

Factors affecting tolerance to herbivory in a calcifying alga on coral reefs

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Abstract Herbivores greatly influence the productivity of algae but their impact can depend on the nuances of the timing, location, and intensity of herbivory. While plants can escape herbivory in spatial refugia, small-scale variations in habitat quality play a critical role in plant tolerance to herbivory and might generate complex trade-offs. On coral reefs, overstory branching corals provide a refuge from fish herbivory but also provide refugia for many small fish that excrete nutrients. Therefore, algae living in this habitat might also benefit from higher nutrient delivery. However, because coral branches occlude sunlight, algal growth rates might be impaired despite experiencing elevated nutrients and lower herbivory. In lab-based experiments, light, nutrients, and simulated herbivory were manipulated in summer and winter to investigate how these processes interact to influence the tolerance of herbivory in the calcifying green algae *Halimeda*, an important producer of reef carbonate sediments worldwide. *Halimeda heteromorpha* which is commonly found associated with branching corals tolerated tissue damage by increasing rates of segment production. Greater tolerance was observed at levels of light resembling

those experienced under the coral's canopy. Nutrient additions increased compensatory segment growth in winter but not summer. Levels of tolerance were seasonal and nutrient dependent. Results show that small-scale variations in habitat quality may influence tolerance to herbivory in *Halimeda*. This suggests that if coral habitats are degraded or lost and oceans continue to warm, a likely negative impact on *Halimeda* populations and its contribution to carbonate sediments could be expected if high levels of herbivory are maintained.

Keywords Compensatory growth · Coral habitats · Grazing pressure · Light attenuation · Coral structural complexity · Limiting resource model (LRM) · Carbonate production

Introduction

The ability of plants to mitigate negative effects of herbivory without a reduction in fitness is termed tolerance (Strauss and Agrawal 1999; Tiffin 2000) and appears to be highly variable in marine plants (e.g. Hay et al. 2011; Honkanen and Jormalainen 2002; Lowell et al. 1991). Mechanisms of tolerance include increasing photosynthetic rates, growth rates or nutrient uptake, phenological changes such as the timing of reproduction, or the reallocation of resources from growth to reproduction (Strauss and Agrawal 1999; Tiffin 2000). However, the ability to tolerate herbivory may depend on the environment in which plants grow (Tiffin 2002) as environmental factors strongly influence plant fitness and, therefore, the expression of these tolerance traits (Sultan 2000).

In the marine environment, light, nutrients and temperature are among the most important environmental

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drivers of macroalgal productivity (Diaz-Pulido et al. 2007) although many species can thrive across a gradient of these resources (Diaz-Pulido et al. 2007; Hay 1981; Mejia et al. 2012). Over broad scales, seasonal changes in the availability of resources such as temperature, light and nutrients are useful in predicting spatial and temporal patterns of macroalgal growth (e.g. Atweberhan et al. 2006; Ballesteros 1991; Ferrari et al. 2012; Vuki and Price 1994). However, in habitats with high structural complexity the quality and quantity of available resources can vary greatly at small spatial scales. For instance, in coral habitats the availability of nutrient resources can be influenced due to damselfish territories (Polunin and Koike 1987) or the presence of dense algal mats which establish nutrient-enriched micro-environments by trapping organic matter (Mason 2009). Similarly, changes in flow due to algal canopy height can affect the recruitment, growth and photosynthetic rates of algae (Carpenter and Williams 1993). Changes in the quality and quantity of available resources at this scale are more difficult to measure, limiting our ability to predict macroalgal productivity at finer scales. Similar impacts of habitat complexity on resource availability can be found in terrestrial habitats. In forest understories for example, the occurrence of sunflecks (intermittent periods of high photon flux density, PFD) resulting from the forest canopy gaps creates light regimes of high spatial and temporal variation that are responsible for much of the photosynthesis of understory plants (Chazdon 1988).

Analogous to a forest canopy, corals of high structural complexity occlude light (Kaniewska et al. 2008) by up to 80% at mid-day (Castro-Sanguino et al. 2016), and while this potentially reduces the photosynthetic productivity of understory algae, these plants are often found in abundance. For example, the marine calcareous algae *Halimeda*, an important producer of reef carbonate sediments worldwide (Nelson 2009), often grows in association with branching corals like *Acropora* (Kraft 2007). A few studies have demonstrated that *Halimeda* can accumulate more biomass when growing associated with structurally complex corals (Castro-Sanguino et al. 2016; Kerr and Paul 1995), similar to other algae such as *Lobophora* (Bennett et al. 2010). One hypothesis to account for this pattern is that by providing spatial refugia from large herbivores, structurally complex corals could benefit macroalgal growth and recruitment (Bennett et al. 2010; Castro-Sanguino et al. 2016; Kerr and Paul 1995). However, the habitat provided by structurally complex corals may also negatively affect algal growth by reducing light. Light is important for *Halimeda* growth and calcification (Borowitzka and Larkum 1977; Littler et al. 1988; Yñiguez 2007). While different *Halimeda* species occupy a wide range of light conditions on reefs, some species at shallow depths exhibit specific responses to changes in light with some species being more shade-adapted than

others when growing in cryptic shaded habitats (Littler et al. 1988). Therefore, coral habitats could benefit algal growth by reducing light intensity within shallow habitats. Similarly, algae can take advantage of nutrient pulses but this response is species specific (Lapointe et al. 1987; Littler et al. 1988). Nutrient enrichment positively affects growth and photosynthetic performance in *Halimeda* (Littler et al. 1988; Mayakun et al. 2012; Smith et al. 2004; Teichberg et al. 2013). Therefore, an increase in nutrient availability within the coral's canopy could also benefit the growth rates of algae. It has been suggested that nutrient availability also increases *Halimeda* tolerance to herbivory (Mayakun et al. 2012). Because tolerance to herbivory varies with different levels of resources (Wise and Abrahamson 2005), we might expect different levels of algal tolerance of herbivory at this finer scale where levels of light and nutrients vary.

Damage by herbivores can also alter the response of plants to the environment either by intensifying resource limitation or causing a different resource to become limiting (Wise and Abrahamson 2005). In coral habitats herbivores such as smaller fish and mesograzers (e.g. crabs, molluscs, sea urchins and amphipods) that have access to algae under the coral's canopy can also influence algal populations (Poore et al. 2012, 2014) and could affect algal fitness and its response to the environment. Although *Halimeda* spp. possess morphological (calcification) and chemical (secondary metabolites) traits to resist herbivory (Hay et al. 1994; Paul and Fenical 1983; Paul and Van Alstyne 1992), some *Halimeda* can be intensely grazed by large fish (mainly parrotfish, Mantyka and Bellwood 2007) and by small invertebrates such as gastropods and crabs (Armitage and Fourqurean 2009; Hillis-Colinvaux 1980; Paul and Van Alstyne 1988). A reduction in herbivory by large fish inside microhabitats provided by corals has been hypothesized as the main driver of algal growth (Bennett et al. 2010; Kerr and Paul 1995). Yet, the contribution of light intensity on *Halimeda* response to grazing within shaded microhabitats such as those provided by branching *Acropora* has not been assessed. Similarly, the role of seasonality in determining the tolerance of *Halimeda* to herbivory is less well understood (Castro-Sanguino et al. 2016; Drew and Abel 1988; Hillis-Colinvaux 1980). Because an increase in growth rate has been associated with increasing temperatures during summer (e.g. Ballesteros 1991; Castro-Sanguino et al. 2016; Drew 1983; Hudson 1985; Multer 1988) we hypothesize that seasonality may also influence the capability of *Halimeda* to compensate for tissue loss. Species-specific responses and potentially interactive responses to light, nutrients and seasonality (Drew and Abel 1988; Littler et al. 1988) make it difficult to predict when and where *Halimeda* will be more affected by changes in the environment. Therefore, understanding the potentially interactive effects

of the resources that might limit algae in a particular environment is central to understanding tolerance of herbivory.

