações significativas de NPP. Porém, *H. cuneata* conseguiu maiores taxas de calcificação na presença de *H. wrightii* do que na ausência, mostrando a capacidade da grama mitigar os efeitos da OA através da assimilação rápida de carbono inorgânico dissolvido (DIC) do microambiente. A temperatura não causou nenhum efeito observável sobre *H. cuneata* nem *H. wrightii*, em virtude da provável plasticidade ou adaptação destes organismos tipicamente tropicais, à magnitude de variação da temperatura observada em ambientes recifais rasos. Concluímos que as populações de *H. cuneata* que coexistem com populações de *H. wrightii* podem ter maiores chances de sobrevivência em níveis de CO_2 esperados para o ano 2065. Deve-se ressaltar que as previsões acerca do futuro dos ambientes costeiros, considerando as mudanças climáticas observadas e previstas, devem levar em consideração não só a variabilidade inter e intra específica, uma vez que a plasticidade fenotípica, combinada com pressões seletivas determinadas, pode levar à expressão de diferentes morfotipos a partir do mesmo genótipo. Por outro lado, é imprescindível considerar que as interações biológicas têm um papel fundamental nas respostas fisiológicas, as quais proporcionam uma alta resiliência para as populações envolvidas. Assim, para poder melhorar planos de conservação costeira, é essencial fornecer previsões realistas que são baseadas em estudos multivariados, para que ambientes costeiros, contendo espécies chaves, possam ser efetivamente preservadas.

Palavras chave

Alga calcária, grama marinha, acidificação oceânica, aumento de temperatura, rendimento fotossintético, calcificação

ABSTRACT

Increased anthropogenic CO_2 and temperature in the ocean have and will continue to have enormous impacts on a diverse group of marine organisms across the globe. Coastal primary producers are especially sensitive because of their important role in the assimilation of inorganic carbon in coastal marine ecosystems. However, there are still many open questions regarding the responses that calcareous and fleshy photoautotrophs will have, while simultaneously considering metabolic interactions between species. We conducted experiments that manipulated multiple variables (CO_2, temperature and species present) in a novel marine mesocosm facility in Brazil in order to test the effects of OA and OW manifested in the physiology of calcareous algae (*Halimeda cuneata, Lithophyllum sp.*, *Lithothamnion sp. and Mesophyllum sp.*) and seagrass (*Halodule wrightii*). First, we evaluated the effects of OA and OW on the photosynthetic performance of three dominant and morphologically distinct coralline algae taxa of a tropical rhodolith bed. We observed species-specific physiological responses for the calcareous algae, while the seagrass displayed a neutral physiological response. The fluorescence analysis revealed species-specific F_v/F_m responses for each rhodolith. *Lithophyllum sp.* showed no changes in F_v/F_m, regardless of the treatment, *Lithothamnion sp.* experienced reduced F_v/F_m under high temperature and *Mesophyllum sp.* suffered a drop in F_v/F_m related to the coupling of high CO_2 and temperature. We suggest that the varying responses in photosynthetic performance are indicators of different physiological strategies that may be rooted in the level of morphological complexity of each species of rodolith. If this hypothesis is corroborated by specific studies, we can predict a reduction in the morphological complexity of rhodolith beds, imposed by the selective pressure of extreme values of CO_2 and temperature. We then tested the calcification and net primary production (NPP) responses of the green calcareous alga, *H. cuneata*, and seagrass, *H. wrightii*, as well as the metabolic interaction between the two species. At high CO_2, *H. cuneata* had a positive NPP response and a negative calcification response, while *H. wrightii* didn't present any significant variation in NPP. However, *H. cuneata* attained greater calcification rates in the presence of *H. wrightii* than in its absence, showing the capacity of the seagrass to ameliorate OA effects via quick assimilation of dissolved inorganic carbon (DIC) from the microenvironment. Temperature had no effect over *H. cuneata* nor *H.*

wrightii, probably due to phenotypic plasticity or adaptation of these typically tropical organisms to the magnitude of change in temperature observed in shallow reef. We conclude that *H. cuneata* populations coexisting with *H. wrightii* populations may be more likely to survive CO_2 levels predicted for 2065. It should be noted that predictions about the future of coastal environments, considering observed and projected climate change, shouldn't only take inter and intraspecific variability into account, since phenotypic plasticity combined with certain selective pressures can lead to the expression of different morphotypes from the same genotype. On the other hand, it is essential to consider that biological interactions play a key role in physiological responses, which provide high resilience for the involved populations. Thus, in order to improve coastal conservation efforts, it's essential that we provide realistic predictions that are based on multivariate studies, so that coastal environments, including key species, may be effectively preserved.

Keywords

Calcareous algae, seagrass, ocean acidification, ocean warming, photosynthetic performance, calcification

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LISTA DE ABREVIATURAS

OA	Acidificação oceânica
OW	Aumento da temperatura oceânica
DIC	Carbono inorgânico dissolvido
HCO_3^-	Bicarbonato
CO_2	Dióxido de carbono
CO_3^{2-}	Carbonato
F_v/F_m	Rendimento quântico máximo
SE	Erro padrão
ANOVA	Análise de variância
ΔF/Fm’	Rendimento quântico efetivo
PAR	Radiação fotossinteticamente ativa
SNK	Student Newman Keuls
NPP	Produção primária líquida
PERMANOVA	Análise de variâncias permutacional
MDS	Multidimensional scaling
PS II	Fotossistema II
[DO]	Dissolved oxygen concentration
A_T	Total alkalinity

SUMÁRIO

1. INTRODUÇÃO GERAL

À medida que a atual população humana, com cerca de 7 bilhões (UN DESA, 2015), continua crescendo, sua demanda de energia vem aumentando. Para suprir todas as necessidades de consumo deste contingente populacional cada vez maior, a utilização de combustíveis fósseis tem sido a principal solução de fonte de energia. De 1750-2011, 2040 ± 310 Gt de CO_2 foram liberados para a atmosfera. Os oceanos absorveram 30% deste valor, causando uma diminuição de 0,1 no pH de suas águas superficiais, o que corresponde a um aumento de 26% na acidez da água (IPCC, 2014). O processo de absorção de CO_2 pelo oceano envolve reações químicas nas quais o CO_2 reage com a água do mar para produzir ácido carbônico e liberar íons de bicarbonato e prótons, dessa maneira, reduzindo o pH da água (Turley et al. 2006). Apesar da capacidade do oceano em absorver o CO_2 antropogênico ser estimada em 70-80%, levaria séculos para que isso realmente acontecesse, devido a demora na exportação do carbono da superfície para águas profundas (Maier-Reimer & Hasselmann, 1987). Assim, a magnitude das emissões de CO_2 fazem com que o oceano diminua sua capacidade tampão ideal e a camada superficial do mar fique sobrecarregada de CO_2.

Sistemas marinhos costeiros, aqueles presentes em águas rasas na zona entremarés da plataforma continental, são alguns dos ecossistemas mais importantes tanto ecologicamente quanto socioeconomicamente (Harley et al. 2006). Ecossistemas nessas áreas como recifes de coral, bancos de gramas marinhas e bancos de macroalgas são chaves na remoção e sequestro de carbono através de processos metabólicos como a fotossíntese e a calcificação (Koch et al. 2013). Esses ambientes servem como a interface entre atmosfera e oceano, bem como regiões costeiras densamente urbanizadas e mar aberto. Sendo assim, são os primeiros a sofrer as consequências de alterações antropogênicas, que podem ocorrer em escalas globais e/ou locais.

Produtores primários, calcários e não calcários, são diversos nessas regiões onde a radiação solar é abundante, sendo os responsáveis pela transformação da energia solar em energia química e pela remoção de grandes quantidade de carbono inorgânico da água e assimilação nas plantas e sedimentos. Com o incremento de CO_2, a maioria das plantas marinhas estarão em ambientes saturados em carbono (Gattuso &

Buddemeier, 2000), e com a consequente redução do pH e alteração do estado químico da água associado, terão repercussões para vários processos fisiológicos intrínsecos (Harley et al. 2006). É esperado que as macroalgas "foliosas" e gramas marinhas tenham respostas fisiológicas positivas ou neutras, enquanto as macroalgas calcárias tenham uma redução no desempenho fisiológico geral (Koch et al. 2013; Kroeker et al. 2013). De fato, há evidências de que muitos organismos marinhos que dependem do metabolismo de bicarbonato já estão sendo ameaçados com grandes mudanças em sua biologia (Barton et al. 2012). Nos interessa saber como os processos fisiológicos serão afetados nas populações destes organismos sob elevado CO_2 e temperatura, considerando também as interações entre espécies. Dessa forma, podemos prever possíveis mudanças em comunidades e tomar as medidas adequadas a fim de conservar as populações chaves que estão em perigo.

2. OBJETIVOS

2.1. Objetivo Geral

A presente dissertação se propõe a investigar os efeitos do aumento de CO_2 e temperatura de forma isolada e acoplada na fisiologia das algas calcárias e gramas marinhas, avaliando o papel da variabilidade específica e das interações metabólicas no seu comportamento ecofisiológico.

2.2. Objetivos Específicos

- Comparar o efeito de OA e OW no rendimento fotossintético das três espécies dominantes de rodolitos (*Lithophyllum sp.*, *Lithothamnion sp.* e *Mesophyllum sp.*).
- Avaliar as respostas de produção primária líquida (NPP) e calcificação em *H. cuneata* e a resposta de NPP em *H. wrightii*.
- Determinar se a presença de *H. wrightii* ameniza os efeitos negativos de OA e OW sobre a fisiologia de *H. cuneata*.

CAPÍTULO 1

O capítulo 1 da presente dissertação não é uma publicação efetiva. A intenção do autor na elaboração dos capítulos da dissertação é para a defesa e obtenção de título.

Species-specific rhodolith photosynthetic responses to ocean acidification and warming potentially rooted in morphological complexity

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Abstract

Rhodoliths, or free-living non-geniculate coralline red algae, make up key calcifier communities along a large, yet understudied range on the South Atlantic coast. They act as some of the major carbonate biofactories on our planet, where Brazil possesses the largest rhodolith bed in the world. Although climate change stressors threaten these marine algae and environments, they are underrepresented in ocean acidification (OA) and warming (OW) studies. In order to better understand the diversity in responses of these reef builders to global stressors, we conducted a mesocosm experiment that tested the combined and isolated effects of elevated temperature and CO_2 on the photosynthetic performance of three rhodolith species, *Lithophyllum sp.*, *Lithothamnion sp.* and *Mesophyllum sp.*, that dominate beds along the tropical Atlantic coast and possess different levels of morphological complexity, from minor to major, respectively. Chlorophyll *a* fluorescence of PSII was used as a non-invasive measurement technique to obtain photosynthetic performance. Each species, and respective morphological type, responded uniquely: *Lithophyllum sp.*, less complex morphology, showed no changes, *Lithothamnion sp.*, intermediate complexity, experienced reduced F_v/F_m under only high temperature and *Mesophyllum sp.*, more complex morphology, presented low $\mathrm{F_v/F_m}$ under the coupling of high CO_2 and temperature. Our results suggest that the species-specific nature of the responses are due to differences in temperature optimums and carbon assimilation mechanisms, and that simpler morphologies may provide high resilience against global

stressors.

Keywords

Rhodolith, Ocean Acidification, Ocean Warming, photosynthetic performance, optimum temperature, morphology

1. Introduction

Rhodoliths are the non-geniculate, free-living forms of coralline algae (Corallinophycidae, Rhodophyta) that form aggregations on the ocean floor also known as rhodolith beds. Rhodoliths provide a heterogeneous substrate that sustains a highly diverse community of marine organisms (Foster, 2001). These benthic communities are long-lived and occur from the subtidal zone to depths of 268 meters across (Littler et al., 1985). In the South Atlantic, rhodolith beds are assumed to occur along the entire tropical and subtropical continental shelf (Kempf, 1970), where some of the most common species in this region are from the genera: *Lithothamion*, *Mesophyllum* and *Lithophyllum* (de Castro Nunes et al., 2008; Amado-Filho et al., 2012b), providing a lengthy geographical distribution despite the latitudinal gradients of environmental factors and geomorphological particularities. Notably, marine research has confirmed that Brazil possesses the most extensive continuous rhodolith bed on the planet, covering 20,900km^2 on the Abrolhos shelf off of Bahia state (Amado-Filho et al., 2012a). This environment plays a key role in the carbonate cycle, producing 0.025 Gt of Ca_2CO_3 yr^{-1} (Amado-Filho et al., 2012a). Their huge abundance, wide distribution and high diversity are, in part, a result of different morphological strategies and ecophysiological behaviors that have been selected for throughout their history. The physical, chemical and biological pressures present in the marine environment act as the forces that select the most fit strategies. High levels of disturbance (i.e. herbivory and hydrodynamics) select less complex morphologies in macroalgae, specifically coralline algae (Steneck, 1986; Steneck & Dethier, 1994). Understanding the selection of simpler morphologies under natural disturbances can also give important insight for the interpretation of the responses of these organisms to anthropogenic disturbances (i.e. pollution and global stressors).

