



Field biology of *Halimeda tuna* (Bryopsidales, Chlorophyta) across a depth gradient: comparative growth, survivorship, recruitment, and reproduction

Peter S. Vroom^{1*}, Celia M. Smith¹, James A. Coyer², Linda J. Walters³, Cynthia L. Hunter⁴, Kevin S. Beach⁵ & Jennifer E. Smith¹

¹Department of Botany, University of Hawai'i at Manoa, 3190 Maile Way, Honolulu, HI 96822, U.S.A.

²Department of Marine Biology, University of Groningen, Kercklaan 30, PO Box 14 9750 AA Haren, The Netherlands

³Department of Biology, University of Central Florida, 4000 Central Florida Blvd., Orlando, FL 32816, U.S.A.

⁴Waikiki Aquarium, 2777 Kalakaua Ave., Honolulu, HI 96815, U.S.A.

⁵Department of Biology, University of Tampa, 401 W. Kennedy Blvd., Tampa, FL 33606, U.S.A.

*Corresponding author: current address: National Marine Fisheries Service, Kewalo Research Facility, 1125B Ala Moana Blvd., Honolulu, HI 96814, U.S.A.

Fax: 808-592-7013, E-mail: Peter.Vroom@noaa.gov

Received 6 August 2002; in revised form 12 May 2003; accepted 26 May 03

Key words: *Halimeda tuna*, coral reef, alizarin, Florida Keys, *Erichthonius brasiliensis*, *Dictyota*

Abstract

Growth, survivorship, recruitment, and reproduction of *Halimeda tuna*, a dominant green alga in many reef systems of the Florida Keys, were monitored at a shallow back reef (4–7m) and deep reef slope (15–22 m) on Conch Reef. Despite lower light intensities and similar grazing pressures, amphipod infestations, and epiphyte loads at both sites, the deeper site exhibited significantly higher growth rates in summer months over a 4-year period than found for the shallow population, possibly because of higher nutrient levels at depth and photoinhibition of shallow plants. Sexual reproductive events occurred simultaneously across the entire reef, with up to 5% of the population at both sites developing gametangia. New upright axes formed from zygotes, asexual fragmentation, or vegetative runners. Plants appear to have persistent basal stumps that survive harsh environmental conditions, even if upright, photosynthetic axes are removed. Sexual reproduction and ‘smothering’ by epiphyte overgrowth are hypothesized to be two causes of death for individuals.

Introduction

Species of the genus *Halimeda* are important sediment producing algae in many tropical reef regions (Flügel, 1988; Hine et al., 1988; Johns & Moore, 1988; Pizzimenti & Silva, 1997; Hillis, 2001). Species are also known to be among the deepest living photosynthetic organisms, being found at depths of up to 130 m (Littler et al., 1985, 1986; Blair & Norris, 1988). Because of the unique unicellular structure exhibited by *Halimeda* and its relatives (Vroom & Smith, 2001), some species have served as model systems for un-

derstanding fundamental ecological and physiological processes (Wilbur et al., 1969; Borowitzka & Larkum, 1976a,b,c, 1977; Drew & Abel, 1988a, b; Wolanski et al., 1988; Freile et al., 1995). Yet despite this research, basic aspects of the ecology of most *Halimeda* species remain poorly understood, often because opportunities for conducting long-term field research have been limited (Hay, 1997).

The importance of *Halimeda* sediments to reef communities is well-documented (Rao et al., 1994), yet recent Caribbean-wide increases of macroalgal cover, possibly caused by the die-back of the herb-

Table 1. Research dates

Year	Sampling periods
1994	September 28–October 30
1997	June 2–June 14
1997	September 16–September 25
1998	July 2–July 10
1998	August 19–September 2
1999	October 18–November 18
2000	August 28–September 22

ivorous urchin, *Diadema antillarum* (Hughes et al., 1987), anthropogenic nutrient increases (Lapointe, 1997; Miller et al., 1999), and overfishing have led researchers to speculate that increases in *Halimeda* populations may be biological indicators of declining reef health. Few multi-year field studies have been undertaken to test this or related hypotheses (Shulman & Robertson, 1996; Lirman & Biber, 2000). This study presents the first long-term comparisons of growth rates between two *Halimeda* populations along a depth gradient within the same reef system, and presents the first growth rate data for *Halimeda tuna* (Ellis and Solander) Lamouroux, in the Caribbean.

