

Standing stock variations, growth and CaCO₃ production by the calcareous green alga *Halimeda opuntia*

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The present paper investigates standing stock variations of Halimeda opuntia on a sandstone reef of the South-west Atlantic Ocean, in order to better understand the role of this seaweed as a CaCO₃ producer. The study was conducted over two 3-month periods, using photo quadrats to analyse the coverage area, and destructive sampling to quantify area-specific biomass and CaCO₃ percentage. The alga occupied 2.4% of the substrate (4464 m²), growing as clumps with an average biomass of 1.59 kg m⁻², resulting in a standing stock of 7097.8 kg of alga. This standing stock varied with environmental conditions, particularly wind speed. Assuming an exponential model for these variations, H. opuntia produced at least 13,050.14 kg (54.37 g m⁻² day⁻¹) of carbonate sediments. There was a positive correlation between changes in standing stock and coverage, but not with area-specific biomass. This suggests that net algal growth results in the occupation of new spaces, with minimal increases in height or segment density. Therefore monitoring coverage should complement traditional individual-based methods for estimating Halimeda growth and production. Combined, these approaches should result in more accurate models of the role of this alga on marine carbonate budgets.

Keywords: Chlorophyta, calcium carbonate, sediments, sandstone reefs, South Atlantic

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INTRODUCTION

Among marine calcium carbonate (CaCO₃) producers, calcareous green algae of the genus *Halimeda* J.V. Lamouroux are one of the major contributors of CaCO₃-rich sediments in tropical regions (Freile *et al.*, 1995; Rees *et al.*, 2006). These algae have widespread distribution, usually attain high coverage, and have brittle segmented thalli with high percentages of calcium carbonate (Hillis-Colinvaux, 1980; Kooistra *et al.*, 2002). In many tropical continental shelves, large fractions of calcareous sediment deposits are composed by *Halimeda* dead fragments (Milliman, 1977; Alexandersson & Milliman, 1981). Due to its importance, the genus has been the subject of many ecological studies, and direct estimates of growth rates and CaCO₃ production are available for many of its species (Multer, 1988; Vroom *et al.*, 2003; Mayakun *et al.*, 2014).

Within the genus, the rock-dwelling *Halimeda opuntia* (L.) J. V. Lamouroux is one of the most common species worldwide (Kooistra *et al.*, 2002; Bandeira-Pedrosa *et al.*, 2004). Due to its abundance, this alga may play a key ecological role on marine hard-bottom communities (Hillis-Colinvaux, 1980; Bandeira-Pedrosa *et al.*, 2004). Nevertheless, direct measures of CaCO₃ production by this species are rare (e.g. Drew, 1983; Hudson, 1985; Multer & Clavijo, 2004), and the

recognition of its importance on sediment dynamics is mostly based on indirect data, derived from sedimentological studies of carbonate build-ups (Enos & Perkins, 1977; Hine *et al.*, 1988; Johns & Moore, 1988).

The paucity of studies on *H. opuntia* is partially due to its complex morphology, which differs from many of its congeners by the tendency of assuming sprawling forms, with indeterminate growth (Verbruggen & Kooistra, 2004; Yñiguez *et al.*, 2008). In such cases it is often not feasible to isolate individuals within a population, hindering quantitative measurements of CaCO₃ production, since these estimates are commonly based on monitoring mean individual growth (e.g. Bach, 1979; Payri, 1988; Garrigue, 1991; Freile & Hillis, 1997; Vroom *et al.*, 2003). The scarcity of quantitative studies in many locations prevents more accurate analyses on the role of *H. opuntia* on global and regional sediment budgets.

In the tropical South-west Atlantic Ocean, the continental margin is a mixed carbonate-siliclastic platform (Testa & Bosence, 1998, 1999; Vital *et al.*, 2008, 2010; Bastos *et al.*, 2015; Gomes *et al.*, 2015). In shallower waters, calcareous accumulations are frequently found near reef habitats (Segal & Castro, 2011; Castro *et al.*, 2012; Silva *et al.*, 2013). These carbonate deposits are mostly composed of skeletal debris of reefal CaCO₃ producers, such as cnidarians, molluscs and algae, including *Halimeda* (Leão *et al.*, 2006; Pereira *et al.*, 2013). Understanding the ecology of the *H. opuntia* in this context is therefore paramount to comprehending regional sediment and carbonate dynamics, which so far have been poorly studied (e.g. Gherardi, 2004; Araújo & Machado, 2008; Amado-Filho *et al.*, 2012; Carneiro & Morais, 2016).