Several hypotheses predict how the availability of resources may alter such tolerance. The compensatory continuum hypothesis (CCH) predicts a general pattern of greater tolerance of herbivory in low-stress and resource-rich environments (Maschinski and Whitham 1989). However, there is increasing evidence of plants displaying greater tolerance of herbivory when growing under relatively stressful conditions (Wise and Abrahamson 2005). The most recently proposed hypothesis, the Limiting Resource Model (LRM), allows prediction of any possible outcome in tolerance (greater, lower or equal) among environments by considering which resource is limiting the plant's fitness, which resource is primarily affected by herbivory, and how resource acquisition is affected by the type of herbivory damage (Wise and Abrahamson 2005). Only few studies have shown the applicability of the LRM to predict tolerance to herbivory in marine algae (Hay et al. 2011).

Here, we used *Halimeda heteromorpha* (hereafter referred as *Halimeda*), which is commonly found associated with branching corals at Heron Island, on the southern Great Barrier Reef to test whether *Halimeda* exhibits different levels of tolerance of herbivory in microhabitats provided by branching corals. We used segment production as the mechanism of tolerance to herbivory and tested the premises of the LRM in aquaria-based experiments. We simulated fish herbivory by clipping *Halimeda* branches and investigated how resource limitation (variations in light availability, nutrients and temperature due to seasons) affects *Halimeda* segment production. We asked whether the alga can compensate for damage caused by simulated herbivory, whether changes in light affect this response, and whether tolerance to simulated herbivory is nutrient or season dependent in *Halimeda*. By considering the potential interactive effects of light, nutrients and seasons on *Halimeda* tolerance to herbivory, the outcomes of this study will be useful in predicting potential impacts of habitat degradation or changes in habitat quality on *Halimeda* abundance, productivity and its contribution to sediments budgets on shallow coral reefs.

Materials and methods

Collection and manipulation of samples

Individual thalli of *H. heteromorpha* were collected from *Acropora* habitats formed by monospecific stands of *A. formosa*. These *Acropora* habitats are found at 4–6 m depth on the reef slope of Heron Bommie located on the western side of Heron Island (23°26'29"S, 151°54'17"E). In addition,

water samples from inside the *Acropora* canopy (i.e. just above the substrate at the bases of *Acropora* branches) and outside *Acropora* habitats (5–10 replicates per habitat) were collected using plastic syringes with Millipore inline filters to estimate nutrient concentration in water. Thalli were transported to the laboratory in plastic bags, rinsed in clean seawater and cleared of epiphytes and replicate individual branches (i.e. multiple branches per thalli) from different individual plants were cut (180 branches per season). Given seasonal differences in thalli size (Castro-Sanguino et al. 2016), branches consisted of 10 ± 0.1 segments (0.22 g wet biomass) in winter and 15 ± 0.2 segments in summer (0.34 g wet biomass). Branches were kept for a week in outdoor aquaria receiving filtered seawater from the reef flat through a flow-through sea water system and under ambient light (mean light levels at mid-day of $331 \pm 66 \mu\text{mol m}^{-2} \text{s}^{-1}$ PAR). In each season, a subsample of branches ($n=5$) was used to determine nutrient content of *Halimeda* tissue. After one week of acclimation, branches were weighed (initial weight = W_i) and randomly allocated to treatments in replicate indoor aquaria with each aquaria containing branches from different individuals.

Experimental design

A factorial experimental design was used to test for the effect of light exposure and herbivory on *Halimeda* under two nutrient treatments (ambient and enriched) and two seasons (summer: January–February and winter: July–August) to assess seasonal differences in its growth rate (Fig. 1a). Three replicate aquaria were randomly allocated to a treatment combination of light exposure (three levels) and nutrients (two levels) for a total of 18 aquaria per season (Fig. 1a). Because of logistical constraints, the experiment was split into two runs each season (i.e. 9 tank replicates per run per season) to complete the number of replicates desired (i.e. 3 light \times 2 nutrients combinations \times 3 replicates = 18). Each experimental run lasted 2 weeks and was conducted with new *Halimeda* branches and new randomisation of aquaria for treatments.

Environmental conditions in aquaria

Each indoor flow-through aquaria received filtered seawater from the reef flat. Aquaria were maintained with a unidirectional constant flow of approximately 5 cm s^{-1} using MP60 VorTech power heads and a water exchange rate of 2 L per minute (see Fig. 1b for details). In each aquarium, light was controlled using a combination of metal halide and blue actinic lights for a 12:12-h photoperiod. Because the colony structure of branching species such as *Acropora* can influence significantly solar irradiance reducing light levels relative to ambient levels found