Today the global carbon cycle cannot be discussed without considering anthropogenic alterations. Significant changes in ocean surface pH and temperature are expected by 2100 (RCP 6.0, IPCC, 2014). The reduction in ocean pH is to be accompanied by large shifts in carbon speciation, and rising temperatures will create additional environmental and biological changes (Doney et al., 2012). With expected physiological alterations, shifts are foreseen for functioning of organisms, causing shifts in the size, distribution range, and seasonal abundance of populations, which is ultimately predicted to greatly affect coastal ecosystem functioning, goods and services (Doney et al., 2012; Harley et al., 2006).

Negative physiological responses to global OA and OW have been the most common findings for calcareous algae (Kroeker et al., 2010), and specifically coralline algae (Anthony et al., 2008; Diaz-Pulido et al., 2012; Martin & Gattuso, 2009; Johnson et al., 2014), yet some neutral or positive responses have been reported (Noisette et al., 2013). Photosynthetic performance is one of the most relevant photoautotroph response parameters to consider when predicting overall species health. Meta analyses report a significant negative effect of OA alone on the photosynthesis of calcareous algae (Kroeker et al., 2010), however increased variation and sensitivity is expected of physiological responses when considering OW simultaneously (Kroeker et al., 2013). Species-specific discrepancies in photosynthetic responses of calcareous algae in the same location at the same time can be due in large part to diversity of carbon assimilation mechanisms and related anatomy (Koch et al., 2013; Price et al, 2011), whereas inter and intra-specific response differences observed along geographic gradients are expected to be additionally influenced by local abiotic variables such as irradiance (Gao et al., 2012), water flow (Comeau et al., 2014), and nutrients (Araujo et al., 2014; Björk et al., 1996). Experimental assays with varying durations (Kroeker et al., 2013) and measurement methodologies have also been shown to cause variation in observed responses. It’s important to highlight that a rather large portion of the published body of work that sustains these findings have considered temperate species (Wernberg et al., 2013; Yang et al., 2015). Despite the threat of coastal stressors (Bjork et al., 1996) and direct exploitation (Riul et al., 2008) that will affect many key primary producer species, no published results about the effects of OA and OW on calcareous algae are available for the tropical and warm temperate South Atlantic (Yang et al., 2015). We aim to begin to fill these gaps by investigating how three dominant species of rhodoliths from a bed off the northeastern Brazilian coast will respond to OA and OW using non-intrusive photosynthetic measurement techniques. We hypothesize that photosynthetic performance responses will reveal species-specific variations, with less complex morphology presenting higher resilience to climate change stressors.

2. Material and Methods

2.1. Study Area

The Marine Mesocosm is one of Projeto Coral Vivo's research facilities, located on Araçaípe beach in Arraial d'Ajuda, Bahia, Brazil. It was designed to test the effects of elevated CO_2 and elevated temperature (and other factors) on marine organisms, while most closely mimicking the adjacent reef conditions. As described by Duarte et al. (2015), the system is composed of 16 experimental tanks, four header tanks and four mixing tanks (Appendix A Fig. 1). Seawater is pumped from 500 meters off the coast, brought to the system, and enters into four 5000 l mixing tanks, where the four treatments are applied. Two of the tanks receive an elevated temperature and thus each contains a 2 m-long heater. The two tanks that receive an increased CO_2 concentration are injected with CO_2 gas and mixed to homogenize the concentration. The water from each mixing tank is then pumped to its corresponding 310 l header tank, which is located in the control house. There, treatments are programmed and monitored, based on the current CO_2 concentration and temperature of the adjacent reef, by an Arduino open source platform (Reef Angel), four temperature sensors and four pH sensors. After treatment regulation, the water from each header tank is distributed among 4 randomized experimental tanks (120 l) where the marine specimens are located, totaling 16 tanks.

2.2. Sampling

Three dominant rhodolith species (Fig. 1) from the rhodolith bed 500m off of Aracaípe beach in Arraial d'Ajuda, BA, Brazil (16°29'29"S 39°4'7"W) were sampled (32 specimens per species) at 2.5m using SCUBA and were brought to shore to the marine holding aquariums of the Marine Mesocosm for the delicate and uniform sorting and removal of plant and animal epibionts. The three dominant rhodolith species were identified as *Lithophyllum sp*., *Lithothamnion sp*. and *Mesophyllum sp*. Samples of each species were adequately deposited in the FLOR Herbarium (0059441-443, 0059435-437, 0059438-440) at the Federal University of Santa Catarina (Florianopolis, SC, Brazil).

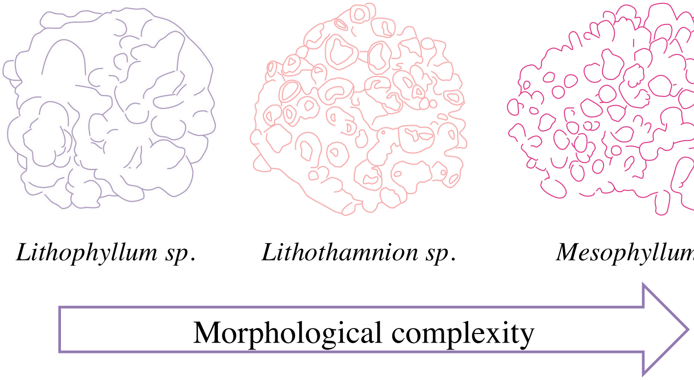

Figure 1. Gradient of morphological complexity of three dominant rhodolith species. Image credit: Marina Sissini.

2.3. Experimental design

A 23 day-long mesocosm experiment was conducted with a bifactorial design that was composed of the four following treatments: 25.7°C ± 0.1 SE & pH 8.13 ± 0.03 SE, 29.5°C ± 0.5 SE & 8.13 ± 0.03 SE, 25.9°C ± 0.1 SE & pH 7.90 ± 0.04 SE and 28.5°C ± 0.2 SE & 7.92 ± 0.04 SE in accordance with the moderate RCP 6.0 scenario for 2100 (IPCC, 2014). 16 plastic trays (40x17x4cm) were filled 3cm high with sand originating from the rhodolith bed and three rhodolith specimens, one per species, were placed equidistantly on top. One tray was placed in each experimental tank for a 15-day acclimation period at 26°C and a pH of 8.13. Treatments commenced upon completion of the acclimation period and reached expected levels within 24 hours. Abiotic parameters were measured daily in each experimental tank: salinity (Refractometer: Instrutherm RTS-101ATC), dissolved oxygen & temperature (Portable dissolved oxygen meter: Instrutherm MO-900), incident irradiance (Quantometer: apogee MQ-200) and pH (pHmeter: Gehaka ISO 9001). Measurements of photosynthetic performance were taken during acclimation (before treatments commenced) and on the last day of treatments, day 23.

2.4. Photosynthetic Performance Measurements

A diving Pulse Amplitude Modulation Fluorometer (Diving PAM; Walz Germany) was used to monitor the chlorophyll *a* fluorescence of Photosystem II (PS II). The following two fluorescence-based parameters were utilized. Effective quantum yield ($\Delta F/F_m$') is the quantum yield of photochemical energy conversion in PS II under illuminated conditions (when reaction centers are partially occupied by photons) (Klughammer & Schreiber, 2008). $\Delta F/F_m$' describes the photochemical efficiency of the organism under present conditions, while it is currently photosynthesizing under incident irradiance. Maximum quantum yield (F_v/F_m) is the quantum yield of photochemical energy conversion in PS II under dark conditions (when reaction centers are completely unoccupied) (Klughammer & Schreiber, 2008). F_v/F_m gives the maximum photochemical efficiency that the organism is capable of under present conditions when the photosynthetic apparatus is completely relaxed. During the acclimation period (pre-treatments), we conducted non dark-acclimated (under incident irradiance) pulses in order to obtain effective quantum yield ($\Delta F/F_m$') measurements at intervals of 2 hours over the course of three days (during light hours), in order to characterize the daily photosynthetic performance cycle of the rhodoliths. We also performed dark-acclimated (20 min) light pulses the day before treatments commenced and on the last day of the experiment, day 23, in order to obtain maximum quantum yield (F_v/F_m) responses to OA and OW treatments.

2.5. Statistical Analysis

Cochran's test for homocedasticity of variances and Shapiro-Wilk's test for normality were performed on all data. Statistica 10.0 was used to perform a one-way Analysis of Variance (ANOVA) on F_v/F_m data to evaluate the differences among species, considering that all species were under the same experimental conditions. A two-way ANOVA was also performed on F_v/F_m data for each species, separately, with two factors (CO_2: two levels and temperature: two levels) to evaluate within-species responses. $\Delta F/F_m$' data was log-transformed to meet ANOVA assumptions and a two-way ANOVA was performed with two factors (species: three levels and time: six levels), to evaluate differences between species and times in the daily photosynthetic performance cycle. Student Newman Keuls (SNK) post-hoc tests were subsequently performed after ANOVAs. We consider differences to be significant when $p<0.05$.

3. Results

3.1. F_v/F_m

Each species and respective morphology presented a particular physiological response considering the global stressors evaluated (Table 1, 2). *Lithophyllum sp*. experienced no changes in F_v/F_m due to OA nor OW and thus performed similarly under all treatments (Fig. 2A, Table 3). There was a significant effect of temperature for *Lithothamnion* sp. (Table 3). F_v/F_m values of *Lithothamnion sp*. under future temperature (29°C) at 23 days were 37% lower than at ambient temperature (Fig. 2C). *Mesophyllum sp*. showed a significant effect of CO_2 and temperature (Table 3). F_v/F_m was reduced by 55% (with respect to the control) when temperature and CO_2 were coupled (Fig. 2D), which was the lowest value reached among the species, even lower than the lowest daily effective quantum yield for this species (Fig. 3).

Table 1: Results from one-way ANOVA of F_v/F_m data from all rhodolith species on day 23 with one factor (species: three levels). Significance when $p<0.05$. n=12.

Source of variation	F_v/F_m			
	df	MS	*F*	*p*
Species	2	0.074	5.056	**0.0121**
Residual	33	0.015		

Table 2: Average values of F_v/F_m and Student Newman Keuls (SNK) post-hoc results from the one-way ANOVA for *Lithothamnion sp.*, *Mesophyllum sp.* and *Lithophyllum sp.*.

Species	Average F_v/F_m	SNK *Lithothamnion sp.*	*Mesophyllum sp.*	*Lithophyllum sp.*
Lithothamnion sp.	0.346		0.8838	**0.0116**
Mesophyllum sp.	0.339	0.8838		**0.0213**
Lithophyllum sp.	0.479	**0.0116**	**0.0213**	

Table 3. Results from the 2-way ANOVAs of F_v/F_m values from day 23 of *Lithophyllum sp.*, *Lithothamnion sp.* and *Mesophyllum sp.* Each 2-way ANOVA used two factors (CO_2: two levels and temperature: two levels). Significance when $p<0.05$. n=3.