To examine the dynamics of *Halimeda* populations and assess overall reef health, research was conducted on Conch Reef, Florida Keys from 1994 to 2000 (Table 1). Conch Reef, Florida Keys National Marine Sanctuary, offers the opportunity to study a complex ecosystem with reduced anthropogenic effects and a diverse flora and fauna (Bach, 1979; Coyer, 1995; Overholtzer & Motta, 1999; Sotka et al., 1999). Previous research has revealed a 'typical' tropical reef system with low nutrient concentrations (Szmant & Forrester, 1996; Miller et al., 1999). However, episodic, internal waves deliver cold, possibly nutrient rich water, over certain deeper water areas of the reef (Leichter et al., 1996, 1998; Leichter & Miller, 1999). Our two study sites, one located on a deep reef slope and the other in a shallow back reef region, take advantage of a gradient in reef habitats within a short distance of each other (<1 km, Fig. 1). This situation is ideal for studying ecological responses to different environmental factors such as light quantity and quality (Beach et al., in press).

Past studies of *Halimeda tuna* in the Mediterranean have shown that populations may differ in physiological responses to environmental factors, leading some researchers to hypothesize that different physiological

strains of the species exist in this area (Häder et al., 1996). In order to reduce genetic differences, this study focused on two populations close enough for the possible exchange of genetic information via asexual fragmentation (Walters & Smith, 1994; Walters et al., 2002), but environmentally distinct enough to display different physiological responses. The objectives of this study were to: (1) assess community structure; (2) examine and compare growth rates of *Halimeda tuna* at two environmentally distinct sites on Conch Reef; (3) determine average life-span of *H. tuna* individuals at each site; (4) calculate recruitment of *H. tuna* in these areas, and (5) document reproductive events at both sites. Photosynthesis (Beach et al., in press), asexual fragmentation (Walters et al., 2002), and nutrient physiology (Smith et al., in review) of *H. tuna* populations at each site were also examined. Data from these studies are being pooled to create a detailed predictive model for *H. tuna* growth and fluctuation in population numbers for Conch Reef plants (Vroom et al., in prep.).

Materials and methods

Site descriptions

Conch Reef is a fringing reef dating to the Holocene (Shinn et al., 1989) and is located 5 km off Islamorada in the Florida Keys National Marine Sanctuary. Two sites were studied from 1994 through 2000 (Fig. 1, Table 1). The back reef site (referred to as 'Shallow Conch', 24° 57' 047" N 80° 27' 657" W) ranges in depth from 4 to 7 m, and is characterized by uniform topography. In contrast, the reef slope (referred to as 'Pinnacle', 24° 56' 870" N 80° 27' 276" W), located ca. 700 m to the southeast, exhibits a high level of vertical relief and depths from 15 to 22 m, and is adjacent to an area of rapid drop-off to 30+ m depths. Temperature regimes at these sites characterized over a 6 year period by Leichter & Miller (1999) showed ambient temperatures to vary between 20°C and 30°C over the course of a year, with seasonal warming occurring from May to September, and cooling occurring from October to March. Day length peaked during summer months at 13.7 h · d⁻¹ of sunlight, and decreased to 10.6 h · d⁻¹ during winter months.

Halimeda tuna occurs at both Shallow Conch and Pinnacle along with *H. opuntia* and *H. goreau*. Shallow Conch was accessed daily by surface-divers, whereas Pinnacle was accessed either by surface