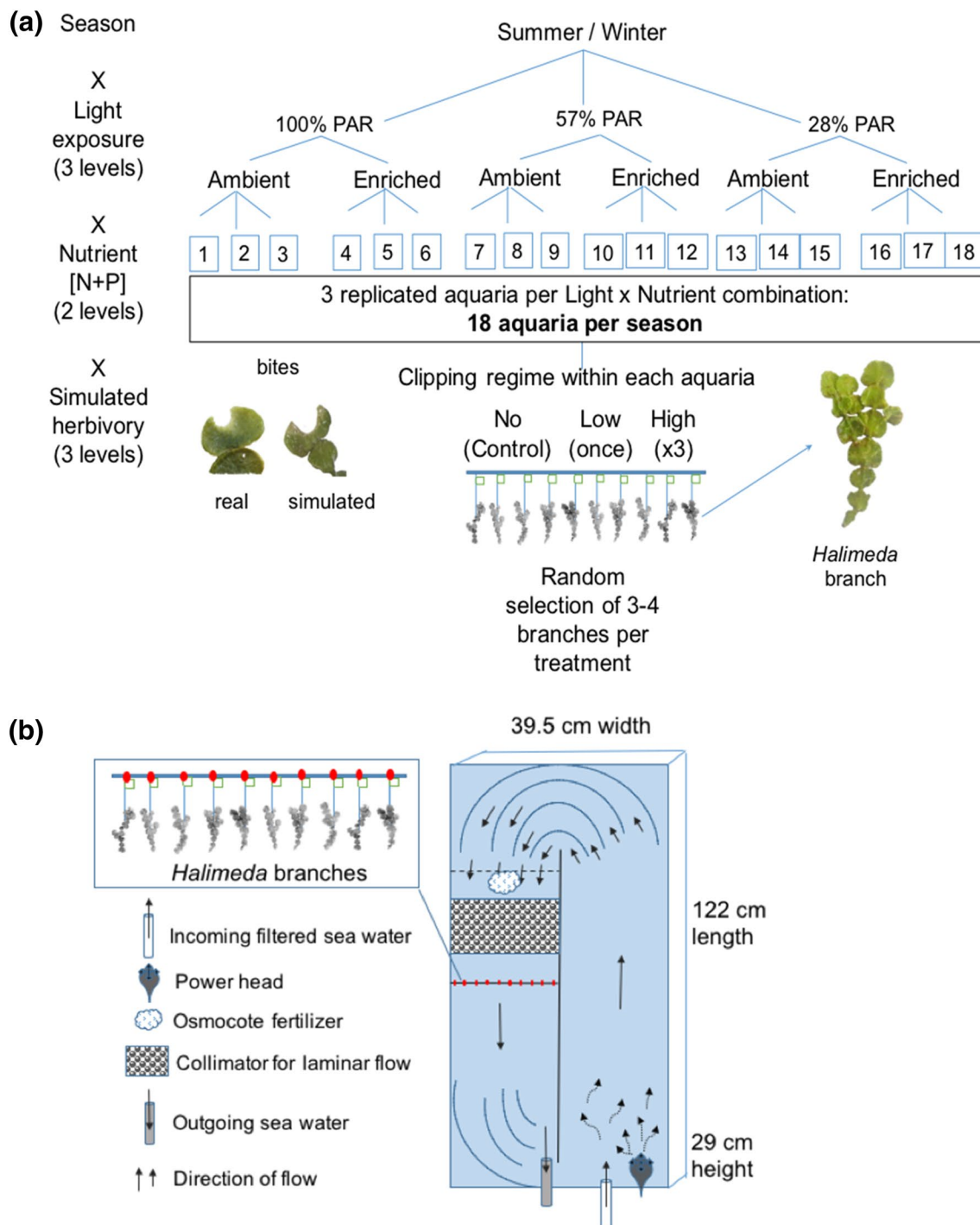


Fig. 1 **a** Diagram of the factorial experimental design used to test the effect of light, herbivory and nutrients on *Halimeda* growth during summer and winter. Representation of a *Halimeda* branch and the “bite” simulated by clipping treatments. **b** Schematic diagram of the

experimental setup detailing tank’s dimensions, position of branches in tanks, nutrient treatments and conditions of water circulation and flow

outside the corals’ canopy (Castro-Sanguino et al. 2016; Kaniewska et al. 2008), we used neutral filters to produce three levels of light: high—100% exposure (mean light levels of $256 \pm 13 \text{ mol m}^{-2} \text{ s}^{-1}$), medium—43%

reduction ($147 \pm 5 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1}$) and low—72% reduction ($74 \pm 4 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1}$). This gradient of light simulated the variation in light regime estimated in the shallow reef slope at 4–6 m depth in open sites (100% exposure)

and at the base of branches of *A. formosa* (43–72% reduction in PAR) where *Halimeda* grows (Online Resource, Fig. S1). Light and temperature were permanently assessed with HOBO Pendant data loggers (Onset, 64 K-UA-002-64). An approximate conversion of Lux to PAR units ($\mu\text{mol m}^{-2} \text{s}^{-1}$) was achieved using a conversion factor of 0.0185 following Thimijan and Heins (1983). Additional measurements of PAR were conducted twice a week with underwater LI-COR sensors (LI-192) for comparison. For nutrient enrichment, 50 g of Osmocote fertilizer (ratio of 14:3.5:9.1 N/P/K) contained in a nylon cloth were deployed in the aquaria assigned to the enrichment treatment (Fig. 1a). Ambient treatments contained only the nylon cloth. Because dissolved nutrients vary with seasons and concentrations can change by twofold during the day due to tide regimes at Heron reefs (Smith and Johnson 1995), the concentration of nutrients in our experiments can dramatically change within hours and between seasons. Therefore, to detect the variation of dissolved nutrients, water samples were taken from random tanks (total of five replicate tanks per treatment) during low and high tide and before and after nutrient additions using plastic syringes with Milipore inline filters. Nutrient concentration in the water was assessed in each experimental run before Osmocote addition, and after addition in the first week and at the end of the experiments. Water temperature and concentration of dissolved nutrients did not vary significantly between runs allowing us to pool them together for analyses as a single experiment. The experiment was conducted in winter 2014 (mean water temperature of $21.3 \pm 1.2^\circ\text{C}$) and summer 2015 (mean water temperature of $27.7 \pm 0.04^\circ\text{C}$).

Experimental setup

Ten *Halimeda* branches were vertically suspended with a nylon line in each indoor aquarium. Branches were suspended in a horizontal line perpendicular to the flow in a way that all branches were equally exposed to the same amount of flow (Fig. 1b). In each aquarium, individual *Halimeda* branches were randomly assigned to one of the three herbivory treatments (3–4 branches per herbivory level) (Fig. 1a). While clipping treatments can change the chemistry of algae, with this response potentially causing stress among branches, the activation of chemical defences appears to be highly localized in *Halimeda*. Paul and Van Alstyne (1992) show that after injuring segments of *Halimeda* spp to mimic herbivory, the activation of chemical defences takes place only in the vicinity of the tissue injury while uninjured segments from the same plant do not show any change in chemical compounds. Therefore, if clipping does not affect adjacent segments of the same branch, it is unlikely that clipping treatments can affect neighbouring control branches. Herbivory was simulated

by removing tissue from two apical segments with a hole-punch. Two hole-diameters and clipping intensities were used to differentiate levels of herbivory. A Low herbivory regime was applied once at the beginning of the experiment (4-mm-diameter tissue removed, equivalent to ~1% thallus biomass), and a High herbivory regime was repeated every 3 days during the experiment (total of 18-mm-diameter tissue removed equivalent to ~7% thallus biomass), with an additional control (no damage). The size and shape of the damage simulated the bite marks observed in situ during summer and winter in different *Halimeda* species occurring on the shallow reef slope of Heron Island in both open habitats exposed to large herbivores and habitats protected from large fish (i.e. in branching habitats and inside fish-exclusion cages) (Fig. 1. See Castro-Sanguino et al. 2016 for details). The frequency of clipping was chosen to test whether tolerance differs with intensity of damage.