Source of variation	F_v/F_m			
	df	MS	*F*	*p*
Lithophyllum sp.				
CO_2	1	0.024	1.848	0.2112
Temperature	1	0.002	0.176	0.6860
CO_2 * temperature	1	0.002	0.168	0.6931
Lithothamnion sp.				
CO_2	1	0.012	1.094	0.3262
Temperature	1	0.073	6.900	**0.0303**
CO_2 * temperature	1	0.004	0.405	0.5425
Mesophyllum sp.				
CO_2	1	0.009	1.527	0.2517
Temperature	1	0.074	12.905	**0.0071**
CO_2 * temperature	1	0.049	8.578	**0.0190**

Table 4. Average F_v/F_m values and SNK post-hoc results from 2-way ANOVAs for a) *Lithothamnion* sp.: temperature effect and b) *Mesophyllum* sp.: CO_2*temperature effect. There was no observed effect of temperature nor CO_2 on *Lithophyllum* sp.

a) SNK

Temperature	Average F_v/F_m	26°C	29°C
26°C	0.424		**0.0305**
29°C	0.269	**0.0305**	

b) SNK

CO_2	Temperature	Average F_v/F_m	8.1 pH & 26°C	8.1 pH & 29°C	7.9 pH & 26°C	7.9 pH & 29°C
8.1 pH	26°C	0.381		0.6516	0.2656	**0.0224**
8.1 pH	29°C	0.352	0.6516		0.2752	**0.0188**
7.9 pH	26°C	0.455	0.2656	0.2752		**0.0076**
7.9 pH	29°C	0.170	**0.0224**	**0.0188**	**0.0076**	

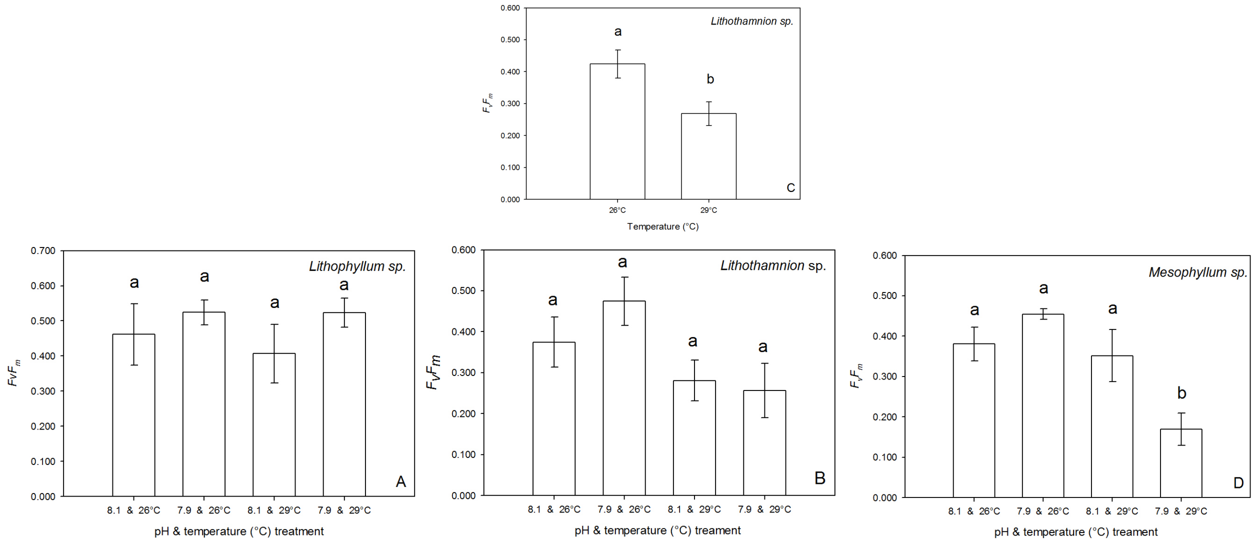

Figure 2. F_v/F_m responses (+ SE) after 23 d of exposure to four treatments for (A) *Lithophyllum sp*., (B) *Lithothamnion sp*. (all treatments: no effects), (C) *Lithothamnion sp*. (temperature treatments: effect present; n=6) and (D) *Mesophyllum sp*.. Different lowercase letters indicate significant differences between treatments from the SNK post hoc test of the 2-way ANOVA. n=3.

3.2. Daily photosynthetic performance cycle

The photosynthetic performance of *Lithophyllum sp*., *Lithothamnion sp*. and *Mesophyllum sp*. before treatments commenced was similar throughout the day (Fig. 3), only varying significantly with time (Table 5). There was a significant difference between values at 11:00 & 13:00, 15:00 and 17:00 (Table 6). The lowest effective quantum yield values for all rhodolith species were observed from 11:00 – 13:00, when irradiance was greatest (617-656 μmol photons m^{-2} s^{-1}). The average of all species during this midday time was 0.175 ± 0.012 SE.

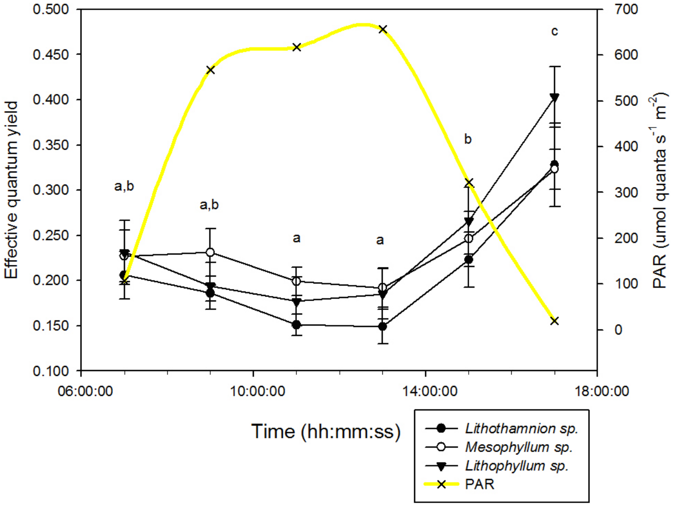

Figure 3. Daily photosynthetic performance cycle. Measurements of effective quantum yield (ΔF/Fm') during the beginning days of the acclimation period for *Lithophyllum sp*., *Lithothamnion sp*. and *Mesophyllum sp*. over the course of the light hours of the day (+ SE). Different lowercase letters show significant differences between times (no effect of species found). n=9.

Table 5. Results from 2-way ANOVA of log-transformed $\Delta F/F_{m'}$ data from the daily photosynthetic performance cycle with two factors (species: three levels and time: six levels). Significance when $p<0.05$. n=9.

Source of variation	$\Delta F/F_{m'}$			
	df	MS	*F*	*p*
Species	2	0.071	2.727	0.0688
Time	5	0.362	13.882	**0.0000**
Species*Time	10	0.013	0.491	0.8937
Residual	144	0.026		

Table 6. Average $\Delta F/F_{m'}$ values from the daily photosynthetic performance cycle and SNK post-hoc results from the 2-way ANOVA.

SNK Time	Average $\Delta F/F_{m'}$	7:00	9:00	11:00	13:00	15:00	17:00
7:00	0.228		0.5255	0.0928	0.0858	0.2910	**0.0000**
9:00	0.204	0.5255		0.1468	0.1973	0.2087	**0.0000**
11:00	0.174	0.0928	0.1468		0.7872	**0.0091**	**0.0000**
13:00	0.175	0.0858	0.1973	0.7872		**0.0058**	**0.0000**
15:00	0.245	0.2910	0.2087	**0.0091**	**0.0058**		**0.0002**
17:00	0.351	**0.0000**	**0.0000**	**0.0000**	**0.0000**	**0.0002**	

4. Discussion

The photosynthetic performance responses of the dominant rhodoliths from the tropical South Atlantic to OA and OW, after just over 3 weeks of treatment, are species-specific. The response observed for *Lithophyllum sp*. is significantly different from those of *Lithothamnion* and *Mesophyllum sp*.. *Lithothamnion sp*. and *Mesophyllum sp*., who possess moderately and more branched morphologies, respectively, were negatively affected, whereas *Lithophyllum sp*., who possesses a minimally branched morphology, showed no shift in its performance due to either variable. While species-specific responses are likely due to differences in carbon-concentrating mechanisms and temperature optimums, we also propose that the morphological complexity of rhodoliths may be negatively correlated with the resilience of their photosynthetic performance to OA and OW. We suggest that the least branched morphology acts as an adaptive strategy against the stress of OA and OW. As observed and predicted by Steneck and Dethier (1994), considering the whole macroalgal community, less complex morphologies can survive under high disturbance levels. They additionally highlight that where productivity potential is high, crustose algae were observed in all evaluated environments, independent of the degree of disturbance. On the other hand, articulated calcified algae, who possess much more complex morphology, only survive under low to intermediate disturbance conditions. Rhodolith community composition and abundance will likely be altered according to the differential photosynthetic capacities under global stressors, possibly favoring simple morphologies, and thus potentially provoking losses of niche availability and consequent species diversity and abundance.

The absence of a shift in the photosynthetic performance of *Lithophyllum sp.* under OA and OW, indicates that this species possesses a strategy that allows it to maintain normal levels of photosynthetic activity, which is a crucial component of the metabolism of primary producers. Temperature and pH can show large vertical and horizontal fluctuations in coastal productive ecosystems (Reusch, 2013), to which the photosynthetic apparatus of *Lithophyllum sp.* may be phenotypically tolerant (plastic) or adapted. This variability has acted over the past 30 million years, the evolutionary age of the genus *Lithophyllum* (Aguirre et al., 2010), and has selected for traits that likely have provided the biological tools to survive periods of negative pressures like warming and acidification. We interpret simple morphology to be a selected trait that may be responsible for the resilience of coralline algae to OA and OW stress. It has been reported that thallus morphology significantly affects photosynthetic performance, and may be of adaptive significance, where the performance of simpler morphologies of green seaweeds (*Ulva lobata*, *Ulva rigida* and *Enteromorpha intestinalis*) was 7.2-10.2 times greater than the performance of a more complex morphology (*Codium fragile*) (Arnold & Murray, 1980). *Lithophyllum spp.* have been observed to acclimate to slow rates of OA (Hofmann & Bischof, 2014) by showing no change in photosynthetic performance and respiration and even increases in calcification responses to acidification (Martin et al., 2013; Noisette et al., 2013). The smooth, minimally branched surface of simple coralline morphologies, like *Lithophyllum sp.*, likely permits the homogeneous absorption of incident irradiance, which may be allowing it to maximize photosynthesis and minimize respiration when presented with high temperature and CO_2 concentrations. This could allow the organism to optimize CO_2 consumption and metabolic rates in order to direct the transformed energy towards physiological maintenance and any potentially adverse effects that OA and OW may have caused on other processes, such as calcification. The ultimate survival of *Lithophyllum* populations surely depends on their ability to calcify and must be considered in future studies concerning correlations between morphology and resilience to OA and OW.

The F_v/F_m decrease in *Lithothamnion sp*., due to a 3°C increase in temperature, regardless of [CO_2], reveals that this species experiences thermal stress. Photoautotrophs typically can increase photosynthetic metabolism with increasing temperature until a specific optimum temperature, or in the case of macroalgae, an optimum range, beyond which, rates drop (Davison, 1991). Supra-optimal temperature is known to inhibit algal photosynthesis by reducing enzyme activity (Davison, 1991) and increasing photorespiration (Berry & Raison, 1981). We suggest that the moderately branched morphology of *Lithothamnion sp.* does not facilitate introduced thermal stress. Due to a more heterogeneous surface, as compared to *Lithophyllum sp*., it is supposed that some areas will receive less irradiance than others because they are positioned on the sides of protuberances. We propose that these areas may be limited photosynthetically when faced with additional stressors, depending on their physiological particularities, and thus hold consumption requirements without contributing to production. Based on our results, *Lithothamnion sp.* is likely sensitive to thermal changes and not DIC changes, as other corallines have been shown to behave (Vásquez-Elizondo & Enríquez, 2016), which may provoke alterations in growth rate or even species abundance. The absence of a CO_2 effect could indicate that *Lithothamnion sp.* possesses a mechanism of carbon assimilation that is able to withstand altered CO_2 concentrations, thus maintaining the same level of photosynthetic performance at both tested CO_2 levels.