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The present study aims to analyse *H. opuntia* population dynamics on a SW Atlantic reef, by measuring temporal variations on coverage and area-specific biomass, as a means to quantify its importance as a space occupier. Furthermore, standing stock changes were used to estimate CaCO_3 production in order to better characterize its role as a producer of carbonate sediments. To our knowledge, this is one of the first quantitative studies on biogenic production of sediments in South Atlantic reefs and the first to relate standing stock and carbonate productivity by *Halimeda*. This different approach results in net instead of gross CaCO_3 production and seems to be suited to species with sprawling, indeterminate habits.

MATERIALS AND METHODS

Study area

The *Parrachos de Pirangi* are ferruginous sandstone outcrops, possibly of tertiary origin (Branner, 1904), located ~ 1000 m, toward 90°E , off the mouth of the Pirangi river in the state of Rio Grande do Norte, Brazil ($05^\circ 58.824'\text{S}$ $35^\circ 06.495'\text{W}$).

The formation is composed of numerous flat sandstone elevations of varying sizes, which may or may not emerge during low tide, forming a discontinuous barrier parallel to the coastline (Figure 1). On previous visits to the study area, *Halimeda opuntia* was found growing only at the intertidal region of the largest of these platforms, an area of $\sim 186,000$ m², that could be divided into three zones: a protected back zone with many

submerged channels, a plateau with flat relief and a wave-exposed crest.

The climate of the region is tropical wet and dry (*Aw* in Köppen classification). The rainiest months are April to June and the driest October to December. Temperatures are usually high and with low amplitudes throughout the year. Average temperature varies from 24.5°C between June and August to 27°C in January–March. The wind speed is higher outside the rainy season, the average speed being the highest between August and October.

Field sampling

Three transects were established parallel to the major axis (roughly parallel to the coastline) of the reef where *H. opuntia* was found, one in each zone: back, plateau and crest. Each transect was 500 m long and separated from the adjacent transect by 75 m.

Six field campaigns were conducted to study the *H. opuntia* population, three in the dry season (August, September and October 2009) and three in the rainy season (March, April and May 2010).

On each campaign, the three transects were visited in periods of diurnal spring tide, when the area is mostly emerged. These transects were used to collect data on biomass and population dynamics (see below).

Area-specific biomass and calcium carbonate

In the present study, biomass was defined as the area-specific mass of the algae (in g cm^{-2}), and was calculated dividing the algal dry weight (DW) by the area of the samples (100 cm²). In each campaign, 15 samples of *H. opuntia* were haphazardly collected, five per transect. Each sample consisted of algal clumps measuring at least 100 cm², scraped from the substrate with the aid of 10×10 cm quadrats. This sample size was chosen to ensure sampling of mature individuals, which should cover the entire quadrat area to allow comparisons among months, and it is similar to the average clump size in the study area (see Results section). The samples were stored in plastic bags containing a solution of 4% formalin in seawater.

In the laboratory, samples were washed and cleaned to remove epibionts and dried at 50°C until their weight was constant. To measure CaCO_3 content, dry samples were decalcified with 0.1M HCl. After the reaction, samples were carefully rinsed with distilled water and the liquid phase was discarded to remove any dissolved salts. The material was once again dried at 50°C until constant weight and the CaCO_3 percentage was calculated as the difference between the DW of the fresh sample and that of the decalcified sample (Van Tussenbroek & Van Dijk, 2007).

Population dynamics

Coverage was defined as the average reef area covered by *H. opuntia* (in m²). To estimate this parameter, 30 quadrats measuring 50×50 cm were evenly distributed over the reef (one quadrat every 50 m, in total 10 per transect) and were photographed using a digital camera with the aid of mounted supports to avoid angle variations. This quadrat size was selected to ensure that no sample was smaller than the largest *Halimeda* clump observed on a pilot survey

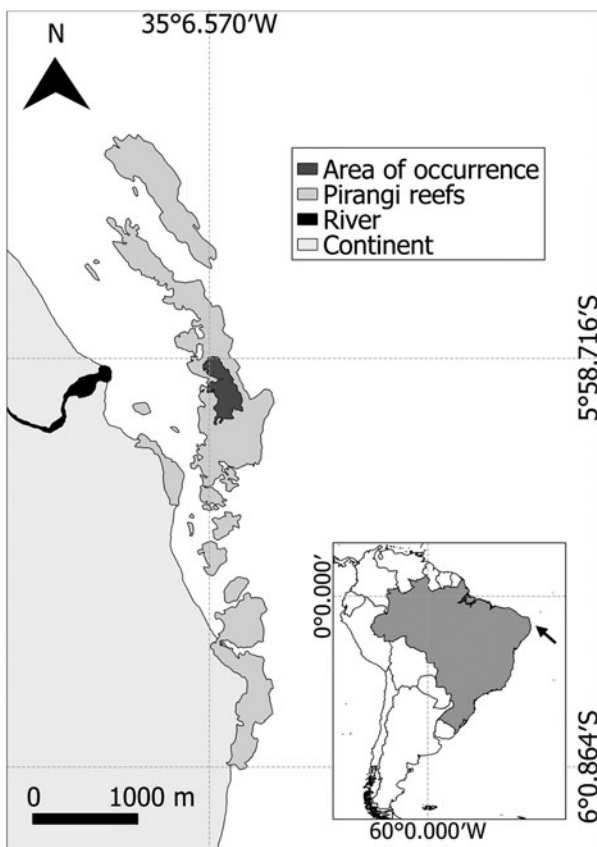


Fig. 1. Location of the study area. Dark grey region in the detail indicates the area of occurrence of *Halimeda opuntia*.