Measurement of response variables of *Halimeda*

In addition to the rate of segment production (number of new segments) which was assessed daily, changes in total wet biomass, photosynthetic performance, and nutrient content were assessed. The wet weight of each branch was measured by blotting the algae dry with paper towels until no change in weight was detectable. Branch wet weight was recorded at the beginning of the experiments (before first clipping) and assessed again after 1 week and at the end of the experiment to estimate changes in biomass over time (to the nearest 0.01 g). The amount of biomass removed by clipping treatments was calculated by weighing the clipped tissue and subtracted from the final biomass to estimate the relative growth rate (as % RGR) of biomass weekly. Relative growth of biomass was estimated using the formula: $(\ln W_f - \ln W_i)/t \times 100$, where W_f and W_i are the final and initial biomass, respectively, and t is the time of the experiment (1 week). At the end of the experiment all branches were air dried for 48 h until constant weight and apical segments were ground to a fine powder for nutrient analyses. Photosynthetic performance of *Halimeda* was quantified using a pulse amplitude modulation (PAM) chlorophyll fluorometer (Diving PAM, Walz, Effeltrich, Germany). The maximum quantum yield of Photosystem II (PSII) (F_v/F_m), which provides a measure of photoinhibition, was measured approximately in the middle section of each branch every 3 days 30 min after lights were turned off to assess potential photodamage to PSII. In the same middle area of the branch, the effective quantum yield ($\Delta F/F_m'$) and the relative electron transport rate (rETR, $\mu\text{mol electrons m}^{-2} \text{s}^{-1}$) which correlate with photosynthetic carbon assimilation (Beer et al. 1998) were measured after 3 h of light exposure (at 09:00 h). In addition, $\Delta F/F_m'$ and rETR measurements were taken before and immediately after

simulating herbivory to assess induced changes in the photosynthetic activity due to tissue damage. Measurements were taken within the first 5 min after clipping in the same segment close to the damaged area or in the closest segment if not enough area was left after damage.

Statistical analyses

We used Linear (and Generalized Linear) Mixed Models (LMM and GLMM) to test the effect of light, simulated herbivory, nutrients, and season alone and in interaction on different *Halimeda* responses (rate of segment production, biomass RGR, photosynthetic performance, and nutrient content). Aquaria were treated as random factor. We looked at the distribution of the response variables to find the appropriate error structure to be used in the model. We selected the best model using the protocol of backwards selection on the explanatory variables. We first fit a model with the explanatory variables of interest and their interactions. We then removed one by one the least significant interactions ($p > 0.05$), refit the model and compared models using the parsimony principle (finding the smallest AIC value). The minimum adequate model was then validated by looking at the residual plots to corroborate assumptions of normality and homogeneity of variance. Induced photosynthetic response due to clipping was fitted with mixed effects models for repeated measures ANOVA. Because data on segment growth was zero-inflated and highly variable, we modelled the growth of segments in two ways. First, we fitted the data (total number of new segments) using a negative-binomial distribution with zero inflation. Then, we transformed the growth response to a binary response (i.e. presence/absence of new segments) to account for the high occurrence of zero values and tested the effect of treatments in the probability of observing positive growth. Multiple comparisons with adjusted p values were performed using the *lmerTest* package for LMM (Kuznetsova et al. 2012) and *multcomp* package for GLMM (Hothorn et al. 2008) when appropriate. Data analyses were performed with the statistical program R (version 3.2.3) (R Development Core Team 2010).

Results

Response in segment growth

The rate of segment production was consistent with the seasonality of segment growth known in *Halimeda*. A greater production of new segments was observed in summer than in winter. At the end of the experiments, we observed nearly threefold more production of segments in summer (Fig. 2). Light had a significant effect in the rate of

segment production and the probability of segment growth (Table 1). While light did not affect segment production during summer, increasing light intensity decreased growth during winter (ANOVA, $F(2,261)=8.83$, $P=0.0001$). However, the response in segment production to light was altered when herbivory was simulated in both seasons (Table 1). Tolerance of herbivory (i.e. segment production in response to clipping) was variable and dependent on the intensity of clipping. While clipping did not affect growth at low levels of light, clipping (low and high) decreased growth at high light levels in both seasons. At mid levels of light, simulated herbivory enhanced segment growth (Fig. 2). *Halimeda* increased production of segments only at medium light with respect to controls (no clipping). However, the response to high levels of clipping differed between seasons (Fig. 2). Repetitive clipping stimulated segments to grow in summer but not winter (Fig. 2). Nutrient enrichment stimulated segment growth during winter (Tukey's post hoc test, $Z=2.6$, $P=0.008$) but not summer with more than twofold increase in segments compared to ambient treatments (Fig. 2).

Changes in biomass

Patterns of biomass growth were consistent with the seasonality in segment production. However, the effect of treatments on biomass growth differed from that of segment production because we observed changes in biomass irrespective of segment growth (Fig. 3). Variation in biomass in the absence of segment addition could result from (1) limitations in the method used to estimate wet biomass or (2) changes in organic matter (e.g. increase in tissue thickness) or changes in CaCO_3 content (calcification). As expected, repetitive clipping simulating high herbivory had a significant (Tukey's post hoc test, $P < 0.0001$) and greater negative impact on biomass than a single clip (simulating low herbivory). At the end of the experiments (i.e. after two weeks), significantly greater losses were found in winter (up to 10% biomass loss) than summer (less than 7% biomass loss) (Fig. 3). *Halimeda* response to clipping was similar among levels of light. Nutrient enrichment increased biomass growth but it did not affect how biomass growth was affected by herbivory treatments (Fig. 3; Table 1). The impact of simulated herbivory on the relative growth rate of *Halimeda* (% RGR) in terms of biomass varied weekly (Online Resource, Fig. S2) and between seasons (ANOVA, $F(2,171)=8.51$, $P < 0.001$).

Photosynthetic response

Overall, artificial light treatments negatively affected *Halimeda* because the photosynthetic activity ($\Delta F/F_M'$) decreased in all treatments during the experiments (Fig. 4).