Mesophyllum sp. photosynthetic performance experienced a synergistic interactive effect between OA and OW, where the coupled increase in temperature and CO_2 resulted in a change that is greater than the sum of the changes due to temperature and CO_2, separately. Previous climate change studies on coralline algae corroborate these synergistic negative effects, specifically showing that physiological responses to

CO_2 are exacerbated by temperature (Diaz-Pulido et al., 2012; Martin & Gattuso, 2009). *Mesophyllum sp*. has a wide tolerance for temperature as is evidenced by its large latitudinal range in the Atlantic (Horta et al., 2011). However, when combined with elevated CO_2 in our study, increased temperature appears to be stressful for *Mesophyllum sp*., resulting in the lowest maximum quantum yield (F_v/F_m) value among all species, and even lower than the effective quantum yield value that corresponds to the average midday hour when irradiance is most intense. *Mesophyllum sp*. morphology is the most complex of the rhodolith species studied, and likely possesses the most within-thallus variability in light adaptation among protuberances, as has been shown for other complex coralline morphologies (Burdett et al. 2012). We suggest that the low light-acclimated areas may be incapable of contributing sufficiently to photosynthetic production due to the synergistic stress of coupled elevated CO_2 and temperature. Due to recent discoveries of intra-specific genetic diversity of *Mesophyllum sp*. (Sissini et al., 2014), potential physiological variations may lead to different responses to OA and OW along the Brazilian coast, thus making predictions of range shifts even more complex for this species.

The presence of neutral and negative photosynthetic responses shows that not all rhodolith species will react the same to OA and OW. While for some species, the carbon assimilation mechanisms become weaker and thermal optimums are surpassed under elevated CO_2 and temperature, others manage to maintain photosynthetic performance at normal levels due to existant adaptations. We suggest that less complex morphology is an adaptation that may be allowing for the resilience of some species of coralline algae to anthropogenic stress. The ultimate, overall health and survival of rhodoliths also depends on other physiological processes such as calcification and respiration. The semi-exposure of the calcification site to the external medium, inability to control surrounding carbonate chemistry and the deposition of High-Mg calcite, the most soluble polymorph of $CaCO_3$, are characteristics of rhodoliths, for example, that can put their calcification in danger under OA (Borowitzka & Larkum, 1987; Morse et al., 2007; Ries et al., 2009). The fact that we are observing some negative photosynthetic responses to elevated CO_2 and/or temperature corroborates the fragile position of some species of coralline algae under climate change that other studies have evidenced (Anthony et al., 2008; Díaz-Pulido et al., 2012; Foster et al., 2013; Johnson et al., 2014; Martin & Gattuso, 2009). Although various coralline species are thought to exist as both crustose coralline algae (CCA) and rhodoliths, these life forms may, however, have different responses to climate change due to the influence of their differing ecological roles (McCoy et al., 2015), and potentially the differing adaptive strategies of their morphologies (Steneck, 1986). Although we can expect many negative responses of coralline species, our results show that resilience may exist among coralline taxa. Even if the adapted species, or morphologies, survive future conditions, the niche availability for these organisms may be far lower than the present. The majority of climate change studies on coralline algae have been with CCA and very few with rhodoliths, making further studies of the latter crucial to the understanding of future populations. Thus, in order to make realistic predictions about the future of rhodolith beds, it is important that studies are multivariate, while ideally addressing the diverse morphological and ecophysiological strategies of these communities.

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CAPÍTULO 2

O capítulo 2 da presente dissertação não é uma publicação efetiva. A intenção do autor na elaboração dos capítulos da dissertação é para a defesa e obtenção de título.

Seagrass metabolic feedback may mitigate negative OA effects for calcareous algae

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Abstract

Ocean acidification (OA) and Ocean Warming (OW) are expected to have enormous impacts on marine coastal organisms across the globe, however, there are still many open questions regarding the responses that photoautotrophs will have, while simultaneously considering metabolic feedbacks between coexisting species. We manipulated multiple variables (CO_2, temperature and seagrass presence) in a novel marine mesocosm facility in Brazil in order to test the magnitude of the effects of OA and OW on the physiology of two common photoautotrophs from shallow marine coastal ecosystems in Brazil: the upright calcareous alga, *Halimeda cuneata*, and the seagrass, *Halodule wrightii*. We also tested whether or not the presence of *H. wrightii* acts as an ameliorating agent for the negative effects of OA on *H. cuneata*. We hypothesize that if large natural variability of temperature and CO_2 in shallow environments of tropical reef systems has selected for phenotypical plasticity and co-evolutionary tools among photoautotrophs, then we expect to observe increased resilience of dominant species against these global stressors. *H. cuneata* reduced calcification rates by 83% when CO_2 concentration rose from 450 to 650 ppm, yet when *H. wrightii* is in close proximity, calcification rates were only reduced by 38%. Elevated CO_2 had a neutral effect on the Net Primary Productivity (NPP) of *H. wrightii* and a positive effect for *H. cuneata*. The observed increase in photosynthesis and decrease in calcification for the calcareous alga at 650 ppm suggests that photosynthesis and calcification become uncoupled under elevated CO_2. The magnitude of temperature change in this study (+2°C) did not have any effect on the calcareous alga nor the seagrass. We conclude that *H. cuneata* populations coexisting with *H. wrightii* populations may present high resilience to OA conditions. Therefore we can speculate that coastal and shallow reef environmental drivers select metabolic interactions between calcifying and non-calcifying organisms as key strategies that provide refuge and acclimation capacity to otherwise sensitive groups.

Keywords

Calcareous algae, seagrass, Ocean Acidification, net primary production, calcification, photoautotroph

1. Introduction

Ocean Acidification (OA) has been a dominant issue in marine sciences in the last years, and it is considered among the global ocean research priorities (Rudd, 2014). This global stressor is caused by the dissolution of anthropogenic CO_2 in seawater. Local stressors, such as upwelling (Feely et al. 2008), degradation of organic matter in coastal areas (Wallace et al. 2014) and river discharge (Araujo et al. 2014), also significantly increase local CO_2 dissolution. Increased concentrations of CO_2 in the ocean, due to the large amount and geographic scale, cause the surface ocean to shift its carbon chemistry and become acidified, losing its buffering ability (Doney et al. 2009). Oceans are thus being deprived of their ability to chemically, physically and biologically sink introduced CO_2 in the deep ocean, which is greatly altering the oceanic carbon cycle (Rost & Riebesell, 2004) and affecting marine ecosystems (Doney et al. 2012; Fabry et al. 2008).

Alongside OA, CO_2 in the atmosphere feeds the Climate Change process, whose other main protagonist is ocean warming (OW). The globally averaged combined land and ocean surface temperature has already increased 0.85°C (IPCC, 2014) and is expected to gradually continue to rise as long as CO_2 emissions climb. However, chronic and acute events such as heat waves are especially responsible for important shifts in marine community structure, which will ostracize sensitive stenothermic populations and/or instigate the pole-ward displacement of distribution edges (Smale & Wernber, 2013). Extreme temperature events can outpace gradual climate trends, producing impacts in key habitats that reduce diversity, complexity and compromise ecosystem services (Wernberg et al. 2013).

Marine photoautotrophs from shallow coastal ecosystems are considered highly susceptible to these global and local stressors (Harley et al. 2006). They are responsible for 10–30 % of the world's marine primary production despite being limited to an area of 7% of the oceans' surface (Andersson & Mackenzie, 2004). Key communities such as seagrasses and calcareous algae beds play unique roles in the removal and storage of carbon from seawater, via photosynthesis and/or calcification (Copertino, 2011, Horta et al. 2016), providing a sink for 85% of the organic carbon and 45% of the inorganic carbon (C_i) in the sediments (Noriega et al. 2013). CO_2 is essential to photosynthesis, yet its excess in seawater reduces pH and threatens the calcification process (Koch et al. 2013). However, these ecosystems naturally experience large vertical and horizontal variations in abiotic parameters, namely dissolved CO_2 and temperature (Reusch, 2014), where they can vary from 400-10,000 ppm CO_2 (Noriega et al. 2013) and 15-30°C (Thompson et al. 2015). Some have suggested that the natural exposure to these fluctuations and presence of phenotypic plasticity may allow organisms to resist or benefit from novel, anthropogenic conditions (Reusch, 2014; Valladares et al. 2014).

The calcareous genus *Halimeda* J.V. Lamouroux (Halimedaceae, Chlorophyta) is a common photoautotroph in shallow coastal environments. It contributes immensely to tropical sand via shedding of bleached calcified segments (Rees et al. 2007) thanks to its calcification rate, which is comparable to that of coral reefs (Nelson, 2009). *Halimeda* morphology separates the calcification site from seawater by a layer of appressed utricles, which make up the coenocyte region where most photosynthetic activity is located (Borowitzka & Larkum, 1976). Thus, in order for seawater conditions to reach the interutricular spaces (IUSs) where calcification occurs, water must pass through the surface boundary layer and adjacent live tissue (de Beer & Larkum, 2001). Under ambient conditions, calcification is closely linked to photosynthesis in *Halimeda* because the photosynthetic process demands CO_2 uptake, which causes a shift in carbon speciation in the IUSs and a consequent pH increase that facilitates calcium carbonate precipitation (Borowitzka & Larkum, 1976). Likewise, CO_2 produced during $CaCO_3$ precipitation can be used by *Halimeda* to enhance photosynthesis (Reiskind et al. 1988), but seems not enough to sustain it, indicating possible C_i-limitation in this genus (de Beer & Larkum, 2001). *Halimeda* calcification is sensitive to pH changes in IUSs (de Beer & Larkum, 2001), and is therefore expected to suffer from OA (Kroeker et al. 2013a). Thus far, the general consensus of OA studies on *Halimeda* is negative to neutral calcification responses and neutral to positive photosynthesis responses to CO_2-enriched seawater, due to species-specificity (Campbell et al. 2014; Comeau et al. 2013; Johnson et al. 2014; Price et al. 2011; Sinutok et al. 2011; Sinutok et al. 2012; Vogel et al. 2015a, 2015b; Wizemann et al. 2015).

Seagrasses are marine angiosperms (Streptophyta) that are widely distributed around the world, but still have a low taxonomic diversity, with only 60 known species (Orth et al. 2006). Six species are currently accepted taxonomically for the genus *Halodule,* with *Halodule wrightii* Ascherson (Cymodoceaceae) described as the most common species for the Brazilian coast (Sordo et al. 2011). *Halodule* beds, generally found in the subtidal zone up to approximately 3m (Oliveira et al. 1983), are key to the shallow, sandy seafloor, due to their contribution to coastal productivity, biodiversity protection and fisheries resources (Sordo et al. 2011). Seagrasses represent important carbon stocks (blue carbon), capable of harnessing more carbon per hectare than tropical rainforests by assimilating large quantities of dissolved inorganic carbon (DIC) from seawater (Fourqurean et al. 2012; Mcleod et al. 2011; Nelleman et al. 2009). Global production of all beds is conservatively estimated at 4.2-8.4 Pg C (potentially reaching 19.9 Pg) or an average rate of 27.4 million tons of carbon immobilized per year (Fourqurean et al. 2012). Although seagrasses have high carbon assimilation rates compared to other photoautorophs, they are still considered to be C_i-limited due to limited CO_2 diffusion rates (Durako, 1993). At present pH, $CO_{2(aq)}$ makes up the smallest pool of total DIC at 1% (Koch et al. 2013; Raven et al. 2005), and is especially limited in the boundary layer surrounding leaf surfaces (Koch, 1994). Thus, abundance and diversity of seagrasses is attributed to the type and efficiency of carbon-concentrating and HCO_3^- uptake systems that result in higher or lower productivity (Bjork et al. 1997; Campbell & Fourqurean, 2013; Uku et al. 2005). When seawater is CO_2-enriched, seagrasses have been shown to increase photosynthetic rates, yet the magnitude of change and affinity to HCO_3- varies (Campbell & Fourqurean, 2013; Palacios & Zimmerman, 2007). The general expected trend for seagrasses is neutral to positive physiological responses to OA (Koch et al. 2013).

There is little known about existing interactions between calcifying and non-calcifying primary producers under the scenario of OA and OW. A metabolic interaction occurs when a primary producer consumes and/or produces dissolved gases via metabolic processes to an extent that it alters the chemistry of the surrounding microenvironment, which in turn affects the metabolism of a neighboring primary producer. These metabolic interactions represent a complex, ecological unit that is, or may be selected by actual and predicted ecological drivers. Changes in benthic macrophyte communities are projected for the future, where altered competition dynamics between fleshy and calcareous algae already have been shown to drive ecosystem shifts under elevated CO_2 conditions (Kroeker et al. 2013b). The weak comprehension of these interactions and the consequent mechanisms that drive biodiversity and ecosystem changes limit our ability to interpret spatial patterns and make long-term predictions. Seagrasses can play important roles in the control of ocean acidification by absorbing large quantities of CO_2 and reducing the acidity of seawater (Barry et al. 2013; Semesi et al. 2009; Unsworth et al. 2012). Diurnal pH fluctuations due to seagrass production have been recorded to reach 0.7-1 pH units (Hendriks et al. 2014; Semesi et al. 2009). Increased pH levels during the day can become locally significant to the point where they have a positive effect on the calcification of surrounding calcareous algae (Barry et al., 2013; Semesi et al., 2009). However, information about how the interactions between calcareous and non-calcareous photoautotrophs will affect their responses to OA and OW is still lacking.