(~1200 cm²). To avoid bias in coverage estimates, only the *Halimeda* observed inside quadrats was considered in the analysis, even if the clumps extended beyond the delimited area. The digital images were analysed on ImageJ software, which was used to visually delimit and measure the area occupied by *H. opuntia*. Since our data did not show significant differences in area-specific biomass (see Results section), this 2D projected area was considered a good proxy to clump size and to the area occupied by the alga on the reef.

For the purpose of calculation, in campaigns where *H. opuntia* was observed on the reef but none of the 30 quadrats contained any clumps we used a percentage occupancy estimate that was 10% of the smallest coverage measured during the study.

Four environmental variables were used to explain eventual variations in population: air temperature (°C), photosynthetically active radiation (PAR) (μmol photons m⁻² s⁻¹), rainfall (mm) and wind speed (m s⁻¹). These variables were chosen to represent those used by Yñiguez *et al.* (2008), with rainfall as an estimator of the nutrient input to the system and wind speed as a measurement of disturbance by hydrodynamic forces, which seems reasonable for the study area (Barros & Rocha-Barreira, 2014). To reduce bias, environmental variables were measured in the 30 days immediately prior to each campaign, therefore: temperature and wind speed were the average daily value along this period; PAR was the average value per hour of sunlight; and rainfall was the rain accumulated in these 30 days.

These data were obtained from the solarimetric station of the Laboratório de Variáveis Ambientais Tropicais (LAVAT) of the Instituto Nacional de Pesquisas Espaciais (INPE), located in Brazil.

Halimeda opuntia net growth

Standing stock was defined as the total amount of algae on the reefs (in kg), which was calculated by multiplying the coverage by the area-specific biomass estimated in each campaign. The net growth rate was the rate of variation of the standing stock between consecutive months.

We have assumed that standing stock variations were exponential. This assumption results in simple but useful models (Hastings, 1996; Yong *et al.*, 2013), that are widely used in seaweed studies (e.g. DeBoer *et al.*, 1978; Littler & Littler, 1985; Pedersen & Borum, 1997; Martins *et al.*, 1999; Barr *et al.*, 2008; Hadley *et al.*, 2015), particularly when density-dependent effects do not need to be considered, such as when population sizes are small or highly variable, which is the case in the present study (see Results section). A more complex approach would require estimates of the carrying capacity of tropical intertidal environments, which are not readily available and would be difficult to determine due to the complexity of this type of ecosystem (Edgar, 1993; Christensen & Pauly, 1998; Monte-Luna *et al.*, 2004). Furthermore, other studies have shown that *H. opuntia* may cover 90–100% of the substrate in some locations (Hillis-Colinvaux, 1980), which indicates that the species is able to flourish into very dense aggregates. As such, density-dependent effects should be an issue only at extreme coverages. Therefore we have considered that exponential models are reasonable approximations, sufficiently accurate to describe the *Halimeda* population under study.

According to this model, the standing stock and the coverage at a given time are calculated by the formulae:

$$S_f^s = S_o^s(1 + r_s)^t \quad (1)$$

$$S_f^c = S_o^c(1 + r_c)^t \quad (2)$$

where S_f^s = final standing stock, S_o^s = initial standing stock, r_s = net growth rate, t = time in number of days, S_f^c = final coverage, S_o^c = initial coverage and r_c = rate of variation in coverage (rate of area growth). Rearranging either equation (1) or (2), the net growth rate is:

$$r = (S_f/S_o)^{1/t} - 1 \quad (3)$$

which is a reliable estimate of seaweed growth when compared with other formulae (Yong *et al.*, 2013). Finally, the average daily growth per square metre (χ) is:

$$\chi = \frac{1}{t} \sum_{i=0}^t \frac{S_i^s r_s}{S_i^c} \quad (4)$$

which represents how much algal mass was lost per day, after adjusting for the respective variations in coverage.

