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Calcification and photophysiology responses to elevated pCO_2 in six *Halimeda* species from contrasting irradiance environments on Little Cayman Island reefs

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ABSTRACT

While elevated pCO_2 associated with ocean acidification has been shown to lower calcification rates in some marine calcifiers, the modulating potential of irradiance is not well studied. Therefore, we examined the interactive effects of irradiance (high and low) and elevated pCO_2 on six species of the macroalgal genus, *Halimeda*, an ecologically important tropical calcifier. Calcification, growth, and aragonite crystal formation were studied in a 42 d aquaria experiment. Species-specific photophysiology and inorganic C saturation levels were ascertained by establishing photosynthesis to irradiance and inorganic C (C_i) relationships potentially linking photophysiology to growth and calcification responses to elevated pCO_2. Because of *Halimeda*'s role as a sediment producer on tropical reefs, the effect of elevated pCO_2 on dissolution of non-living segments was also examined. Net calcification rates varied among species, with no significant pCO_2 or irradiance effects. However, low irradiance enhanced new apical segment growth in all six species. Crystals formed in new apical segments under elevated pCO_2. Non-living segment mineral:organic content and internal calcium carbonate ($CaCO_3$) crystal microstructure remained stable over 21 d at elevated pCO_2. Species-specific photosynthetic responses corresponded to irradiance conditions at collection sites. For example, high irradiance-adapted species photosynthetic rates were enhanced under increased availability of C_i. In contrast, low irradiance-adapted species photosynthetic rates were saturated under current C_i levels. While species-specific photosynthetic responses to elevated pCO_2 occurred, these photophysiology shifts did not result in reduced calcification or microstructural alteration of carbonate crystals at pCO_2 levels predicted for the year 2100 in any of the six *Halimeda* species examined.

1. Introduction

Ocean acidification from elevated pCO_2 is predicted to lower $CaCO_3$ precipitation rates or enhance dissolution on tropical reefs. Reduced calcification may occur through a reduction in the saturation state (Ω) of carbonate minerals (aragonite/calcite) and/or an increase in energetic requirements to pump protons across a steeper gradient of $[H^+]$, a by-product of calcification (Cyronak et al., 2015; Jokiel, 2015). The lowering of ocean pH by uptake of atmospheric CO_2 predicted for the end of the century will also increase total inorganic carbon (C_i) concentrations, specifically HCO_3^- and pCO_2 (Fabry et al., 2008; Feely et al., 2004; Kleypas and Langdon, 2006). While these pH and C_i changes may negatively affect calcification of marine organisms, they may also enhance photosynthesis in marine autotrophs (Gao et al., 1991, 1993; Kübler et al., 1999; Zou and Gao, 2009) including calcifying macroalgae (Cornwall et al., 2012; Hofmann et al., 2014; Semesi et al., 2009). The susceptibility of individual calcifying autotrophs to elevated pCO_2 is likely to be species-specific (Hurd et al., 2009; Koch et al., 2013; Ries et al., 2009). One genus of calcifying macroalgae that has shown both positive and negative species-specific responses to elevated pCO_2 in field and laboratory experiments is *Halimeda* (Campbell et al., 2014, 2015; Comeau et al., 2013; Meyer et al., 2015; Price et al., 2011). *Halimeda* is an ecologically important benthic macroalgae on tropical reefs that contributes to carbonate sediment production (Hillis-Colinvaux, 1980; Milliman, 1974; Payri, 1988; Rees et al., 2007) and accounts for 8% of the global carbonate budget (Hillis, 1997).