Most of the studies regarding the impact of global stressors evaluate the isolated responses of primary producers, using only unifactorial models or eventually considering the role of OA and OW in the fitness of a specific and isolated biological indicator (reviewed in Yang et al. 2015). The isolated effects of temperature and CO_2 on *Halodule* (Campbell & Fourqurean, 2013; Ow et al. 2015) and their isolated and combined effects on *Halimeda* have been addressed (Campbell et al. 2015; Campbell, 2014; Meyer et al. 2015; Price et al. 2011; Robbins et al. 2009; Vogel et al. 2015a, 2015b; Wizemann et al. 2015). Although two studies have considered the effect of seagrass-*calcareous algae* interactions under ambient conditions (Barry et al. 2013; Semesi et al. 2009), no studies address the effects of seagrass-*Halimeda* interactions on OA and OW responses. The species-specific nature of the isolated responses emphasizes the necessity to conduct more studies that address OA and OW together in order to better understand the mechanisms behind the presence/absence of interactions between these stressors. Mesocosm experiments of short duration that simulate rapid heat waves and acidification, as is observed in different regions due to global climate change and local stressors, serve as critical tools for predicting complex ecosystem interactions. The acute variation of CO_2 and temperature in a short assay has great potential for showing how communities and their functioning may change. Thus, this study aimed to quantify and compare the effects of OA and OW on the photosynthesis and calcification of *Halimeda cuneata* Hering and *Halodule wrightii*, which commonly coexist in the shallow tropical waters of the Brazilian coast (Bandeira-Pedrosa et al. 2004b). Also, we determined if a metabolic feedback originating from the high photosynthetic activity of *H. wrightii* could ameliorate the potential negative effects of OA and OW on the physiology of *H. cuneata*. We hypothesize that if large variations in seawater [CO_2] and temperature are tolerated or even beneficial for seagrasses, then metabolic interactions between seagrasses and shallow calcareous algae, such as *H. cuneata*, may mitigate the negative OA and OW effects predicted for calcareous algae.

2. Material and Methods

2.1. Study Area

The Marine Mesocosm is one of Projeto Coral Vivo's research facilities, located on Araçaípe beach in Arraial d'Ajuda, Bahia, Brazil. It was designed to test the effects of elevated CO_2 and elevated temperature (and other factors) on marine organisms, while most closely mimicking the adjacent reef conditions. As described by Duarte et al. (2015), the system is composed of 16 experimental tanks, four header tanks and four mixing tanks (Appendix A Fig. 1). Seawater is pumped from 500 m off the coast, brought to the system, and enters into four 5000 l mixing tanks, where the four treatments are applied. Two of the tanks receive an elevated temperature and thus each contains a 2 m-long heater. The two tanks that receive an increased CO_2 concentration are injected with CO_2 gas and mixed to homogenize the concentration. The water from each mixing tank is then pumped to its corresponding 310 l header tank, which is located in the control house. There, treatments are programmed and monitored, based on the current CO_2 concentration and temperature of the adjacent reef, by an Arduino open source platform (Reef Angel), four temperature sensors and four pH sensors. After treatment regulation, the water from each header tank is distributed among 4 randomized experimental tanks (120 l) where the marine specimens are located, totaling 16 tanks.

2.2. Sampling

Aracaípe beach's (16° 29' 29" S 39° 4' 7" W) fringing reef commences 100m off the coast of the Marine Mesocosm, where the seagrass, *H. wrightii,* and the upright calcareous green alga, *H. cuneata*, coexist in close proximity (Fig. 1). Approximately 160 specimens of *H. cuneata* and a biomass of 480 g of *H. wrightii* were collected at a depth of 2m using SCUBA by carefully removing the entire holdfast and rhizome, respectively, and were brought to the holding aquariums of the Marine Mesocosm for the sorting and removal of plant and animal epibionts. Sediment from the first 10cm of the sampling area was also collected for subsequent planting in the mesocosm. The species-level identification for *Halimeda* and *Halodule* was confirmed as *H. cuneata* and *H. wrightii* using keys from Bandeira-Pedrosa et al. (2004a) and Kuo & Den Hartog (2001), respectively. Samples of the two were adequately deposited in the Herbarium FLOR (0059444, 0059434) at the Federal University of Santa Catarina, Brazil.

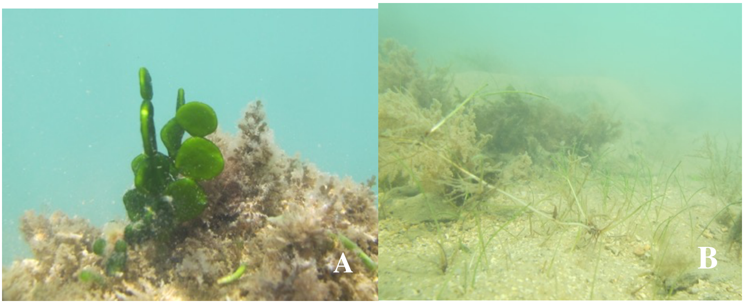

Figure 1. (A) *Halimeda cuneata* and (B) *H. wrightii* within an approximate proximity of 0.5m in the subtidal zone at a depth of 2m off of Aracaípe beach, Arraial d'Ajuda, Bahia, Brazil. Photo credentials: Paulo Horta.

2.3. Experimental design

A 10 day-long mesocosm experiment was conducted to study the effects of OA and OW on *H. cuneata* and *H. wrightii*. The bifactorial design was composed of the four following treatments: 450 ppm & 28°C (control), 650 ppm & 28°C, 450 ppm & 30°C and 650 ppm & 30°C in accordance with the moderate RCP 6.0 scenario (IPCC, 2014). Control temperature and CO_2 concentration refer to present environmental conditions of the adjacent reef. Ten *Halimeda cuneata* individuals were placed upright in a plastic tray (40x17x4 cm) that had 3cm of sand by burrying the holdfasts in the sand. One tray was placed in each of the 16 experimental tanks. While assuring that *H. wrightii* never entered in contact with the air, an approximate biomass of 15 g of seagrass was replanted in each of 2 plastic trays that had 3cm of sediment in each experimental tank. Each of the 16 experimental tanks thus possessed 3 trays, 1 with *H. cuneata* and 2 with *H. wrightii*. Specimens were acclimated for 15 days at an average temperature of 28 ± 0.5°C and a CO_2 concentration of 442 ± 42 ppm. Treatments commenced upon completion of the acclimation period, reached expected levels within 24 hours and were applied for a total of 10 days. CO_2 treatments were maintained at 442 ± 42 ppm and 663 ± 75 ppm, and temperature treatments were kept at 28.0 ± 0.5°C and 30.0 ± 0.5°C.

Abiotic parameters were measured daily in each experimental tank: salinity (Refractometer: Instrutherm RTS-101ATC), dissolved oxygen & temperature (Portable dissolved oxygen meter: Instrutherm MO-900), incident irradiance (Quantometer: apogee MQ-200) and pH (pHmeter: Gehaka ISO 9001). The daily average photosynthetically active radiation (PAR) was monitored with light loggers deployed in tanks at the level where organisms were situated (Fig. 2). Nutrient concentrations were monitored in each treatment every 3 days according to Grasshoff et al. (1983) and were maintained at the following concentrations: 0.17 ± 0.06 μM phosphate (PO_4^{3-}), 1.22 ± 0.07 μM nitrate & nitrite ($NO_3^- + NO_2^-$), and 1.64 ± 0.14 μM Ammonium (NH_4^+).

Short incubations were conducted in order to test individual species responses to elevated temperature and CO_2 as well as metabolic interactions between the species. Measurements of oxygen evolution and total alkalinity allowed us to calculate net primary production (NPP), light calcification rates and dissolved inorganic carbon (DIC) speciation responses. Incubations were performed immediately before commencing treatments and on the last three days of the experiment, starting with the eighth day. Three days of final incubations were necessary to obtain sufficient replication (n=3 per treatment) and were deemed adequate due to the minimization of day-to-day variability of responses by using fixed artificial light (750 μmol quanta m^{-2} s^{-1}). This irradiance intensity was chosen because it was representative of average midday irradiance on days when tests were performed and it resulted in the highest oxygen production, even though it is higher than the average intensity during midday throughout the entire experiment (Fig. 2). Initial incubations were carried out for 1.25 hours during midday immediately before treatment commencement in four experimental tanks representing initial control conditions (28°C & 450 ppm). The eighth experimental day marked the first of three consecutive days of 2.5-hour final incubations beginning at midday. One replicate tank per treatment was incubated each day, totaling 3 replicates (n=3).

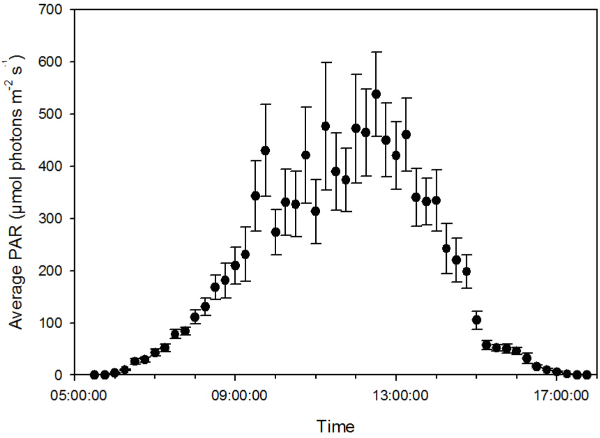

Figure 2. Average daily PAR (+ SE) during the duration of the experiment.

2.4. Incubations

An incubator was constructed with four metallic vapor lamps (220V, REV426A4, Serwal) positioned over four independent dark maroon 28L boxes (22.5x35x50cm, Marfinite). Each box was connected to a separate experimental tank by a 12 mm (diam.) hose so that seawater was constantly renewed in the box. Thus, each box corresponded to a treatment (Appendix A Fig. 2). In each box, or replicate incubation, four transparent 29x39 cm plastic sacks impermeable to dissolved oxygen were manually filled with approximately 500 ml of seawater and three of the four possessed the following conditions: only ~ 10 g (fresh weight) *H. cuneata*, ~10 g *H. cuneata* & ~1.5 g *H. wrightii* and only ~1.5 g *H. wrightii*. The fourth sack contained only seawater in order to monitor any background changes in oxygen concentration due to microorganisms. Initial water samples were taken for measurements of dissolved oxygen concentration ([DO]) and total alkalinity (A_T). Sacks were removed of all visible air bubbles prior to sealing. Lamps were simultaneously turned on and reached an intensity of 750 ± 80 μmol quanta m^{-2} s^{-1} within 5 minutes and remained constant throughout the entirety of the incubation. Upon terminating incubation time, sacks were opened, final water samples were taken for measurements of [DO] and A_T, water volume was measured and *H. cuneata* and *H. wrightii* were removed, dried at 60°C and weighed. Basal segments were then removed from each sample, decalcified with nitric acid (0.6M HNO_3) and weighed to determine the dry decalcified weight.

2.5. Oxygen evolution

Five 12 ml samples of water were collected from each treatment box at the beginning of the incubation. Upon termination, five more were extracted from each sack via a small tube that allows for a 60ml syringe to remove water without it entering in contact with the air. These samples were immediately treated with manganese chloride and alkaline-iodide reagents upon removal and refrigerated for 72 hours, when they were treated with a sulfuric acid reagent and analyzed spectrophotometrically according to the Winkler method adapted by Labasque et al., 2004.

NPP results were obtained by first normalizing O_2 production values by incubation time and volume of seawater, then removing background O_2 fluctuations due to microbial respiration, and then normalizing by the decalcified dry weight.