Statistical analyses

Due to the non-normal skewed nature of abundance data, a Kruskal–Wallis test was used to compare reef zones in terms of *H. opuntia* coverage. An Analysis of Variance (ANOVA) followed by a Tukey HSD test in cases where the P -value was <0.05 was used to examine whether there was a significant variation in area-specific biomass or percentage of CaCO₃. The normality of the data was analysed by Lilliefors test and a log transformation was applied in the cases where data seemed to be non-normal.

The relation of the environmental variables with area-specific biomass, % CaCO₃, coverage and standing stock was examined via Pearson correlation analysis, with the data log-transformed ($\log_{10} + 1$).

A linear regression analysis was used to understand which environmental variable could explain most of the variation in the standing stock. Considering that the environmental variables are often correlated, to prevent multicollinearity we performed a Principal Component Analysis (PCA) of these variables and used the Components with eigenvalue higher than one in the multiple regression.

All tests were performed using R 3.1.1 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Halimeda opuntia population

Throughout the study period, *H. opuntia* was the only species belonging to the *Halimeda* genus that was observed growing on the studied reefs. No evidence was found that these clusters were more common in any particular transect (Kruskal–Wallis $\chi^2 = 3.09$, $P = 0.21$). Therefore, it seems that

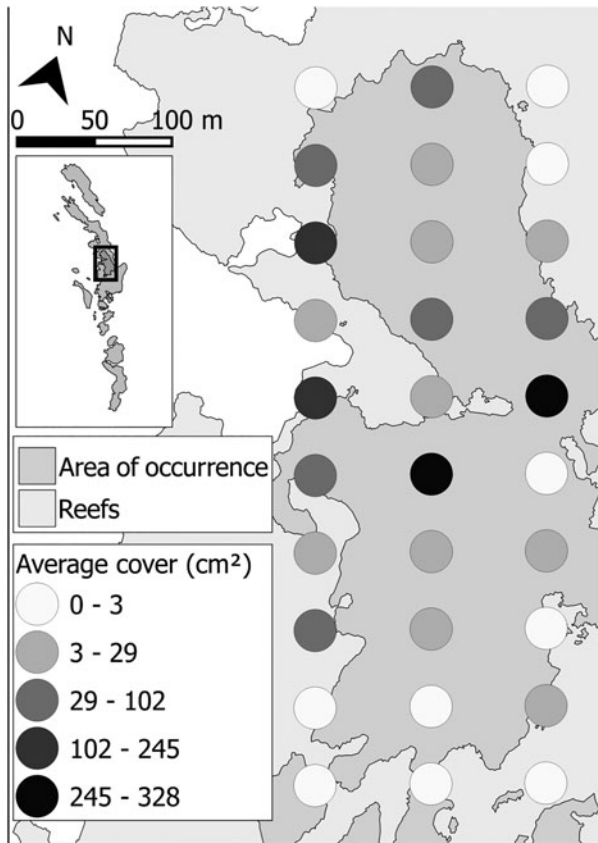


Fig. 2. Area of occurrence of *Halimeda opuntia*. The black circles illustrate the average area of coverage at each point sampled indicating no differences between transects or reef areas.

Halimeda may grow over their entire area of occurrence, without preference for any specific reef zone (Figure 2).

Population parameters are summarized in Table 1. When present on the reef, *H. opuntia* appeared as aggregates with an average of 2.67 ± 0.4 clumps m^{-2} (mean \pm SEM), each clump measuring 99.2 ± 18.0 cm^2 . The average biomass was 1.59 ± 0.13 $kg m^{-2}$, most of it ($84.06 \pm 0.6\%$) being calcium carbonate.

The percentage of $CaCO_3$ differed between months ($F = 24.99$, $P < 0.001$). The Tukey HSD test did not show a clear pattern, grouping August and September (highest % $CaCO_3$); October, March and May (lowest % $CaCO_3$); and April (intermediate % $CaCO_3$), every group differing significantly to each other.

Table 1. Summary statistics (mean \pm SEM) of *Halimeda opuntia* J.V. Lamouroux on Brazilian sandstone reefs.

Month	N° of clumps m^{-2}	Clump area (cm^2)	Biomass ($kg m^{-2}$)	$CaCO_3$ %	Coverage (m^2)	Standing ^a stock (kg)
August	3.60 ± 0.8	127.40 ± 50.3	1.30 ± 0.21	88.43 ± 1.1	8799.80 ± 3971.8	11,439.74
September	4.27 ± 0.9	37.00 ± 10.8	1.40 ± 0.23	90.04 ± 0.6	2758.40 ± 1082.4	3861.76
October	— ^b	— ^b	1.20 ± 0.4	80.65 ± 1.3	275.84^c	331.01
March	0.67 ± 0.7	211.80 ± 136.9	1.40 ± 0.22	81.01 ± 0.9	3096.30 ± 2382.4	4334.82
April	4.27 ± 1.0	107.80 ± 36.4	2.20 ± 0.35	85.13 ± 0.5	6373.30 ± 3236.0	14021.26
May	3.20 ± 0.9	111.50 ± 37.1	2.00 ± 0.4	79.47 ± 1.0	4802.60 ± 2733.1	9605.20
Study	2.67 ± 0.4	99.2 ± 18.0	1.59 ± 0.13	84.06 ± 0.6	4464 ± 1067.3	7097.76

^aCalculated by multiplying mean coverage and mean biomass.