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Sediment production by *Halimeda* is important in shallow reefs and lagoons as well as deep mesophotic reefs. Because of this depth range, species of this genus experience various irradiance conditions (Bandeira-Pedrosa et al., 2004; El-Manawy and Shafik, 2008; Vroom et al., 2003). While members of the *Halimeda* genus are known to occur in low and high irradiance environments, the interactive effects of elevated $p\mathrm{CO_2}$ and irradiance have not been well studied for this genus. The few studies conducted (Peach et al., 2016; Vogel et al., 2015b) found no interactive effects of irradiance and elevated $p\mathrm{CO_2}$ on *H. discoidea* and *H. opuntia*, but all of the individuals were collected from shallow (<3 m) high irradiance environments. *Halimeda* species adapted to low versus high irradiance environments may respond differently to elevated $p\mathrm{CO_2}$ due to irradiance acclimation and differences in seawater carbon chemistry. Deeper waters are typically low in irradiance, but enriched with $\mathrm{CO_2}$ at relatively stable levels (Orr et al., 2005) only experiencing periodic changes in $\mathrm{CO_2}$ when currents upwell $\mathrm{C_i}$-enriched deep water (Feely et al., 2008). In contrast, on shallow reefs and in lagoons $\mathrm{CO_2}$ is variable, and may be limiting throughout the day. Diel fluctuations in pH in shallow reefs and lagoons often occur as a result of photosynthetic $\mathrm{CO_2}$ uptake during the day and respiratory $\mathrm{CO_2}$ production at night (Barry et al., 2013; Beer et al., 2006; Semesi et al., 2009). In *Halimeda*, $\mathrm{CO_2}$ is likely the preferred form of $\mathrm{C_i}$ for photosynthesis (Koch et al., 2013), but a majority of species are also known to utilize $\mathrm{HCO_3^-}$ via carbon concentrating mechanisms (CCMs) (Borowitzka and Larkum, 1976), albeit with a higher energetic cost than diffusive uptake of $\mathrm{CO_2}$ (Giordano et al., 2005; Hepburn et al., 2011; Raven et al., 2014). High irradiance in shallow environments provides solar energy for efficient $\mathrm{HCO_3^-}$ uptake (Cornelisen et al., 2007; Hepburn et al., 2011; Kübler and Raven, 1994) supporting macroalgae with $\mathrm{HCO_3^-}$-based CCMs. Thus, high irradiance-adapted species in shallow habitats may not experience enhanced photosynthesis and growth in $\mathrm{C_i}$-enriched seawater. Further, $\mathrm{HCO_3^-}$ is currently the dominant form of $\mathrm{C_i}$ composing seawater and may not be limiting (Beer, 1994; Beer and Koch, 1996; Israel and Hophy, 2002). In contrast, photosynthesis and growth may respond positively to elevated seawater $\mathrm{C_i}$ levels in low irradiance species that rely solely on diffusive $\mathrm{CO_2}$ uptake that could be enhanced with seawater $p\mathrm{CO_2}$ increases (Kübler et al., 1999; Kübler and Raven, 1994; Zou and Gao, 2009). It is further suggested that upregulation in photosynthesis may compensate for negative effects of elevated $p\mathrm{CO_2}$ on net calcification and precipitation of aragonite, the primary crystal form of calcium carbonate in *Halimeda* (Peach et al., 2016).

The goal of this study was to determine if *Halimeda* species from diverse irradiance environments have variable photophysiology and calcification responses to irradiance and $\mathrm{C_i}$ availability. We hypothesized that high irradiance species (*H. incrassata*, *H. monile*, *H. opuntia*, and *H. tuna*) have efficient $\mathrm{HCO_3^-}$ uptake systems, and thus will not increase photosynthesis or calcification under elevated $\mathrm{HCO_3^-}$ and $p\mathrm{CO_2}$. In contrast, low irradiance species' (*H. copiosa* and *H. goreauii*) photophysiology and calcification will be enhanced with increased availability of $\mathrm{CO_2}$. Because members of the *Halimeda* genus are important sediment producers, we also examined the susceptibility of non-living segments to dissolution in seawater with elevated $p\mathrm{CO_2}$. We predicted that non-living segments would experience a greater loss of inorganic tissue mass in elevated $p\mathrm{CO_2}$ seawater compared to living photosynthetically active segments. Active photosynthetic $\mathrm{CO_2}$ uptake has been shown to enhance conditions for $\mathrm{CaCO_3}$ precipitation (de Beer and Larkum, 2001). These hypotheses were tested in a 42 d aquaria experiment examining live thalli growth and calcification responses to elevated $p\mathrm{CO_2}$ and high and low irradiance. Further, a series of short-term photophysiology experiments were conducted to determine individual species photosynthesis to irradiance (P:I) and $\mathrm{C_i}$ (P:$\mathrm{C_i}$) kinetics. Finally, a 21 d study examined the effects of elevated $p\mathrm{CO_2}$ on dissolution of non-living *Halimeda* segments. The crystalline microstructure of newly produced live apical segments and non-living segments were assessed using scanning electron microscopy (SEM).

