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Increased physiological performance of the calcifying green macroalga *Halimeda opuntia* in response to experimental nutrient enrichment on a Caribbean coral reef

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ABSTRACT

Calcifying green macroalgae of the genus *Halimeda* are widely distributed on coral reefs and may become more abundant under higher nutrient availability. To determine how nutrient enrichment affects the physiological performance of *Halimeda opuntia* (Linnaeus) J.V.Lamouroux in relation to different water depths, we carried out in situ nutrient enrichment experiments in Curaçao, Netherlands Antilles. *H. opuntia* was collected in 5 m and then incubated at 5 and 15 m in clear acrylic cages with or without addition of N and P. Growth, algal tissue composition (internal C, N, and P content, $\delta^{15}N$ signatures, protein content), photosynthetic performance and pigment content were measured after 14 days of incubation. Growth rates and total C increased with nutrients and were higher in 5 m water depth. N and P content were higher and $\delta^{15}N$ signatures were lighter with nutrients in both depths. Photosynthetic performance, concentrations of the main and accessory photosynthetic pigments, and photoprotection also increased with nutrients and showed some response to depth. These results indicate that nutrient enrichment supported a rapid increase in physiological performance of *H. opuntia*, but with differences in depth. In 5 m, more C was allocated to growth, where light levels were sufficient, while in 15 m C was allocated to photosynthetic pigments. These results suggest that nutrient enrichment may influence their abundance and depth distribution on the reef.

1. Introduction

Halimeda spp. are green calcareous macroalgae that are ubiquitous in tropical shallow and deep reefs and play an important role in primary production and carbonate sediment formation (Wefer, 1980; Drew, 1983; Jensen et al., 1985). An increase in their abundance and distribution has been reported coinciding with a decrease in coral cover in reef ecosystems (Lirman and Biber, 2000; Shulman and Robertson, 1996; Nugues and Bak, 2008).

High nutrient supply has been reported as one of the reasons for increases in growth and abundance of *Halimeda* spp. (Lapointe and Thacker, 2002; Smith et al., 2004; Collado-Vides et al., 2005). Studies, however, have shown contrasting response to nutrients in different species of *Halimeda*. Nutrient limitation experiments showed that *Halimeda incrassata* (J.Ellis) J.V.Lamouroux and *Halimeda opuntia* (Linnaeus) J.V.Lamouroux do not rapidly increase growth under high nutrients (Delgado and Lapointe, 1994; Kuffner and Paul, 2001; McClanahan et al., 2002; Fong et al., 2003) suggesting that the genus could be adapted to low nutrient environments. Other studies have shown that when exposed to higher nutrient supply some species of *Halimeda* may increase growth, photosynthetic response, or production (Lapointe et al., 1987; Littler et al., 1988; Beach et al., 2003; Smith et al., 2004), but responses to N and P are different. *Halimeda lacrimosa* M.A. Howe and *Halimeda copiosa* Goreau and E.A.Graham from a shallow reef in the Bahamas and *H. opuntia* from a barrier reef in Belize all increased their photosynthetic response or total production to N but not to P (Lapointe et al., 1987; Littler et al., 1988), while *Halimeda tuna* (J.Ellis and Solander) J.V.Lamouroux and *Halimeda simulans* M.A. Howe were found to increase photosynthesis in response to P (Littler et al., 1988). In deep reefs, biomass of *H. copiosa* in Jamaica was enhanced by land-derived nutrient pollution (Lapointe and Thacker, 2002). Deep populations of *H. tuna* have also been found to be influenced by nutrient pulses from internal tides, showing higher growth rates, nutrient storage, and pigment concentrations than shallow reef populations despite lower light levels in the deep reef (Beach et al., 2003; Smith et al., 2004). Thus, due to varying responses reported in the literature, it is still unclear how the interaction between increased nutrient supply and light availability may control growth and distribution of *Halimeda* spp. along the reef slope.

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One of the confounding factors for differences in macroalgal responses to nutrients with depth may be differences in photoadaptation among species and strategies of photoacclimation and photoprotection involved in photosynthesis (Franklin et al., 1996; Beach et al., 2003; Jensen et al., 1985). A large range in depth distribution exists within the genus of *Halimeda*. Many species, like *H. tuna*, *H. simulans*, *H. lacrimosa*, *H. opuntia*, and *H. copiosa*, have been found growing at very shallow depths in the intertidal and subtidal zone (Littler et al., 1988; Franklin et al., 1996; Kuffner and Paul, 2001; Beach et al., 2003; Smith et al., 2004), while *Halimeda fragilis* W.R. Taylor, *Halimeda gracilis* Harvey ex J.Agardh, *Halimeda discoidea* Decaisne, may grow deeper, down to 160 m (Drew and Abel, 1988; Jensen et al., 1985; Hillis-Colinvaux, 1986; Bandeira-Pedrosa et al., 2004; Kahng and Kelley, 2007). *H. copiosa* and *H. tuna* have also been found in both in shallow and deep reefs (Lapointe and Thacker, 2002; Beach et al., 2003; Smith et al., 2004).

Photosynthetic pigments aid in photoacclimation and photoprotection strategies. While chlorophyll concentrations are often higher in deeper waters to enhance light harvesting where light is more limiting, carotenoids of the xanthophyll-pool are involved in photoprotection to alleviate light stress, and have been found to be important in tropical shallow water macroalgae (Beach et al., 2003; Franklin et al., 1996). *Halimeda* spp. also contain siphonaxanthin and siphonein, special light-harvesting pigments belonging to the group of siphonous macroalgae (Beach et al., 2003) which aid in photoprotection under high light or act as additional light harvesting pigments in the blue–green region with increasing depth (Raniello et al., 2006). Photoinhibition may also play a role under high light, in which macroalgae, including those from the tropics, respond to daily light cycles by decreasing their photosynthetic yield and increasing non-photochemical quenching to dissipate excess energy as heat and prevent damage to the photosystem (Häder et al., 1996; Franklin et al., 1996).