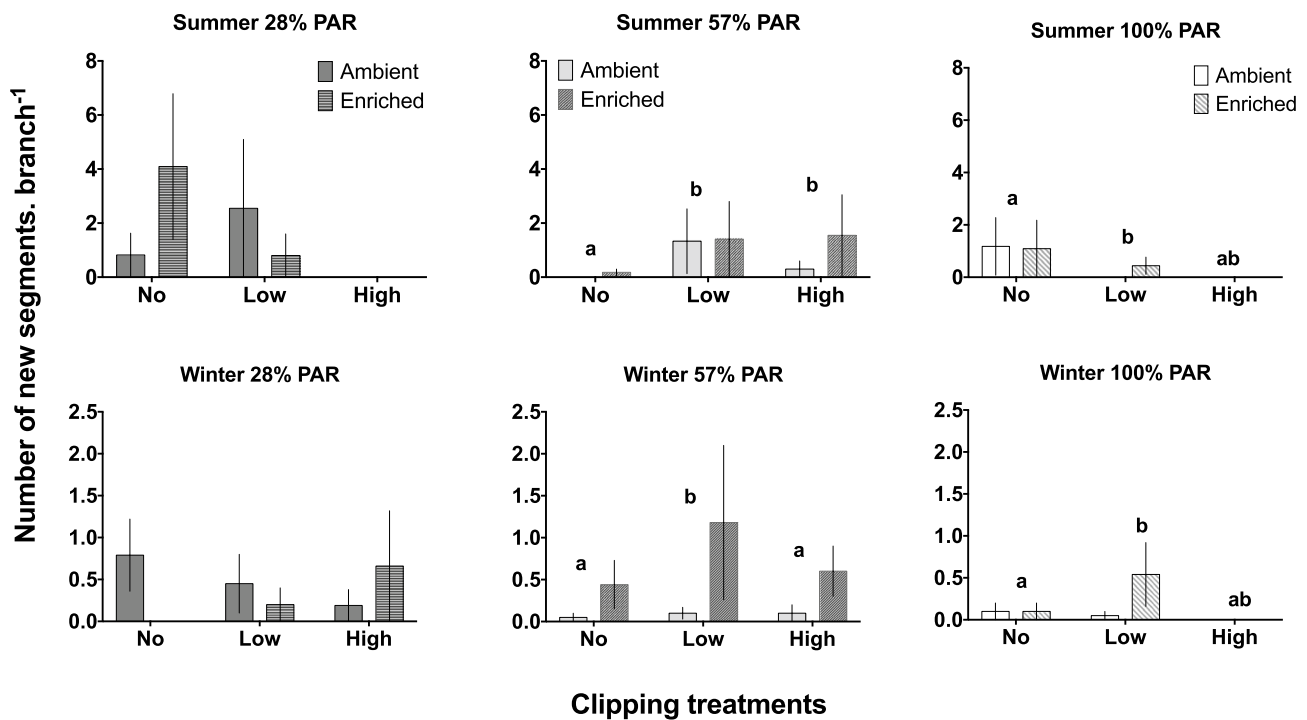


Fig. 2 Segment growth (mean \pm SE) of *Halimeda* in response to clipping treatments: No (Control)—low and high. Panels show the response in summer (top) and winter (bottom) under different light intensity (28, 57 and 100% PAR) and nutrient conditions (ambient

and enriched). Different letters denote significant differences among clipping treatments. Differences tested by Tukey's post hoc analyses. Data represent growth estimated at the end of the experiment (i.e. after 2 weeks)

Moreover, light intensity had a significant negative effect on $\Delta F/F_M'$ as it increased as light decreased (Table 1; Fig. 4a). Maximum quantum yield (F_V/F_M ; measured at dark), which is a measure of the maximum efficiency of light utilization in photosynthesis, also decreased with light intensity by 17% in summer and 23% in winter in high- vs low-light treatments. This result indicates that *Halimeda* experienced light stress (i.e. photoinhibition) in both summer and winter. Our results are consistent with what we expected as plants were collected from shaded habitats within the *Acropora* canopy. For example, during summer we estimated light fluctuated from 12 to 310 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PAR (mean of $67 \pm 38 \mu\text{mol m}^{-2} \text{s}^{-1}$) within the *Acropora* canopy at 4–6 m depth while in nearby open surfaces light ranged from 27 to 516 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PAR (mean of $227 \pm 81 \mu\text{mol m}^{-2} \text{s}^{-1}$) (Online Resource, Fig. S1). Assessment of the photosynthetic electron transport rate (rETR), which is proportional to photosynthetic carbon gain (Beer et al. 1998; Krall and Edwards 1990), decreased with lower light levels (Online Resource, Fig. S3). Nutrient enrichment did not significantly affect the F_V/F_M or rETR response to light but rETR decreased with nutrient enrichment especially under high levels of light (Online Resource, Fig. S4). The photosynthetic response of *Halimeda* (F_V/F_M) was 7% higher in summer than winter (~ 0.73 and ~ 0.68 , respectively)

(Table 1). Simulated herbivory induced a negative response in the photosynthetic potential ($\Delta F/F_M'$ and rETR) of *Halimeda* immediately after clipping (Table 1; Fig. 4b, Online Resource, Fig. S3). However, before treatments $\Delta F/F_M'$ and rETR were 13% and 37% higher in branches subjected to simulated herbivory during summer (Online Resource, Fig. S4). Specifically during summer we found that an increase in photosynthetic capacity was associated with an increase in biomass growth under low clipping levels (see Online Resource, Figs. S2 and S4).

Manipulation of nutrients

Nutrient concentrations obtained from inside and outside the *Acropora* canopy corroborated that nutrient availability is likely to change at small scales due to the presence of *Acropora*. We found a significant increment in nitrate and nitrite within *Acropora* habitats ($\text{NO}_2\text{-N}$: $0.29 \pm \text{SD} = 0.59 \mu\text{g L}^{-1}$; $\text{NO}_3\text{-N}$: $14.35 \pm \text{SD} = 6.27 \mu\text{g L}^{-1}$, $n = 31$) compared with exposed sites with no *Acropora* ($\text{NO}_2\text{-N}$: $0.03 \pm \text{SD} = 0.17 \mu\text{g L}^{-1}$; $\text{NO}_3\text{-N}$: $10.93 \pm \text{SD} = 3.97 \mu\text{g L}^{-1}$, $n = 32$). As expected, due to tide regimes and seasons, nutrient concentrations in tanks were highly variable and the manipulation of nutrients also resulted in a significant increase of dissolved

Table 1 Summary statistics of linear and generalized linear mixed model analyses of *Halimeda* responses to treatments

	Est	SE	Z value	P value
Growth traits				
Segment growth (no. segments branch ⁻¹)—all levels of clipping				
Intercept	2.64	0.29	8.80	<0.0001
Season (Winter)	-1.44	0.29	-4.89	<0.0001
Low clipping	-0.45	0.31	-1.45	0.14
High clipping	-0.06	0.71	-0.09	0.93
Enriched nutrients (+N)	0.55	0.30	1.83	0.06
Low clipping × light (28% PAR)	2.27	0.64	3.53	0.0004
High clipping × light (28% PAR)	1.67	0.91	1.82	0.06
Probability of segment growth (binomial response)				
Intercept	-2.73	0.51	-5.30	<0.0001
Season (Winter)	0.25	0.34	0.73	0.47
Low clipping	0.76	0.43	1.76	0.07
Light (100% PAR)	-1.30	0.67	-1.92	0.05
Enriched nutrients	-0.30	0.58	-0.53	0.59
Light (28% PAR) × Enriched nutrients	1.43	0.77	1.85	0.06
Biomass growth (% RGR)				
Intercept	0.57	2.78	0.20	0.84
Season (Winter)	-12.32	2.11	-5.83	<0.0001
Low clipping	12.01	2.33	5.14	<0.0001
No clipping	14.97	2.33	6.41	<0.0001
Light (57% PAR)	-0.21	2.49	-0.08	0.93
Light (100% PAR)	-3.39	2.49	-1.36	0.18
Enriched nutrients (+N)	4.72	2.11	2.23	0.03
Photosynthetic responses				
Effective quantum yield (relative change)				
Intercept	-0.26	0.02	-11.15	<0.0001
Season (Winter)	-0.05	0.02	-2.21	0.03
Light (57% PAR)	0.15	0.02	5.76	<0.0001
Light (28% PAR)	0.23	0.02	8.81	<0.0001
Change in effective quantum yield due to clipping				
Intercept	0.63	0.01	56.45	<0.0001
Season (Winter)	-0.03	0.01	-3.05	0.004
Clipping (before)	0.04	0.005	7.08	<0.0001
Light (57% PAR)	-0.05	0.01	-3.66	0.0007
Light (100% PAR)	-0.12	0.01	-9.56	<0.0001
Change in rETR due to clipping				
Intercept	15.32	1.05	14.53	<0.0001
Season (Winter)	3.94	1.00	3.90	0.0003
Clipping (before)	1.46	0.23	6.28	<0.0001
Light (57% PAR)	0.47	1.21	0.39	0.69
Light (100% PAR)	4.68	1.21	3.87	0.0004