^bNone of the quadrats had tufts in October.

^cFor calculation purposes, coverage in October was considered to be 10% of the smallest estimated coverage.

Halimeda opuntia net growth and sediment production

Halimeda opuntia occupied an average of 2.4% of its area of occurrence ($4464 m^2$). This area, however, varied between the months, from $2758.4 m^2$ in September to $8799.8 m^2$ in August (Figure 3). In October, as none of the 30 quadrats contained any clumps, we considered a coverage area of $275.8 m^2$ for calculation purposes.

Multiplying average coverage by the area-specific biomass, it was estimated that there was on average 7097.8 $kg DW$ of *H. opuntia* over the reef. Variations in this standing stock correlated positively with coverage ($r = 0.90$, $P = 0.01$), but not with biomass ($r = 0.52$, $P = 0.10$), which have remained relatively stable throughout the study period (Figure 4).

Considering that during the study period the standing stock of *H. opuntia* decreased, the net growth rate estimated by equation (3) was -0.07% of the total amount of algae on the reefs per day. Applying equation (4), this equates to an average net loss of 1.0 $gDW m^{-2} day^{-1}$.

This rate, however, was not constant throughout the study period. The maximum daily net growth observed was 75.2 $g DW m^{-2} day^{-1}$ between March and April. On the other hand, between September and October, there was an average net loss of 72.2 $g DW m^{-2} day^{-1}$ (Figure 5).

If we consider only the periods when the population declined (August–September, September–October and April–May) and that 84.06% of the dry mass of *H. opuntia* was $CaCO_3$, it was estimated that over the study period, this alga alone contributed toward at least $13,050.14$ kg (54.37 $g m^{-2} day^{-1}$) of carbonate sediments to the surrounding reef areas.

Environmental variables and population dynamics

As expected by the climate in the study area, the 30 days preceding the field campaigns between August and October had on average higher wind speeds and PAR, but lower rainfall intensities and air temperature than the 30 days leading collections between March and May (Figure 6).

The correlation analysis showed a strong positive relation between rainfall and both coverage ($r = 0.90$, $P = 0.01$) and standing stock ($r = 0.88$, $P = 0.02$). On the other hand, these two variables had a strong negative relation with wind (coverage: $r = -0.83$, $P = 0.04$; standing stock: $r = -0.88$, $P = 0.02$) (Figure 7). None of the other variables showed

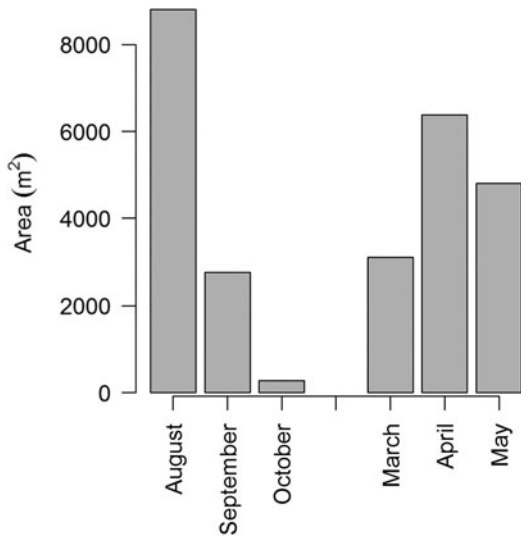


Fig. 3. Area covered by *Halimeda opuntia* in each month of the study.

significant relationships, with the exception of a negative correlation between rainfall and PAR ($r = -0.86, P = 0.03$).

Only the first two components generated by the PCA had eigenvalue greater than one, and together represented 87.34% of the variance of the environmental variables. The plot of environmental variables in the space formed by the two components (Figure 8) showed that PC1 separated PAR and wind speed from rainfall, with minor influence from air temperature. Thus it can be understood as representing climatic variations between rainy and dry season in the study area, which result in increased agitation in shallow marine environments. Based on the same reasoning, PC2 could represent climatic variations of the transition from the coldest to the warmest months, which could lead to desiccation and heat stress.