The macroalgal cover along coral reefs of the south-west coast of Curaçao, Netherlands Antilles has increased over the last decade, primarily in 20 and 30 m (Nugues and Bak, 2008). Additional studies show a high relative abundance of turf algae and other macroalgae in 7–10 m (Vermeij et al., 2010). *H. opuntia* is one of the dominant shallow reef macroalgae found growing in shallow bays and on the reef primarily between 5 and 10 m (Kuenen and Debrot, 1995; M. Teichberg, pers. observations). Other macroalgae, including the Phaeophyceae *Lobophora variegata* (J.V.Lamouroux) Womersley ex E.C.Oliveira and *Dictyota* spp., begin their distribution at this depth and grow down to at least 40 m (Nugues and Bak, 2008; Fricke et al., 2011). Although ambient water nutrient concentrations in coral reefs along the south-west coast of Curaçao are generally low, development and urbanization along the coastline have increased, and some evidence of localized nutrient inputs into the reefs primarily from sewage have been reported (Gast et al., 1999).

The increases in coastal development and macroalgal cover on coral reefs in Curaçao make this site suitable to carry out nutrient enrichment experiments to determine the effects of increased nutrient supply on macroalgal growth, physiology, and depth distribution on coral reefs. In this study, growth and photosynthetic performance of *H. opuntia* were examined under in situ nutrient enrichment at 5 m and transplanted to 15 m water depth. Additionally, the C, N, and P content and N isotopic signatures of the macroalgae were measured as a useful indicator of nutrient conditions in the environment and of their nutrient uptake and storage capacity (Atkinson and Smith, 1983; Lapointe, 1989; Fong et al., 2003). We hypothesized that nutrient enrichment would lead to a higher growth response and photosynthetic performance of *H. opuntia* enabling this species to expand its depth distribution and abundance further down the reef slope than it is normally distributed under nutrient limited conditions. However, we also predicted that there would be differences in photoacclimation strategies with increasing depth with and without nutrient enrichment, as also found by Lapointe (1997) for other reef algae.

2. Material and methods

2.1. Experimental unit and layout

To investigate the effects of nutrients on the physiological response of *H. opuntia* at different depths, we carried out an in situ nutrient enrichment experiment at Piscadera Bay, Curaçao, Carmabi Buoy Zero (69°58′26″ N, 12°07′27″ W). The experiment took place during the dry season between 14 and 27 March 2008. Experimental cages were 20 cm × 15 cm × 20 cm, (L × W × H), made of acrylic plastic sides to allow for light penetration and two mesh sides of 1 mm size openings to allow for water flow while excluding the majority of grazers. This cage design was previously used and tested for its effectiveness in other field experiments (Teichberg et al., 2008, 2010). *H. opuntia* was collected from 5 m, kept in an aquarium with flowing ambient seawater overnight, and then incubated for 14 days in cages at 5 and 15 m with and without nutrient additions ($n = 4$). Two thalli of approximately 5–10 g were placed in each cage. Cages were randomly placed approximately 3 m apart at each depth, attached to a rope to keep it upright and anchored to the sediment with a cement block.

For the nutrient additions, enrichment was maintained throughout the experiment by the addition of OSMOCOTE, slow-release fertilizer pellets (10% NH_4, 9% NO_3, and 6% PO_4) in a perforated polyvenylchloride (PVC) tube secured vertically at the center of the cage to allow for slow release of nutrients over the course of the experimental run. Similar enrichment methods using slow-release fertilizer pellets have been used in benthic habitats, including coral reef ecosystems, and have been proven to be successful at increasing nutrient concentration in the water column above ambient levels (Agawin et al., 1996; Worm et al., 2000; Littler et al., 2006).

2.2. Environmental and experimental conditions

To ascertain the ambient seawater nutrient concentrations at each depth and the experimental nutrient supply available to the macroalgae during the incubations, water was sampled outside and within the cages on 3 days throughout the incubation experiments ($n = 12$). Water samples were filtered through GF/F glass fiber filters, preserved with $HgCl_2$ following methods from Kattner (1999). Concentrations of dissolved inorganic nitrogen (DIN) and PO_4 from the water samples were measured using standard photometric and fluorometric procedures on a continuous flow analyzer SAN++ (Scalar, Netherlands) with determination limits based on blanking procedures of 0.08, 0.06, and 0.06 μM NO_x, NH_4, and PO_4 respectively.

To determine the relative difference in irradiance reaching the macroalgae at 5 and 15 m water depths at the study site, irradiance was measured at the surface and at 5 m depths during the midday, when irradiance was highest. Measurements were averaged among several days throughout the incubation period using a LI-COR data logger (LI-1000, Li-Cor, Lincoln, USA) equipped with a LICOR 190 (air) and a 192 (underwater) quantum sensor (cosine corrected), which measures the total range of Photosynthetic Active Radiation (PAR, 400–700 nm). Irradiance was not directly measured at 15 m, but was calculated from the Lambert–Beer light attenuation curve (Kirk, 1994) applying the K_d value calculated from light profiles measured down to 15 m in a subsequent study in this location (Fricke et al., 2011). Percent surface irradiance at each depth was then calculated from the total irradiance measured above the water surface. Shading of the cage was minimal with a reduction