Only the final model with significant interactions after backward selection process is shown. Est: parameter estimates (\pm SE) and statistical significance ($p < 0.05$) of growth traits (segment growth rate, growth probability, relative biomass change) and photosynthetic responses (effective quantum yield and ETR)

nutrients: a nearly threefold increase in the concentration of phosphate [$\text{PO}_4\text{-P}$] in water (from $6.2 \pm \text{SE} = 0.8$ to $16.7 \pm 3.2 \mu\text{g L}^{-1}$ in summer and from $9.3 \pm \text{SE} = 0.7$ to $15.3 \pm 2.3 \mu\text{g L}^{-1}$ in winter), a 8- to 20-fold increase in ammonium [$\text{NH}_4\text{-N}$] (from $8.7 \pm \text{SE} = 0.9$ to

$67.5 \pm 18.3 \mu\text{g L}^{-1}$ in summer and from $2.2 \pm \text{SE} = 0.6$ to $43.2 \pm 10.3 \mu\text{g L}^{-1}$ in winter), a twofold increase in nitrate [$\text{NO}_3\text{-N}$] (from $48.3 \pm \text{SE} = 4.9$ to $121.4 \pm 24.4 \mu\text{g L}^{-1}$ in summer and from $37.4 \pm \text{SE} = 1.9$ to $71.3 \pm 9.5 \mu\text{g L}^{-1}$ in winter), and a less than twofold increase in nitrite [$\text{NO}_2\text{-N}$]

Fig. 3 Top panel shows final changes (i.e. after 2 weeks) in branch biomass as a function of segment addition. Bottom panel shows the effect size for the response to simulated herbivory (No–low–high) and season on the RGR of *Halimeda* (as mean percentage change in biomass) under different nutrient conditions. Points represent observed values at the end of the experiment (i.e. after 2 weeks) and bars connected by lines represent the transformed fitted values of the model with 95% CI for the estimated effects. Different letters denote significant differences among treatments for both seasons. Differences tested by Tukey's post hoc analyses

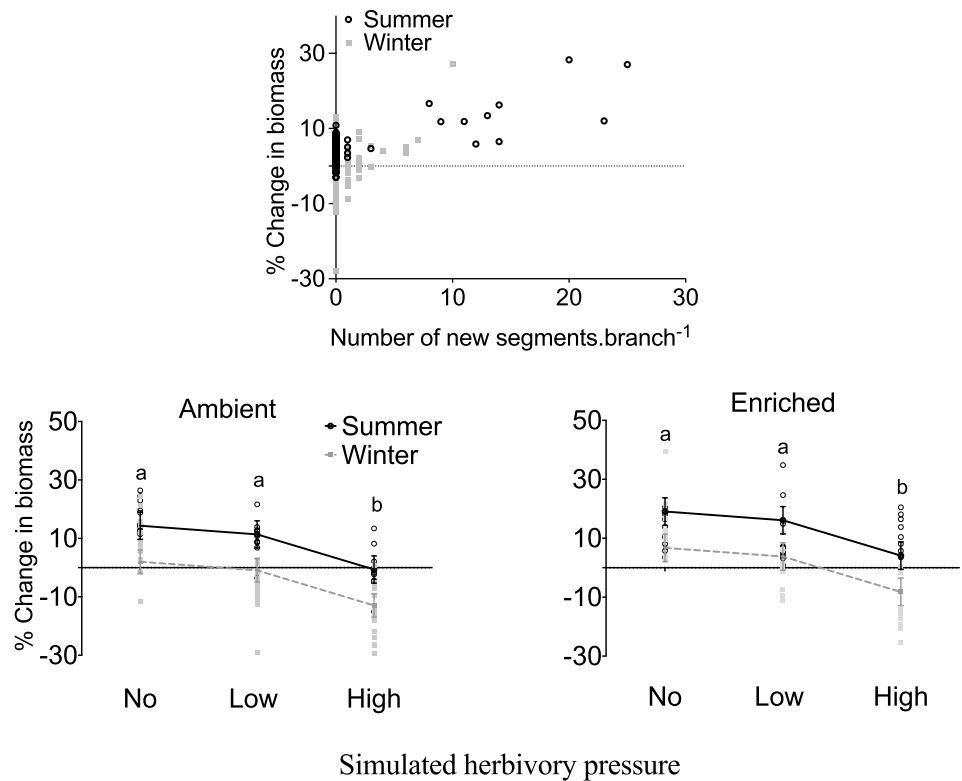
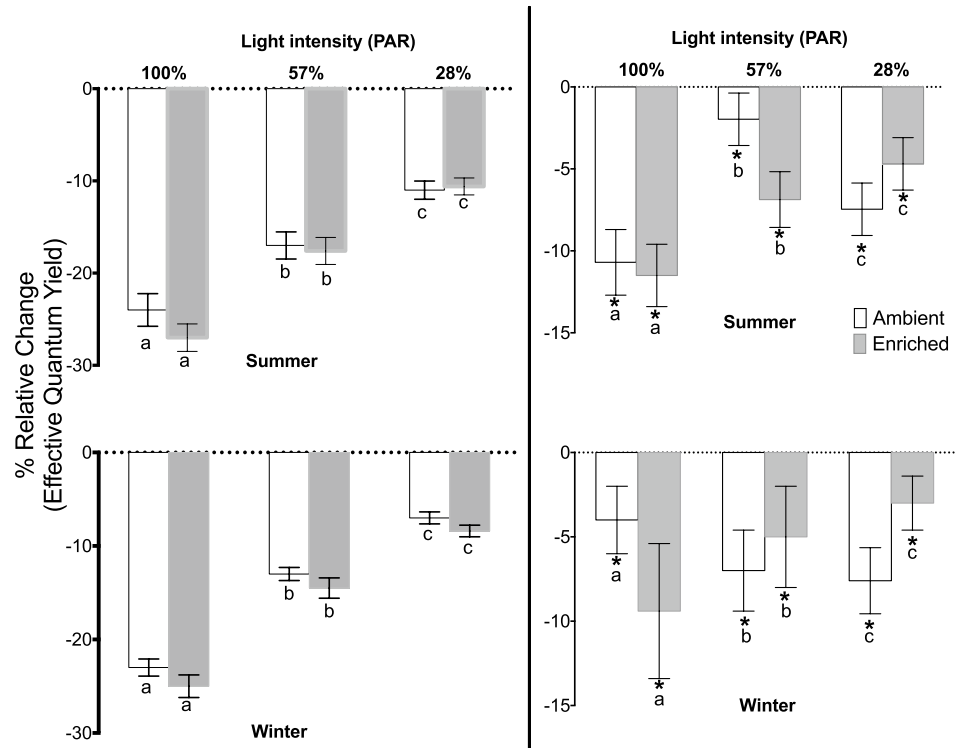