The regression analysis of the standing stock with the first two PCs generated a model with a low but non-significant P -value ($F = 9.17, P = 0.052$). However, considering that the t -test showed a significant coefficient for the first PC

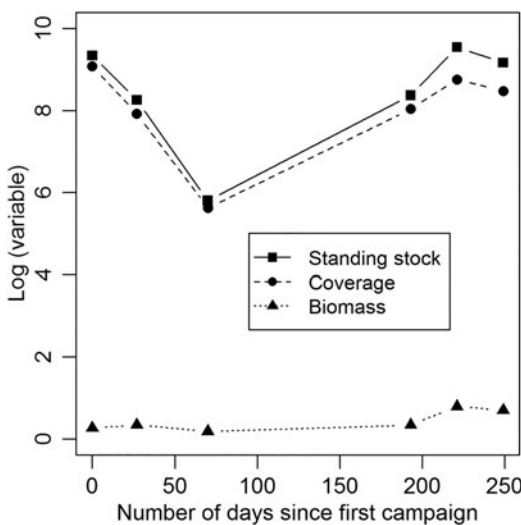


Fig. 4. Temporal variation in standing stock, coverage and biomass of *Halimeda opuntia* on Brazilian reefs.

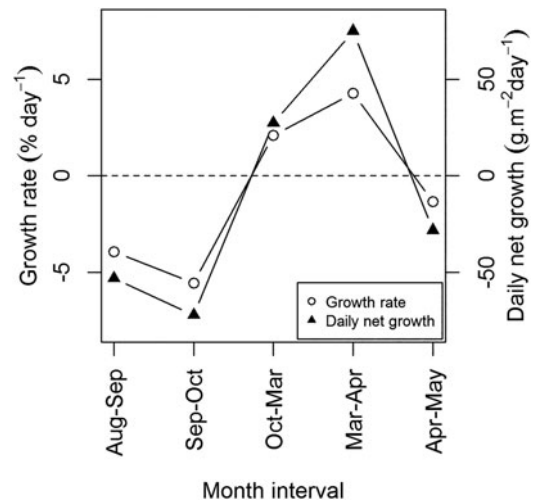


Fig. 5. Temporal variation of average growth rate and daily net growth of *Halimeda opuntia*. Daily net growth represents how much algal mass was lost per day after adjusting for the respective area variations.

($t = -4.14, P = 0.02$) and that the two components are by definition orthogonal (non-correlated), we interpreted this non-significant F -test as a consequence of the inclusion of the PC2, which seems to be unrelated to the standing stock ($t = 1.06, P = 0.36$) and, as so, could negatively affect the result of the model. Because of that, we excluded this second component from the model and repeated the regression only with PC1.

This second linear regression with only the first principal component and the standing stock was significant ($F = 16.65,$

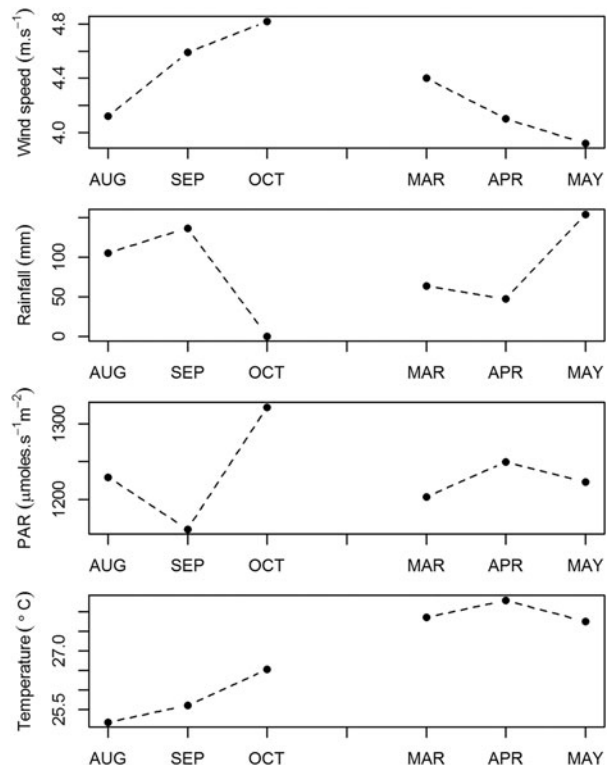


Fig. 6. Average of six environmental variables in the 30 days prior to the campaigns to study *Halimeda opuntia* on Brazilian reefs.

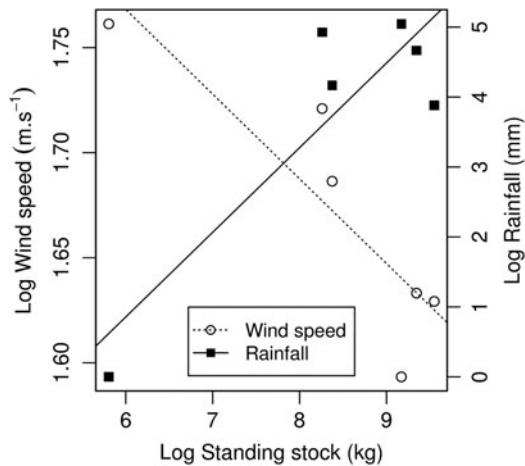


Fig. 7. Scatterplot showing a negative relationship between *Halimeda opuntia* standing stock and wind speed ($r = -0.88$, $P = 0.02$), but a positive relation with rainfall ($r = 0.88$, $P = 0.02$).