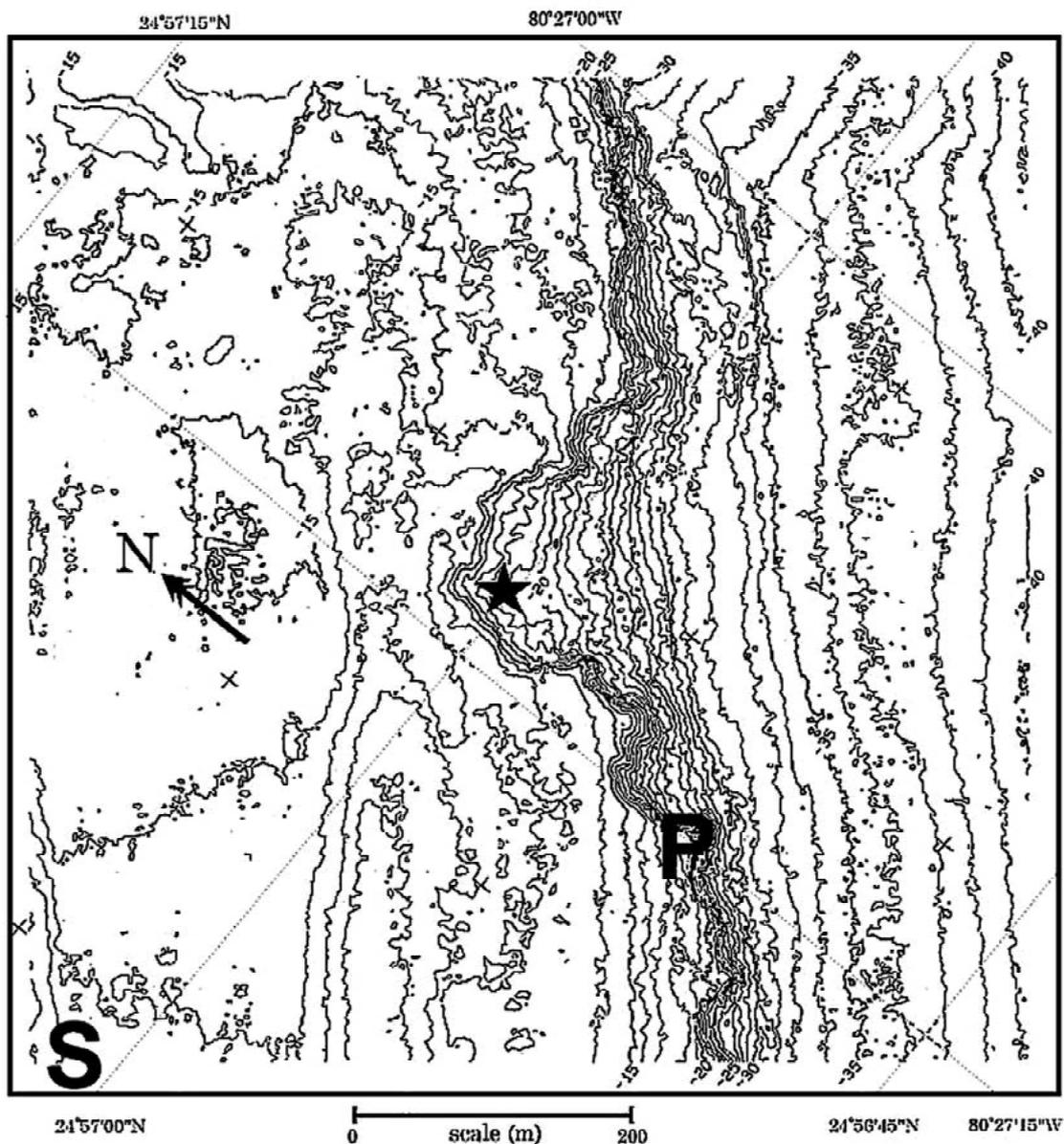


Figure 1. Map of Conch Reef, Florida Keys showing Shallow Conch and Pinnacle sites. S = Shallow Conch, P = Pinnacle, star = Aquarius. Figure from <http://www.uncwil.edu/nurc/aquarius/img/concbath.jpg>.

divers or saturation divers living in the nearby Aquarius habitat (see <http://www.uncwil.edu/nurc/aquarius/>).

Density and morphometrics

Discrete, upright axes of *Halimeda tuna* were considered unique thalli and enumerated using 10, 1 m² quadrats located randomly along 3 permanent 50 m transects that were separated by 25 m. Beginning in

1999, the number of axes per thallus, number of segments per thallus, and height of each individual were counted at both sites. An axis was defined as any branch arising within 3 segments of the base of the thallus (Fig. 2) whereas a segment was defined as a calcified internodal region separated by an uncalcified nodal region (Fig. 2, see Hillis-Colinvaux, 1980).