2. Materials and methods

2.1. Aquaria live thalli study

2.1.1. Species and sampling locations

Thalli ($n = 80$) of six *Halimeda* species were collected off the north coast of Little Cayman Island from shallow to deep reef sites (Fig. 1) each with distinct irradiance levels (Fig. 2). All sites were located north of the Little Cayman Research Centre (LCRC; 19°41′44″ N, 80°3′39″ W). *Halimeda incrassata* was collected in a back reef lagoon (Site A, 19°41′46″ N, 80°03′39″ W), *Halimeda tuna* was collected from a spur and groove reef (Site B, 19°42′3″ N, 80°03′25″ W), *Halimeda monile* was collected from a soft-bottom substratum groove adjacent to the spur and groove reef (Site B, 19°42′3″ N, 80°03′25″ W), *Halimeda opuntia* was collected on the seaward edge of a sand groove at the fore reef ledge (Site C, 19°42′9″ N, 80°03′25″ W), and *Halimeda copiosa* and *Halimeda goreauii* were collected under ledges of the fore reef in deep shade (Site C, 19°42′9″ N, 80°03′25″ W). After collection, specimens were transported to the lab at the LCRC and immediately placed in aquaria receiving ambient seawater in a flow-through system. After a 5 d acclimation period, specimens were randomly placed in experimental aquaria, with one sample replicate per $p\mathrm{CO_2}$ and irradiance treatment combination.

2.1.2. Experimental design and treatments

The aquaria experiment was run as a completely randomized split-plot design with $p\mathrm{CO_2}$ as the whole-plot factor and irradiance as the split-plot factor. The $p\mathrm{CO_2}$ treatment was delivered from two header tanks (~600 L) receiving a constant supply (~120 L min^{-1}) of ambient seawater from the back reef ~250 m offshore of the LCRC on the north side of Little Cayman Island (Site A, Fig. 1). The elevated $p\mathrm{CO_2}$ treatment was maintained by mixing $\mathrm{CO_2}$ (100%) and air using mass flow controllers (Sierra Instruments Inc., Smart-Trak-100). The elevated $p\mathrm{CO_2}$ treatment cycled with diel reef metabolism averaging ~597 to 1087 μatm consistent with year 2100 predictions for $\mathrm{CO_2}$ emission scenarios (~475–1300 μatm, RCP 2.6 to 8.5, IPCC, 2013), while the ambient controls averaged ~375 to 657 μatm. Seawater from treatment and control tanks was continuously delivered (~1.1 L min^{-1}) to aquaria (11 L, $n = 10$) resulting in total replenishment of 6 times h^{-1}. The aquaria experiment was conducted using natural irradiance adjusted with shade screen to an average saturating irradiance of 250 μmol photons m^{-2} s^{-1} at mid-day. Low and high irradiance treatments were achieved by covering one half of each aquarium with an additional layer of shade screen and leaving the other half uncovered. Irradiance levels for the high irradiance treatment averaged 250 ± 28 μmol photons m^{-2} s^{-1} and were reduced by ~80% in the low irradiance treatment averaging 56 ± 10 μmol photons m^{-2} s^{-1}. Irradiance levels

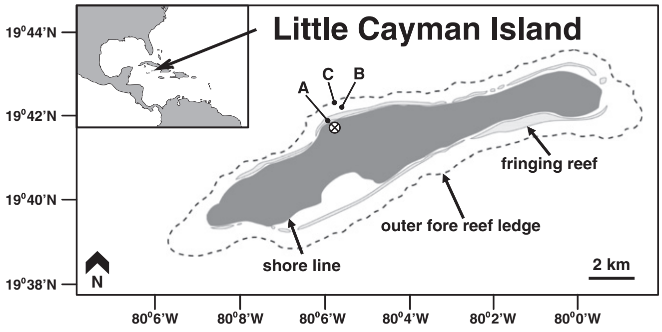

Fig. 1. Location of the experimental facility shown as encircled multi symbol at the Little Cayman Research Centre (LCRC) on the northern coast of Little Cayman Island in the Caribbean (inset). The three collection sites were: (A) a shallow (<2 m) back-reef lagoon ~250 m from LCRC, (B) a spur and groove fore-reef (~15 m) ~700 m northeast of LCRC and (C) an outer fore-reef ledge (~18 m) ~900 m northeast of LCRC.