$P = 0.02$). Both the intercept ($P < 0.001$) and the linear coefficient ($P = 0.01$) were also significant. The model formula was:

$$S^s = 3.65 - 0.36PC$$

where S^s = standing stock and PC = first principal component. The 95% confidence limits for the intercept are from 3.321 to 3.990, and for the linear coefficient from -0.608 to -0.116 .

DISCUSSION

The role of *Halimeda* on calcium carbonate production is well established, and the genus is considered an important player in the global carbonate budget (Freile *et al.*, 1995; Rees *et al.*, 2006). In accordance with the genus standards, the present study has detected variations in abundance of *Halimeda opuntia* which suggests that it is a fast growing

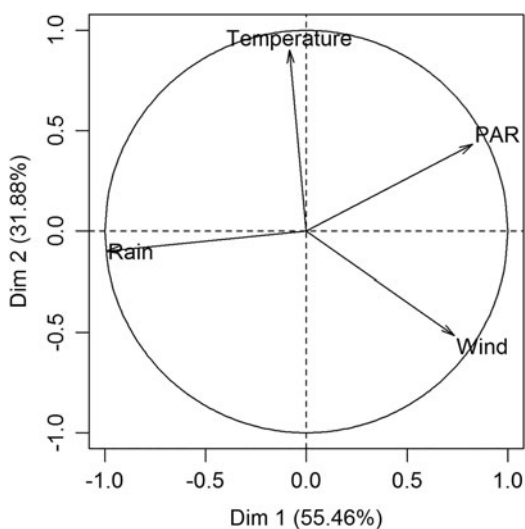


Fig. 8. Plot of four environmental variables on the space formed by the first and second Principal Components Analysis. PAR, photosynthetically active radiation.

species and an important sediment producer in tropical reefs. Even though it did not cover large areas of the substrate (i.e. we have estimated a coverage of 2.4% of the area), the variations in standing stock were responsible for at least 13,000 kg of CaCO_3 -rich sediments to the reef habitat and its surroundings.

Regarding the influence of environmental conditions on *H. opuntia*, our results agree with the model proposed by Yñiguez *et al.* (2008), which have indicated that disturbance is one of the main factors structuring its populations. In the present study the population declined in times of stronger winds, which determine more intense waves and currents, and consequently result in increased disturbances (Vital *et al.*, 2008, 2010). There was also a significant correlation with the first principal component, which was interpreted as summarizing agitation-mediated disturbances of abiotic origin. Such conditions are also known to negatively affect seagrass beds in the SW Atlantic (Barros & Rocha-Barreira, 2014). Intense wind-driven disturbance seems to affect coverage by breaking algal clumps, while preventing fragment resettlement (Yñiguez *et al.*, 2008).

Despite this likely importance of wind-driven disturbance in structuring *H. opuntia* populations, environmental variables are typically correlated in the study area, and it is not always easy to isolate the effects of a single factor on the population. The PCA analysis suggests that temperature may have had only a minor effect on *Halimeda* standing stock, whereas rainfall significantly benefited the population. During rainy seasons, the average wind speed usually decreases and this benefit could have been the result of a reduced disturbance. Nevertheless, considering that our data did not show a linear correlation between rain and wind, we believe that the positive effect of rainfall was mainly due to increased nutrient inputs. The CaCO_3 percentage of thalli supports this interpretation, since algae tended to be less calcified in rainy months (Table 1), indicating an active production of new segments. Such a reduction in calcium carbonate would not be expected if the larger coverage was due mainly to a less disturbed season. Furthermore, there is a river near the reefs, which is a natural source of fresh water to the system, increasing nutrient supply during rainy seasons (Rodrigues *et al.*, 2009; Seitzinger *et al.*, 2010; Dias *et al.*, 2013).

Moreover, new studies are needed to assess the effects of PAR on *H. opuntia*. In our analysis, this variable did not have an influence on the population. Nevertheless high levels of radiation may have inhibited photosynthesis (Littler *et al.*, 1988; Payri, 1988), reducing growth rates. This could have led to an accentuation of the destructive effects of disturbance, even if PAR did not affect the population directly.