Fig. 4 Relative change (mean \pm SE) in *Halimeda* effective quantum yield ($\Delta F/F_M'$). Panels show response in summer (top) and winter (bottom) and under ambient (white bars) and enriched (grey bars) nutrient conditions: **a** response to light intensity after 2 weeks of experiments and **b** induced response immediately after clipping. Different letters denote significant differences among light treatments. Asterisks (*) denote significant changes in $\Delta F/F_M'$ after clipping. Differences detected by Tukey's post hoc analyses



(from $4.4 \pm SE=0.5$ to $6.1 \pm 0.7 \mu\text{g L}^{-1}$ in summer and from $0.37 \pm SE=0.1$ to $0.37 \pm 0.07 \mu\text{g L}^{-1}$ in winter) (Online Resource, Fig. S5). Nutrient enrichment resulted in a doubling of phosphorus in algal tissue in summer and

a 1.5-fold increase in winter (ANOVA, $F(1,35)=5.40$, $P=0.02$), but no significant changes in nitrogen content were observed between seasons (ANOVA, $F(1,30) = 1.60$, $P = 0.21$) (Online Resource, Fig. S5). Consequently, N:P

ratios decreased in *Halimeda* tissues with nutrient enrichment (ANOVA, $F(1,29) = 10.39$, $P = 0.03$). Clipping did not affect tissue nutrient concentrations (ANOVA, $F(1,32) = 1.90$, $P = 0.17$) but light treatments had variable effects on tissue nutrients. The content of N and C in tissues was always lower at mid light levels than in full exposed or shaded treatments. We saw a significant effect of light on carbon content (ANOVA, $F(2,30) = 8.51$, $P = 0.001$), which increased at low light. The content of N increased at mid light in winter and at low light in summer (Online Resource, Table S1).

Discussion

The response of *Halimeda* to simulated herbivory indicates that this species has the capacity to tolerate high levels of tissue removal by herbivores, replacing biomass rapidly under some conditions. Here, greater tolerance was observed in summer compared to winter, and at mid light levels compared to higher light levels. Therefore, the capacity to tolerate herbivory was influenced by resource availability (e.g. levels of light) consistent with the LRM model. The availability of light and nutrients had contrasting impacts on *Halimeda* tolerance of herbivory (i.e. segment growth rate) over different seasons. Because the morphology of the coral's colony influences the amount of light and/or nutrients available to the algae (Kanievska et al. 2008), variations in the architectural complexity of *Acropora* (e.g. in branch length and space between branches) may impact *Halimeda* in different ways. Our experiments suggest that fine-scale variations in resource availability such as those found among branching *Acropora* beds may influence the ability of *Halimeda* to compensate for tissue losses associated with fish grazing. These results may explain the contrasting effects of *Acropora* canopy on *Halimeda* biomass and morphometrics observed in the field among seasons and between locations (Castro-Sanguino et al. 2016).

Simulated herbivory can be a poor substitute for real herbivory (Strauss and Agrawal 1999) creating different responses in plants compared to natural herbivory (e.g. Hay et al. 2011). However, the response of plants to herbivory depends on the type of herbivory influencing plant's fitness (e.g. Moran and Bjorndal 2005; Vergés et al. 2008). *Halimeda* is consumed by a variety of herbivores including small invertebrates that scrape the superficial tissue to large herbivores such as fish that crop the algae by removing discrete bites of segments (Hillis-Colinvaux 1980). Because losses in biomass due to small invertebrates are assumed to be small compared to losses due to large herbivores and the impact of fish herbivores is assumed to be greater outside spatial refugia (Castro-Sanguino et al. 2016; Vergés et al.

2011), here we consider damage of fish only with our simulated herbivory intending to mimic the bite scars from fish observed in the field.

As expected, our results indicate that temperature (seasons) was the most influential factor consistently affecting segment production and biomass accumulation. An increase in segment growth rate and biomass was associated with higher temperatures during summer similar to previous observations (e.g. Ballesteros 1991; Castro-Sanguino et al. 2016; Drew 1983; Hudson 1985; Multer 1988). Therefore, the phenology of growth in *Halimeda* had an impact on the degree of tolerance to herbivory. During winter, compensation of biomass for low levels of tissue damage was not evident until the second week (14 days of treatment) while in summer *Halimeda* compensated for small and more extensive tissue losses (single and repetitive clipping) in 1 week. Our results support our hypothesis that seasonality influences the capability of *Halimeda* to compensate for tissue loss. Similar results were observed in Japan in the kelp *Undaria pinnatifida*, which showed greater compensation for tissue loss during the growing season (Gao et al. 2013). The influence of the timing of damage has also been observed in the compensatory capabilities of higher plants (e.g. Maschinski and Whitham 1989). Seasonality in the ability to compensate for biomass loss has great implications for the maintenance of *Halimeda* populations as *Halimeda* sexual reproduction is also driven by seasonality, occurring mainly during summer (Drew and Abel 1988). Moreover, in the face of ocean warming, an increase in temperature during winter could confer an increased tolerance of herbivory on *Halimeda*. Further studies on the thermal tolerance of *Halimeda* during winter are needed to test this hypothesis.

In a natural environment, algae are exposed to short-term variations in light intensity during the day due to changes in solar elevation, turbidity or tides, among other factors (Sagert and Schubert 2000). Therefore, a constant exposure to specific light levels during the experiments could be damaging for *Halimeda* and may explain the overall decline in photosynthetic activity. Light affected the production of new segments only during winter, with more segments being produced at lower light levels than at full light exposure. This result suggests that (1) light attenuation inside the *Acropora* canopy does not negatively affect *Halimeda* growth, and (2) the high experimental levels of light during winter ($256 \pm 13 \mu\text{mol m}^{-2} \text{s}^{-1}$) may be exceeding those tolerated by this species. However, higher levels of light were recorded during winter in exposed habitats where *Halimeda* grows (up to $407 \mu\text{mol m}^{-2} \text{s}^{-1}$). The influence of light on *Halimeda* segment production has been shown for other *Halimeda* species (Vroom et al. 2003; Yñiguez et al. 2010). If *Halimeda* is adapted to the low-light conditions found within the *Acropora* canopy, then a

sudden and prolonged exposure to high levels of light could have been damaging to the algae. High light exposure may cause photoinhibition and a decrease in photosynthetic performance could limit growth. In our study, light was the dominant regulator of the photosynthetic efficiency of *Halimeda*, increasing as light decreased. Photosynthetic performance declined (up to 30%) with increasing light intensity. Similar patterns have been observed in other species of *Halimeda* from shallow depths, which have shown to light saturate at irradiances below the average incident irradiance on sunny days (Littler et al. 1988; Vroom et al. 2003). Our results suggest that in the likely event of a reduction in *Acropora* (e.g. after storm damage or bleaching), exposure to high levels of light could negatively impact *Halimeda* growth at least temporarily (few weeks) until the plant acclimates to the new light environment. The consequences of habitat loss for the contribution of *Halimeda* carbonate sediments are unknown.