2.6. Alkalinity and calcification

One 180 ml water sample was collected from each treatment box (n=3) at the beginning of the incubation, and upon termination, one was extracted from each replicate sack via the small, connected tube (as detailed above in *Oxygen Evolution*). Water samples were immediately refrigerated until analysis. Alkalinity measurements were performed according to Yao & Byrne (1998) using a custom USB4000 spectrophotometer (Ocean Optics, Dunedin, USA). Alkalinity data, along with other measured abiotic parameters, were inserted into the program CO2SYS (Pierrot et al. 2006) in order to obtain the DIC speciation of seawater pre- and post-incubation. DIC species include bicarbonate (HCO_3^-), carbon dioxide (CO_2) and carbonate (CO_3^{2-}), where Global DIC denotes all DIC species together. Consumption of each DIC species and Global DIC was calculated by subtracting the post-incubation value from the pre-incubation value.

Calcification rates were calculated for *H. cuneata* using the following equation, adapted from Yao & Byrne (1998):

$$g = -0.5 \frac{\Delta A_T V}{DW \Delta t} \qquad (1)$$

Where $g = \mu$mol $CaCO_3$ g^{-1} h^{-1}, ΔA_T = change in total alkalinity, V= volume of seawater in incubation sack, DW=dry weight of *H. cuneata* and Δt = incubation time (hr).

2.7. Statistical analysis

Tests of homocedasticity of variances (Cochran) and normality (Shapiro-Wilk's) were applied to all data. We performed a 3-way Analysis of Variance (ANOVA) on two variables, calcification and log-transformed NPP data, with three factors (temperature: two levels, CO_2: two levels and species present: three levels) and significance when $p<0.05$ in Statistica 10.0. Student Newman Keuls (SNK) post-hoc tests were used on significant effects. DIC evolution data were not normal nor homocedastic, thus we proceeded with non-parametric statistics, applying a Permutational Multivariate Analysis of Variance (PERMANOVA) and Monte Carlo test to all data in order to obtain a global evaluation of physiological parameters. We used three factors (temperature: two levels, CO_2: two levels and species present: three levels) with statistical significance when unique permutations *(p) and p(MC) (Monte Carlo)* <0.05, using the software PRIMER 6.1.13 & PERMANOVA+ 1.0.3 (Clarke & Anderson, 2010). A Bray–Curtis dissimilarity matrix was generated using all samples with log(x+1) transformed data (Clarke & Warwick, 1994). Multidimensional Scaling (MDS) graphs were created to show similarities between species as well as among all treatments.

3. Results

The light calcification and photosynthesis of *H. cuneata* were negatively and positively affected by high CO_2, respectively, and the presence of *H. wrightii* reduced the potentially harmful effects of OA on *Halimeda* calcification (Table 1, Fig. 3). The +2°C temperature increase had no effect on the physiology of either organism (Table 1).

Table 1. Significant effects from the 3-way ANOVA of Calcification and Net Primary Production (NPP) values with species, temperature and CO_2 treatments as factors. NPP log10 transformed. Significance when p<0.05.

Source of variation	Calcification				NPP			
	df	MS	*F*	*p*	df	MS	*F*	*p*
Species (sp.)	1	95.697	1.514	0.2363	2	6.466	150.650	**<0.0001**
Temperature (temp.)	1	75.249	1.191	0.2914	1	0.052	1.206	0.2830
CO_2	1	1707.147	27.009	**0.0001**	1	0.04333	1.0096	0.3250
Sp.*temp.	1	7.430	0.118	0.7362	2	0.03246	0.7562	0.4803
Sp.*CO_2	1	288.016	4.557	**0.0486**	2	0.15435	3.5963	**0.0430**
Temp.*CO_2	1	61.193	0.968	0.3398	1	0.02694	0.6277	0.4360
Sp.*temp.*CO_2	1	0.289	0.005	0.9469	2	0.03054	0.7115	0.5010

Table 2. Average a) Calcification and b) NPP values and Student Newman Keuls (SNK) post-hoc results from 3-way ANOVA of Calcification and NPP values for Species*CO_2 effects. H&H = *Halimeda* and *Halodule*.

a) SNK

Species	CO_2	Average Calcification	*Halimeda* & 450 ppm	*Halimeda* & 650 ppm	H&H & 450 ppm	H&H & 650 ppm
Halimeda	450 ppm	29.02		**0.0006**	0.5318	**0.0324**
Halimeda	650 ppm	4.90	**0.0006**		**0.0010**	**0.0303**
Halimeda and *Halodule* (H&H)	450 ppm	26.09	0.5318	**0.0010**		**0.0459**
Halimeda and *Halodule* (H&H)	650 ppm	16.15	**0.0324**	**0.0303**	**0.0459**	

b) SNK

Species	CO_2	Average NPP	*Halimeda* & 450 ppm	*Halimeda* & 650 ppm	H&H & 450 ppm	H&H & 650 ppm	*Halodule* & 450 ppm	*Halodule* & 650 ppm
Halimeda	450 ppm	1.20		**0.0117**	**0.0002**	**0.0003**	**0.0001**	**0.0001**
Halimeda	650 ppm	2.12	**0.0117**		**0.0143**	**0.0361**	**0.0001**	**0.0002**
H&H	450 ppm	5.13	**0.0002**	**0.0143**		0.4088	**0.0001**	**0.0002**
H&H	650 ppm	4.15	**0.0003**	**0.0361**	0.4088		**0.0002**	**0.0001**
Halodule	450 ppm	44.27	**0.0001**	**0.0001**	**0.0001**	**0.0002**		0.8804
Halodule	650 ppm	43.38	**0.0001**	**0.0002**	**0.0002**	**0.0001**	0.8804	

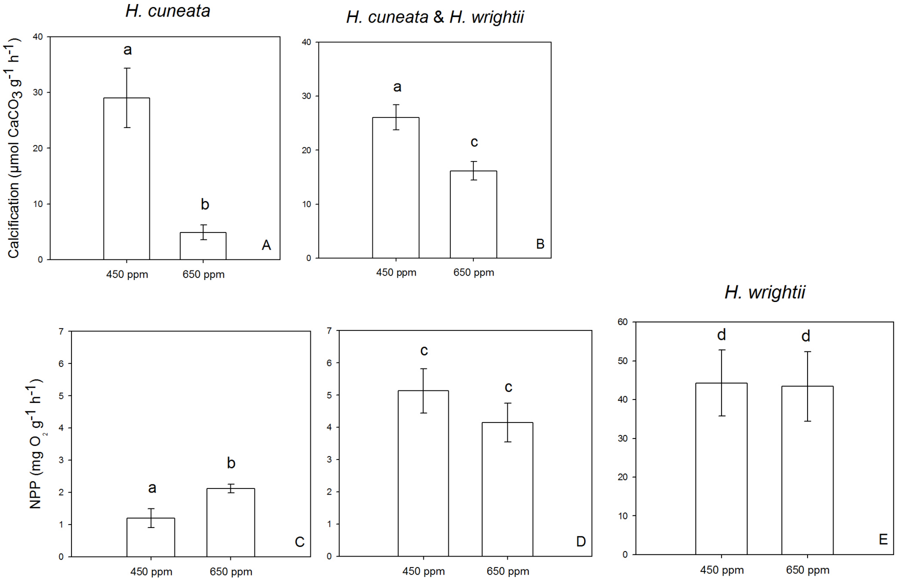

Figure 3. (previous page) Light Calcification responses for Species*CO_2 effects are shown for (A) *H. cuneata* and (B) *H. cuneata* in the presence of *H. wrightii*, and Net Primary Production (NPP) responses for Species*CO_2 effects are represented for (C) *H. cuneata* alone, (D) *H. cuneata* with *H. wrightii* and (E) *H. wrightii* alone. Calcification values were normalized by calcified dry weight (+ SE) whereas NPP values were normalized by decalcified dry weight (+ SE). Different lowercase letters (a,b,c…) mark significant differences within response variables (rows) from the SNK post-hoc test from the 3-way ANOVA. Responses were the result of incubations of *H. cuneata* and *H. wrightii* under ambient (450ppm) or elevated (650ppm) CO_2 levels. n=6.

Table 3. PERMANOVA results of Calcification, NPP, Global DIC consumption and CO_2 consumption values, where all data was log(x+1) transformed and significance was considered when unique *p(perm)* (p value using permutations) and *p(MC)* (p value using Monte Carlo sampling) were <0.05.

Source of variation	df	SS	MS	Pseudo-F	Unique *p (perm)*	perms	*p(MC)*
Species	2	10174.00	5087.20	168.750	**0.0001**	9942	**0.0001**
Temp.	1	47.66	47.66	1.581	0.2128	9945	0.2119
CO_2	1	284.94	284.94	9.452	**0.0028**	9943	**0.0031**
Sp.*temp.	2	69.06	34.53	1.145	0.3274	9947	0.3335
Sp.*CO_2	2	278.87	139.43	4.625	**0.0146**	9951	**0.0122**
Temp.*CO_2	1	56.93	56.93	1.888	0.1713	9934	0.1698
Sp.*temp.*CO_2	2	96.98	48.49	1.609	0.2069	9943	0.2094

Table 4. Summary of pair-wise test results from the PERMANOVA for Calcification, NPP, Global DIC consumption and CO_2 consumption values for Species*CO_2 effects. Significance when unique *P(perm)* (p value based on permutations) and *p(MC)* (p value based on Monte Carlo sampling) <0.05.

Species *CO_2		t	Unique *p(perm)*	perms	*p(MC)*
Species					
I) Halimeda					
	a) 450ppm, 650ppm	2.7801	**0.0031**	8950	**0.0036**
II) Halimeda & Halodule (H&H)					
	b) 450ppm, 650ppm	1.8661	**0.0368**	8924	**0.0449**
III) Halodule					
	c) 450ppm, 650ppm	1.5522	0.1103	8935	0.1068
CO_2					
I) 450ppm					
	a) Halimeda, H&H	2.7395	**0.0023**	8967	**0.0036**
	b) Halimeda, Halodule	10.237	**0.0007**	8845	**0.0001**
	c) H&H, Halodule	11.983	**0.0012**	8882	**0.0001**
II) 650ppm					
	a) Halimeda, H&H	4.0461	**0.0021**	8932	**0.0002**
	b) Halimeda, Halodule	13.587	**0.0024**	8802	**0.0001**
	c) Halimeda & H&H	15.685	**0.0013**	8701	**0.0001**

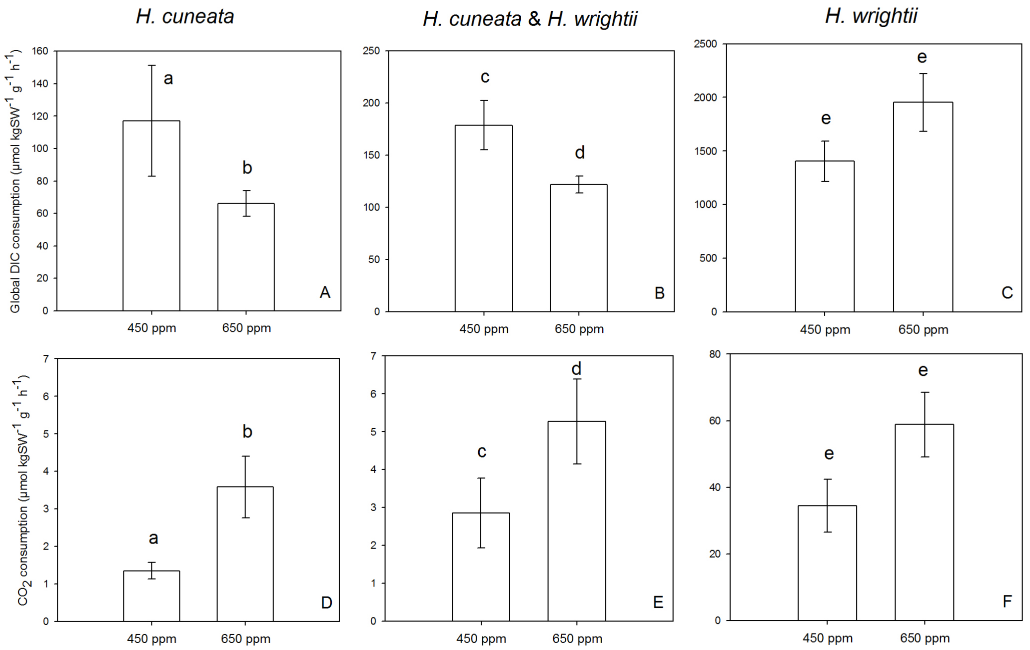

Figure 4. (previous page) Global Dissolved Inorganic Carbon (DIC) consumption responses (+ SE) for Species*CO$_2$ effects shown for (A) *H. cuneata* alone, (B) *H. cuneata* with *H. wrightii* and (C) *H. wrightii* alone. CO$_2$ removal responses (+ SE) for Species*CO$_2$ effects given for (D) *H. cuneata* alone, (E) *H. cuneata* with *H. wrightii* and (F) *H. wrightii* alone. Different lowercase letters (a,b,c…) mark significant differences within response variables (rows) from the PERMANOVA.