Regarding *H. opuntia* growth, most of the previous data came either from *in vitro* analyses (Drew & Abel, 1985; Payri, 1988) or from monitoring individual colonies on shallow subtidal environments (Drew, 1983; Hudson, 1985; Multer & Clavijo, 2004). These studies have shown that the species grows through spasmodic events, which may be strongly seasonal, wherein algae may produce segments luxuriantly, but unevenly over their thalli. The present study adds information on intertidal populations growing on reef flats, and it suggests temporal variations on coverage, but a relatively stable area-specific biomass of individual clumps. This indicates that most of the growth results in an increased coverage, the algae occupying new spaces with relatively minor increases in height or segment density.

Since *H. opuntia* may assume a laxer morphology in shallow subtidal habitats, exhibiting looser and longer branches (Hillis-Colinvaux, 1980; Littler & Littler, 2000), new studies are needed to estimate area-specific biomass changes in these environments. Furthermore, new analyses are needed to understand growth in younger and smaller individuals, since we have restricted our biomass observations to mature algae (with at least 100 cm²). Nevertheless, due to the limited height that is achievable by this species in field conditions, and based on descriptions by other authors (e.g. Hillis-Colinvaux, 1980; Multer and Clavijo, 2004), sprawling morphotypes seem to be the rule rather than the exception for the species. Hence our results should be extendable to other populations under similar environmental conditions, irrespective of their average clump sizes.

Furthermore, previous studies have also pointed to the same relationship between variations in standing stock and coverage in *Halimeda*, with a relatively minor importance of individual sizes or biomass (Van Tussenbroek & Van Dijk, 2007). This seems to be particularly true for populations under disturbance – which seems to be the case in the present study – where fragmentation may be a rapid source of new individuals (Walters & Smith, 1994; Yñiguez *et al.*, 2008), since segment resettlement seems to be an important mechanism for dispersion in *Halimeda* (Walters *et al.*, 2002). Nevertheless, population growth by reproduction and space colonization remains poorly measured in this alga. Most analyses of production are based on counting the number of new segments produced by previously marked individuals over a given period of time (Bach, 1979; Payri, 1988; Garrigue, 1991; Freile & Hillis, 1997; Vroom *et al.*, 2003; Carneiro & Morais, 2016). This approach, because it does not consider the effect of propagation, may have been underestimating *Halimeda* contributions to both CaCO₃ and sediment production.

New studies are also necessary to assess the role of sexual reproduction in the process of space occupation and CaCO₃ production by this alga. All *Halimeda* species undergoes holocarpic sexual reproduction, in which the cellular content is converted into gametes, resulting in algal death, total dismantling of thallus and segment release (Hillis-Colinvaux, 1980). The literature on *Halimeda* suggests that only a small fraction of the population is actively reproducing at any given time (Clifton & Clifton, 1999; Vroom *et al.*, 2003). Nevertheless, some authors have pointed to the coincidence between periods of sexual reproduction and population declines (Clifton & Clifton, 1999). During our field campaigns, no specimen was found with clear signs of sexual activity, such as visible gametangia. However, the fast population reduction, as well as the rapid subsequent recovery, observed around October may have been caused, at least in part, by episodes of sexual reproduction. As such, due to their potential to produce segment release, these events may be an important source of CaCO₃ in reef environments.

The exponential function used in the analysis of standing stock variations is simple and may be further improved to better capture the complexity of this alga. However, it seems to be adequate to describe a typical population that is far from the ecosystem's carrying capacity (Weiner *et al.*, 1998). This seems to be the case in the present study, due to the low mean coverage observed. Moreover, we have only aimed to quantify standing stock variations and test some hypotheses on their causes, and not to develop full models of *H. opuntia* population dynamics.

Additionally, estimating population net growth through coverage variations, besides being suited to *H. opuntia* and other sprawling species, is a way of detecting increases in population size due to reproduction and colonization, refining CaCO₃ production estimates by these algae. Furthermore, this approach may overcome other limitations of individual methodologies, such as the impossibility of detecting population reductions, which may happen even if some algae are actively growing (Multer & Clavijo, 2004). On the other hand, this methodology lacks the capacity to quantify total growth, which could, at least theoretically, be done with individual measurements of growth. Therefore, instead of an alternative methodology, estimates of coverage variation should be used to complement *Halimeda* growth measurements, resulting in more precise estimates of its CaCO₃ and biomass production.

In conclusion, the quantitative analyses on the present study, based on standing stock measurements, support the view that *H. opuntia* has an active role in sediment production in tropical intertidal reef environments. Nevertheless, they also indicate that reproduction and dispersion should be better quantified in studies of calcium carbonate production. Since growth in such environments seems to be strongly related to substrate occupation, as opposed to increases in algae height or area-specific biomass, more accurate measurements of *Halimeda* standing stock and new models of population dynamics may be useful tools in estimating current CaCO₃ production by this alga, improving the knowledge on their role on marine carbonate budgets.

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