Simulated herbivory affected the response of *Halimeda* to light. While we observed that photosynthetic efficiency slightly decreased 5–10% immediately after simulating herbivory, damaged branches had higher photosynthetic activity (rETR and $\Delta F/F_M'$) than undamaged controls. Enhanced photosynthetic activity in the remaining tissue is one of the most common mechanisms in terrestrial plants to promote tolerance to herbivory (Strauss and Agrawal 1999; Tiffin 2000). Here, tolerance (i.e. segment growth in response to clipping) was greater at mid light levels than at low light levels. Experiments that manipulated light availability in *Dictyota ciliolata* and *Sargassum filipendula* show similar results of greater growth under less shaded conditions (72% of ambient light) than under the more shaded conditions (19% of ambient light) (Cronin and Hay 1996). While the relationship between compensatory growth and photosynthetic efficiency has been found in terrestrial plants, it has not been widely studied in marine plants (Hay et al. 2011; Vergés et al. 2008). Whether this response mitigates the negative effect of damage on fitness and also supports the synthesis of induced chemical defences is still unclear (Tiffin 2000). In our study, increases in photosynthetic activity were also associated with increased biomass growth, but this association was only evident in summer under low simulated herbivory and ambient nutrient conditions. These results partially support the hypothesis that the response in photosynthetic efficiency may help mitigate the negative effect of damage on fitness. However, increased photosynthesis in response to simulated herbivory was not associated with an increase in biomass growth under intense clipping and enriched nutrient conditions. Simulated herbivore damage has resulted in the immediate “activation” of chemical defences in *H. macroloba*, *H. opuntia* and *H. incrassata* in laboratory experiments (Paul and Van Alstyne 1992), and thus increases in photosynthetic rates with

simulated herbivory may support production of chemical defences.

Nutrient enrichment affected the rate of segment production in winter but not summer. When simulating herbivory during winter, nutrients increased segment production at mid levels of light regardless the intensity of clipping similar to observations of *H. macroloba* (Mayakun et al. 2012). At high light levels, nutrients did not affect growth in the absence of herbivory but when applying low clipping, nutrients increased segment growth. Therefore, the negative impact of clipping was ameliorated by the addition of nutrients, and tolerance was greater in the higher nutrient environment. Similar results have been observed in terrestrial plants where nutrient enrichment can overcome the negative effects of herbivory during the slow-growing season (Maschinski and Whitham 1989). Nutrient enrichment enhanced P but not N content in *Halimeda* tissues. Increasing P in tissues may suggest that P is more important than N for macroalgal growth (Lapointe et al. 1987; Littler et al. 1988).

In this study, the timing and intensity of clipping influenced *Halimeda* tolerance to herbivory. The ability of *Halimeda* to compensate for small but not for larger tissue losses can be explained by differences in the time available for compensation. Low clipping was inflicted at the beginning of the experiment while repetitive clipping occurred until the beginning of the second week resulting in less time for compensation. However, in situ, *Halimeda* can reduce grazing pressure by rapidly activating chemical defences (Paul and Van Alstyne 1992). If clipping induced chemical defences in *Halimeda* and the production of these compounds is costly for the plant, a trade-off between growth and production of chemical compounds would occur (Paul and Van Alstyne 1988) and this may explain why *Halimeda* could not compensate intense clipping with biomass growth.

Application of the limiting resource model (LRM) to predict tolerance in *Halimeda*

Contrary to sand-dwelling species, epilithic *Halimeda* species such as *H. heteromorpha* (rock dwellers) rely on water-column nutrients due to their limited rhizoidal structures (Littler and Littler 1990). Therefore, clipping of segments simulating fish herbivores damage is likely to simultaneously affect carbon assimilation and nutrient uptake in *Halimeda*. Light and nutrients influenced *Halimeda* growth in our experiments. Application of the LRM model requires identification of whether light or nutrients are limiting growth. Light did not limit segment production during summer but it did during winter. The next step is to evaluate the impact of herbivory on light acquisition. The LRM predicts that when herbivory causes the focal resource to

become limiting (i.e. increased photosynthetic activity with clipping), plants should show greater tolerance in the “high resource” or otherwise benign environment. Here, a “high-resource” environment is that of low stress for *Halimeda* growth (i.e. light levels that resemble in situ conditions). Light limitation due to clipping (low and high) was only evident in summer. Here, the LRM successfully predicted greater tolerance to herbivory at mid and low levels of light compared to high light levels with low amount of clipping. However, tolerance was affected when higher levels of clipping were used. Similarly, the LRM predicts that when herbivory does not affect light acquisition like we observed in winter, plants should show equal tolerance at different levels of light. The LRM was successfully predicting equal tolerance to high clipping at all levels of light during winter but again the level of tolerance was affected when lower levels of clipping were used. The contrasting responses with high herbivory treatments could result from the short time allowed for compensation after repetitive clipping as explained above.

In the absence of herbivory treatments, nutrients were not limiting the rates of segment growth in our experiments during summer but they were limiting growth during winter. However, clipping of segments can affect nutrient uptake. During winter, clipping did not affect the positive effect of nutrient enrichment. Clipped branches also benefited from nutrient enrichment causing nutrient limitation. Similarly during summer segment production did not benefit from nutrient enrichment and clipping did not affect this response. Therefore, in accordance with the LRM, we observed equal tolerance to herbivory in both low- and high-nutrient environments.

Conclusion

The LRM was successful in predicting different levels of tolerance depending on the impact of herbivory treatments and the environment where *Halimeda* grew. However, the contrasting responses to different levels of clipping limited the applicability of this model to explain tolerance under some circumstances. Our experimental results show that the ability of *Halimeda* to cope with tissue damage is dependent on the magnitude and timing of damage. While *Halimeda* is less tolerant during winter than summer, *Halimeda* could benefit from nutrient pulses during winter to increase tolerance. The results of this study suggest that the ability to compensate for losses due to herbivory is likely to be affected if *Halimeda* is exposed to intense light and higher levels of herbivory. Therefore, if habitat complexity is reduced and *Halimeda* cannot adapt to the environmental conditions found in exposed habitats, then a likely negative impact on *Halimeda* populations could be expected if

herbivory is high. These findings highlight the importance of preserving habitats provided by branching corals for maintaining *Halimeda* productivity in habitats of intense herbivore pressure.

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Compliance with ethical standards

Conflict of interest The authors have declared that no competing interests exist.

Ethical approval This research does not contain any studies with human participants or animals performed by any of the authors. Research was conducted under GBRMPA permit #G13/36037.1.

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