3.1. Calcareous algae response

There was a significant effect of CO_2 on the calcification and photosynthesis of *H. cuneata* (Table 1). OA compromised the calcification response of *H. cuneata* as it suffered an 83% decrease in light calcification at 650 ppm (Fig. 3A). NPP of *H. cuneata* was positively affected by OA, as it increased by 77% at 650 ppm (Fig. 3C). The consequences of these metabolic responses can be identified in the resulting seawater chemistry. All DIC parameters (HCO_3^-, CO_2, CO_3^{2-} and global DIC consumption) showed a significant interactive effect between CO_2 and species present (Table 3). HCO_3^- and global DIC consumption portrayed very similar patterns due to the fact that bicarbonate makes up 90% of the global DIC pool. Therefore, we use global DIC to describe general patterns. At 650 ppm *H. cuneata* calcification decreased and photosynthesis increased, and there was a 43% decrease in global DIC consumption and a 177% increase in CO_2 consumption (Fig. 4A & 4D).

3.2. Seagrass response

Halodule wrightii experienced no significant change in NPP from 450 to 650 ppm (Fig. 3E). NPP of *H. wrightii* is observed to be ~ 37 times greater, per gram of dry photosynthetically-active biomass, than *H. cuneata*. Due to the much higher NPP of *H. wrightii*, compared to *H. cuneata*, it consumed 12 times more global DIC at ambient CO_2 and 29.6 times more under OA, which was responsible for the metabolic feedback when the species coexist. When *H. wrightii* was alone, global DIC and CO_2 were consumed equally at 450 ppm and 650 ppm (Fig. 4C & 4F).

3.3. Metabolic Interaction

When *H. wrightii* and *H. cuneata* coexist at 650 ppm, the metabolic interaction between the two mitigated the negative impact of OA and caused the light calcification rate of *H. cuneata* to be reduced by only 38% (as opposed to 83%; Fig. 3B). In other words, at 650 ppm, the presence of *H. wrightii* raised the light calcification rate of *H. cuneata* 3.3-fold. When *H. cuneata* and *H. wrightii* are together, joint NPP does not differ significantly from 450 to 650 ppm (Table 2). However, since we did not have a way to separate the NPP of each species when together, we chose not to elaborate on potential changes in photosynthesis due to the presence of the other species.

The MDS based on overall responses of *H. cuneata*, *H. wrightii* and *H. cuneata* & *H. wrightii* (Fig. 5A) formed three main clusters due to dissimilarity between species responses to OA and OW. The MDS based on all treatments (Fig. 5B) formed various clusters, specifically illustrating dissimilarities between *H. cuneata* responses at 450ppm and 650 ppm, and between the three groups that differ in which species is present.

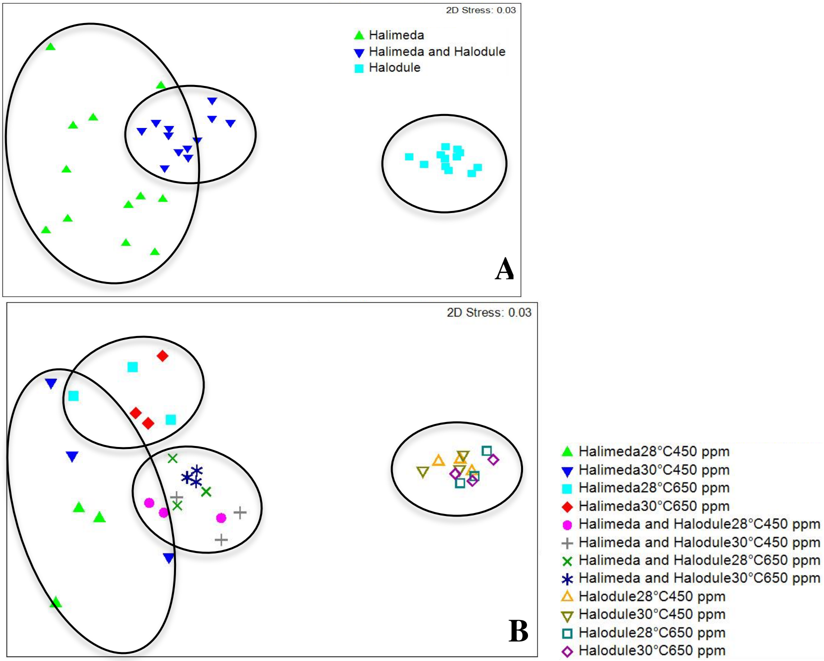

Figure 5. Multidimentional scaling (MDS) analysis showing similarities between A) species and B) all treatments (species x temperature x CO_2 concentration) relative to treatment conditions.

4. Discussion

OA, caused by local (Wallace et al. 2014) and/or global stressors (IPCC, 2014), threatened the physiology and the biological role of the upright calcareous alga, *H. cuneata*. Even in an experiment of short-duration, this species presented decreased light calcification due to elevated CO_2 concentrations. However, the presence of the seagrass *H. wrightii* mitigated the negative effect of OA and *H. cuneata* calcification remained at or above values observed by Campbell et al. (2014) for *Halimeda* species at ambient conditions, more than 3-fold higher than when the seagrass is absent. These results are corroborated by the findings of Barry et al. (2013) and Semesi et al. (2009), which showed that *in situ* seagrass photosynthesis influences rates of calcification in calcareous macroalgae. We confirmed that under high CO_2 concentrations, seagrass could change coastal micro-environments by relieving seawater of C_i and enhancing the calcification of otherwise ill-fated, surrounding *Halimeda*.

Temperature had no isolated nor combined (with CO_2) effect on the calcification or photosynthesis of *H. cuneata* nor on the photosynthesis of *H. wrightii*. We conclude that an elevation of 2°C was simply not sufficient to invoke physiological responses for these populations since daily-weekly variation in temperature in this area approaches this value. Phenotypic plasticity or even genetic adaptations likely account for the absence of physiological response to this magnitude of temperature variability. We do not suggest that increased temperature has no absolute effect on photoautotroph physiology. Actually, OA studies that incorporated an increase of 3-4°C showed synergistic effects on calcareous algae mortality (Diaz-Pulido, 2012; Martin & Gattuso, 2009). In a meta-analysis testing responses of marine organisms to OW and OA in isolation and combination, it was concluded that as a rule, coupling stressors presented a stronger biological effect (Harvey et al. 2013). Thus, we still deem it essential to provide a combined temperature and CO_2 experimental design that is appropriate for the geographical area being tested in order to perceive realistic physiological responses to OA and OW.

4.1. Calcareous algae response

We interpret the decreased light calcification rate of *H. cuneata* under elevated CO_2 to be the result of the acidified microenvironment around the calcification site. Global DIC consumption decreased at 650 ppm, likely due to the reduced use of HCO3-, CO_2 and CO_3^{2-} for calcification. The calcification of many benthic macrophyte organisms is slowed, stopped or even reversed to the point of decalcification when seawater is acidified (Kroeker et al. 2010). *Halimeda* has been reported to reduce or even stop calcification upon exposure to seawater enriched with CO_2, which has been correlated with decreased growth in some cases (Johnson et al. 2014; Meyer et al. 2015; Price et al. 2011; Sinutok et al. 2011, 2012). Since nocturnal calcification naturally tends to be significantly lower (or even negative) than day calcification in *Halimeda* (Borowitzka & Larkum, 1976), and upon exposure to elevated CO_2 has been shown to be reduced even more (Meyer et al. 2015; Vogel et al. 2015a, 2015b), based on our results we can expect very low to no net calcification from *H. cuneata* under increased CO_2. Additionally, *Halimeda* grows new segments at night when herbivorous fishes are inactive (Hay et al. 1988). If OA reduces light calcification, the protection of these new segments may be compromised. Prolonged reduction in calcification is bound to have ramifications for organismal energetic balances, growth, entire thallus integrity and increasing susceptibility to breakage and grazing (Campbell et al. 2014).

Responses have been observed to be species-specific among calcifiers due to the diversity of calcification mechanisms, including the form of $CaCO_3$ precipitated by the organism (Vogel et al. 2015a), as well as anatomical diversity, such as varying sizes and arrangements of utricles and IUSs among *Halimeda* species (Price et al. 2011). *Halimeda cuneata* precipitates aragonite (Borowitzka & Larkum, 1976), one of the most soluble forms of $CaCO_3$, putting it at a higher risk than calcite-precipitating (except high-Mg calcite) calcifiers under OA (Bach, 2015). More heavily calcified species of *Halimeda* are more susceptible to OA than the less calcified (Price et al. 2011), where *H. cuneata* is considered moderately calcified in the *Halimeda* lineage of the genus (Verbruggen & Kooistra, 2004). Conservation priority should be given to such sensitive species.

Although calcification is compromised under elevated CO_2, *H. cuneata* increased its NPP by using excess anthropogenic DIC as substrate. Previous experiments that studied the effects of OA on *Halimeda* photosynthesis report mostly neutral to negative responses (Johnson et al. 2014; Meyer et al. 2015; Price et al. 2011; Sinutok et al. 2011, 2012). However, due to the species-specific nature of responses within the genus, in the present study, *H. cuneata* likely achieved an increase in NPP under elevated CO_2 conditions because it no longer was CO_2-limited, and therefore depended less on the energetically expensive active transport of HCO_3^- as substrate (Tait, 2014). We predict that *H. cuneata* prefers to utilize CO_2, as shown by increased CO_2 consumption, and does so until it becomes sparse, at which point HCO_3^- is used, but at a less efficient rate due to energetic costs (Borowitzka & Larkum, 1976). It is worthy to mention that under elevated CO_2, the increase in photosynthesis and growth of some calcareous algae is not likely when light is limiting due to low intensities (Tait, 2014). Thus, the intensity we used in incubations, 750 μmol m^{-2} s^{-1}, appears to be a non-limiting quantity of solar radiation for *H. cuneata* for it to be able to increase photosystem and carbon fixation activity in the presence of excess DIC.

Borowitzka & Larkum (1976) suggested and De Beer & Larkum (2001) provided evidence that links photosynthesis and calcification in *Halimeda* at ambient CO_2 conditions. However, due to our observed decrease in calcification, we can infer that the increase in photosynthesis is not capable of countering the negative effects of increased anthropogenic CO_2, thus corroborating the hypothesis that photosynthesis and calcification can become uncoupled under high CO_2 (Koch et al. 2013). Since IUSs are in dynamic equilibrium with the external medium (Borowitzka & Larkum, 1976), we suggest that under elevated CO_2, IUSs are constantly replenished with DIC and high [H+] at a much quicker rate than photosynthesis can remove DIC, and thus *H. cuneata* calcification remains partially inhibited. Excess H+ not only directly inhibits the calcification metabolic pathway (Borowitzka & Larkum, 1976), but also induces the formation of reactive oxygen species (ROS) in the cytoplasm, which is known to produce various consequences that are capable of increasing respiration rates, displacing ATP and redistributing it to different processes other than calcification (Torres et al. 2008).

4.2. Seagrass response

The observed lack of significant changes in the photosynthesis of *H. wrightii* due to elevated CO_2 or temperature corroborates results from recent meta-analyses that show neutral-positive photosynthetic responses of tropical seagrasses to elevated CO_2, as most are naturally CO_2-limited (Koch et al. 2013; Kroeker et al. 2010). Due to its much higher ambient photosynthetic rate (44.27 ± 8.5 (SE) mg O_2 g^{-1} h^{-1}), as compared to *H. cuneata* (1.2 ± 0.3 (SE) mg O_2 g^{-1} h^{-1}), *H. wrightii* removes 12-29.6 times more DIC from seawater. DIC removal increases seawater pH (Invers, 2001), HCO_3^-/H^+ ratio, CO_3^{2-} availability and aragonite/calcite saturation states (Bach, 2015). Large diurnal pH fluctuations due to seagrass photosynthesis are reported for shallow coastal systems and have been recorded to reach 0.7-1 pH units

(Hendriks et al. 2014; Semesi et al. 2009). We do not, however, report the seagrass density of the studied *Halodule* bed, which is a factor that is shown to affect the magnitude of *Halimeda*-seagrass interactions (Davis & Fourqurean, 2001). Our results support that *Halodule* populations are projected to withstand moderate OA scenarios, yet local irradiance, temperature and nutrient conditions may very well determine the density of seagrass beds and the degree to which the seagrass can increase production. Interspecific variation in photosynthetic responses among seagrasses due to diverse HCO_3^- assimilation mechanisms (Beer et al. 2006) will also put certain species at an advantage over others. The extent to which populations are acclimated to temporarily elevated conditions may determine long-term health.

4.3. Metabolic Interaction

Halimeda cuneata achieves higher calcification rates in the presence of *H. wrightii* under OA because the seagrass photosynthetically removes a significant fraction of anthropogenic DIC from the seawater and thus decreases H^+ concentration, or increases pH, which permits *H. cuneata* calcification to increase. Thus, *Halodule* populations may allow the survival of coexisting *H. cuneata* populations. It is due to the aforementioned ability of *H. wrightii* to de-acidify seawater that our results suggest that *H. cuneata* could survive 650 ppm of CO_2. Another *Halimeda* species, *H. renchii,* was also shown to increase calcification rates 1.6-fold in seagrass beds, however at ambient CO_2 levels (Semesi et al. 2009). We validate that under OA, the presence of seagrass is able to promote calcification rates comparable to, yet still lower than, those at ambient CO_2 conditions, however is not capable of increasing calcification rates any higher. The fate of calcareous algae under OA may very well lie in the accompanying photoautotroph community composition. Fleshy macroalgae are reported to directly and indirectly compete with calcareous algae under ambient and CO_2-enriched conditions (Hofmann et al. 2012; Kroeker et al. 2013b; Russell et al. 2009), and competition for nitrogen between seagrass and *Halimeda* was even reported under ambient field conditions (Davis & Fourqurean, 2001). OA allows seagrasses to optimize carbon assimilation, potentially putting seagrasses at an advantage (Campbell & Fourqurean, 2013). However, for calcareous macroalgae that can also increase primary production under OA, like *H. cuneata*, we propose that seagrasses aid in seawater buffering more than they compete. In each locale where *Halimeda* occurs, the specific photoautotroph community composition is likely to alter seawater chemistry in slightly different ways. If surrounding species achieve seawater pH increase, allowing *Halimeda* to properly calcify, perhaps competition between those surrounding photoautotrophs will determine future community shifts.

It is still unknown whether and which calcareous algae will adapt to OA & OW (Hofman & Bischof, 2014). The presence of physiological tolerance or increased performance to changes in CO_2 and temperature in the marine environment can be due to trans-generational plasticity, phenotypic buffering, or plasticity within generations (or 'classical' plasticity) and may develop into 'true' evolutionary adaptation via changes in DNA (Reusch, 2014). Genetic variation for traits important for OA and OW seem to be prevalent in near-shore plants (Reusch, 2014). Based on our results, we suggest that the large natural variability of temperature and CO_2 in shallow coastal environments has selected for phenotypic plasticity and co-evolutionary tools involving the metabolic feedback between *Halimeda* and *Halodule*, thus potentially providing high resilience to OA. Plasticity to OA and OW will help maintain populations under changing environments (Chevin et al. 2010). If *Halimeda* species have adequate genetic variability to generate phenotypes with different CO_2 tolerances and optima, then it is probable that inter or intraspecific variability in fitness will be observed, where OA winners are likely to be those coexisting with seagrasses. The potential evolutionary adaptability of this metabolic interaction makes it likely that these organisms may survive future oceanic conditions in coexistence.

4.4. Conclusion

Our findings show that seagrass *H. wrightii* and upright calcareous macroalga *H. cuneata* may survive acute increases in OA in coexistence. Longer experimental evaluations of this metabolic interaction are required to provide support for our hypothesis in the long-term, as well as the potential manipulation of other limiting factors such as light and nutrients to better understand the dynamic of the interaction and individual metabolisms. There are few global studies that address marine plant interactions in the realm of OA and OW (Tait, 2014). This study represents just the beginning of many multivariable studies needed to analyze how metabolic feedbacks may influence the physiological effects of OA and OW on marine photoautotrophs, crucial for understanding shifts in coastal primary producer communities. Not only will the responses we observed aid in understanding possible community composition shifts and trophic cascades, but offer predictions on the adaptability of *Halodule* and *Halimeda*, and will surely provide solid criteria for the determination of marine protected areas and management options in attempts to save foundation species from the threats of Climate Change.

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3. DISCUSSÃO GERAL E CONCLUSÃO

O presente estudo observou respostas à acidificação oceânica e ao aumento da temperatura que são específicas para cada espécie de alga calcária, enquanto a grama marinha não mostrou mudanças na sua fisiologia, ilustrando a variabilidade fisiológica dos organismos marinhos costeiros. No experimento envolvendo a comunidade de rodolitos, a observação simultânea de respostas negativas e neutras no rendimento fotossintético nos indica que algumas espécies irão sofrer enquanto outras manterão seu estado fisiológico. *Mesophyllum sp.* mostrou uma interação sinérgica negativa entre o CO_2 e a temperatura, um efeito corroborado globalmente para várias espécies de algas calcárias não articuladas (Díaz-Pulido et al. 2012; Martin & Gattuso, 2009). A variação nas respostas pode ser devido as pressões evolutivas que resultaram em diferentes mecanismos de assimilação de carbono e faixas ótimas de temperatura. No entanto, propõe-se também que a seleção ambiental do nível de complexidade morfológica poderia ser correlacionada negativamente com a resiliência fotossintética dos rodolitos contra estressores globais. Acredita-se que a abundância e composição da comunidade de rodolitos poderá alterar de acordo com as necessidades de calcificação, possivelmente favorecendo *Lithophyllum sp.* e outras espécies com morfologias simples.

Para entender melhor o efeito que terão as mudanças ambientais antropogênicas na fisiologia e eventual sobrevivência dos produtores primários que fazem parte de comunidades costeiras, o segundo capítulo adicionalmente considerou a interação metabólica entre as algas calcárias e gramas marinhas. A resposta isolada de *H. cuneata* foi uma drástica redução na calcificação sob elevado CO_2, indicando uma séria desvantagem para o metabolismo dessa espécie de alga calcária. A grama marinha, *H. wrightii*, apresentou os mesmos níveis de produção primária sob níveis atuais e elevados de CO_2, porém sendo muito maior quando comparada com *H. cuneata*. Foi através dessa alta taxa de fotossíntese que a presença de *H. wrightii* removeu grandes quantidades de DIC da água, mitigando a acidez e possibilitando o aumento na calcificação de *H. cuneata*.

Concluímos que a fisiologia das algas calcárias é susceptível a OA e OW. Porém, algumas algas e a grama marinha apresentaram respostas que sinalizam plasticidade fenotípica, morfologías resilientes e até possíveis adaptações que irão ajudar na sobrevivência de algumas espécies. No entanto, a anásile da interação entre uma alga calcária e grama marinha que coexistem permitiu a observação da amenização dos efeitos da OA e OW na fisiologia da alga calcária. Sem a presença da interação metabólica, as algas calcárias poderão ter dificuldades na manutenção de um talo suficientemente calcificado para resistir a hidrodinâmica e pressão de herbivoria do local (Campbell et al. 2014). A variedade nas respostas entre as algas calcárias e a grama marinha é devido a não só aos diversos mecanismos fisiológicos (Campbell & Fourqurean, 2013), mas também às limitações de outros recursos como nutrientes e radiação solar, os quais nem sempre são adequadamente administrados nos estudos de OA e OW. Ainda existe uma grande importância de incorporar múltiplas variáveis e considerar as interações entre espécies nos estudos de OA e OW para melhor prever as futuras mudanças nas comunidades de produtores primários costeiros e preserver as populações chaves que também representam interesses socio-econômicos.

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APÊNDICE A

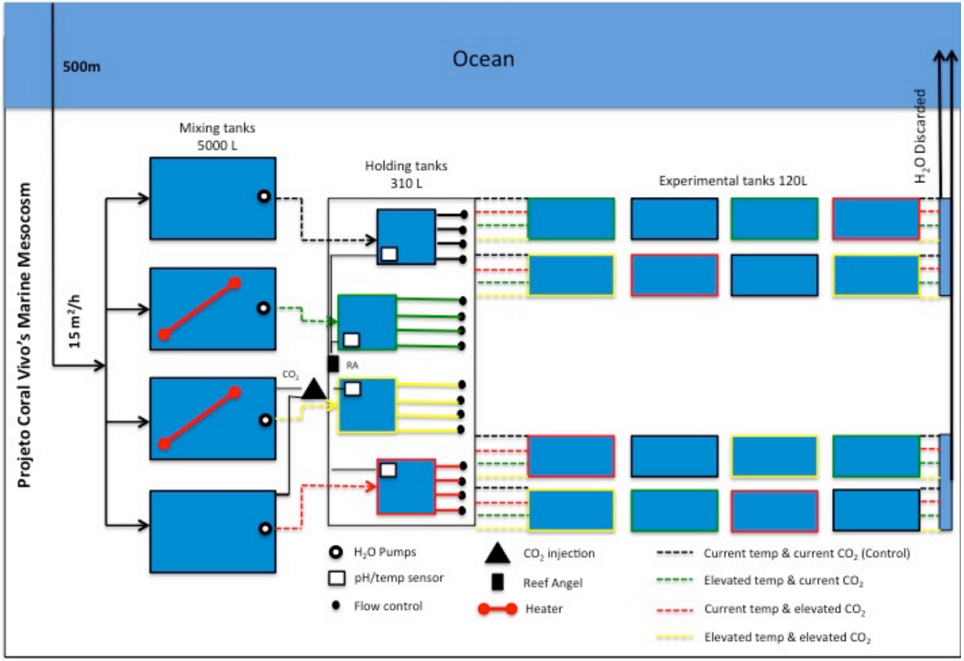

Figure 1. Schematic of Projeto Coral Vivo's Marine Mesocosm, which was used for the experiments in Chapter 1 and Chapter 2. Adapted from Geniane Schneider.

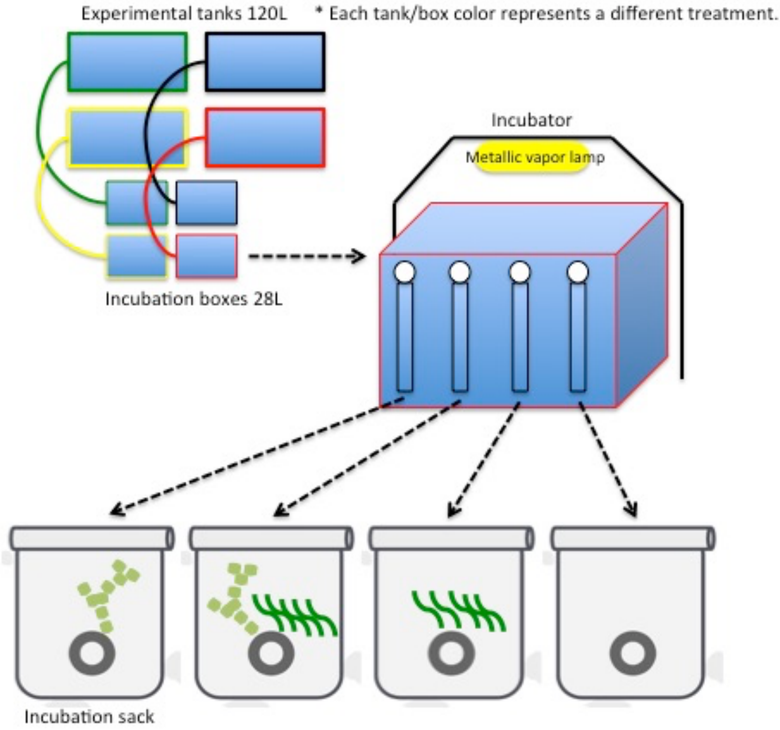

Figure 2. Schematic of incubation setup for Chapter 2 experiment.