After the 2000 sampling period, the length, width, and thickness of dried segments from the growth study

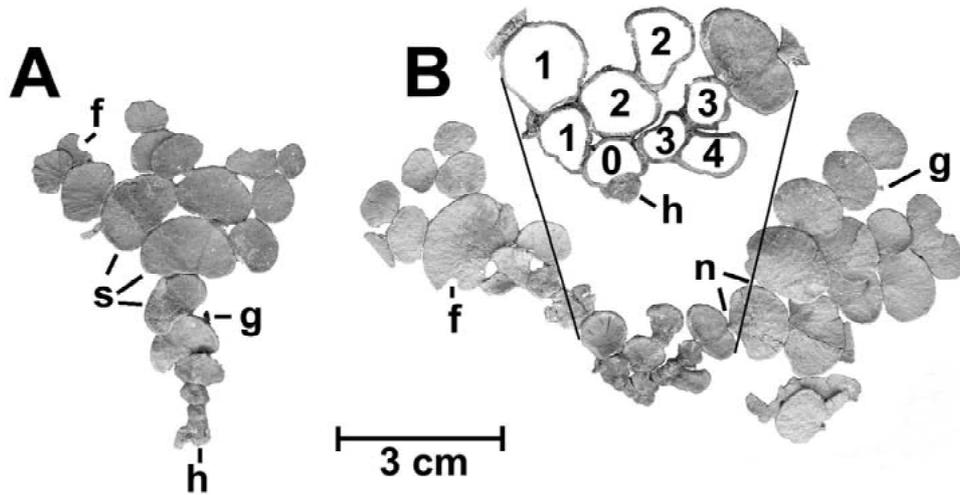


Figure 2. Single- and multi-axis *Halimeda tuna*. (A) Single axis individual of *Halimeda tuna* exhibiting a small rhizoidal holdfast (h). Segments are defined as calcified internodal regions (s). This specimen shows one newly forming, uncalcified segment (g), and one clear fish bite (f). (B) Multitaxis individual of *H. tuna* with uncalcified nodes (n), holdfast (h), new segment (g), and fish bite (f) indicated. In this study, an axis was defined as any branch arising within 3 segments from the base of the thallus. A blow-up of the basal region, with all segments within 3 segments from the base numbered, shows 4 distinct axes. A basal segment (0) is shared by axes 1 and 3 (both large with many segments), and axis 2 (containing only 2 segments). Axis 4 (only 1 segment) arises from axis 3.

(below) were recorded for individuals from both sites. Because the structural integrity of the calcified segments remains the same after drying (no significant difference in size between dried and fresh individuals, data not shown), these numbers are equivalent to the actual size of living segments in the field.

To determine percent $\text{CaCO}_3 \cdot \text{thallus}^{-1}$, individuals were decalcified by placing thalli in a 5% HCl solution 3x or until bubbling ceased. Individuals were rinsed with fresh water and dried at 65 °C until a constant weight was achieved. Dry weights were recorded and subtracted from initial dry weights to determine percent of CaCO_3 per thallus.

Growth measurements

New growth was measured by staining individuals of *H. tuna* with alizarin Red-S (see Fig. 8 in Vroom & Smith, 2001), a biological stain that dyes calcium carbonate pink and has been widely used to determine growth rates of calcareous algae (Wefer, 1980; Multer, 1988; Payri, 1988), coral (Dodge, 1984), and fish (Benoît & Pepin, 1999). Because staining temporarily retards growth in some corals (Dodge, 1984), growth rates of alizarin-stained individuals were compared to growth rates of unstained individuals (see re-growth/recruitment quadrats below) to insure that alizarin growth rates were not an experimental artifact.

At both sites, selected individuals with no to few epiphytes were 'tented' with a 4-l plastic bag (enclosing ca. 500 ml of seawater) and secured to the base of a plant with a rubber band. A 1.5 ml Eppendorf tube filled with a 1% alizarin solution was placed into each bag prior to attachment. After bags were attached, the tubes were opened through the bag to release the dye (ca 0.003% final concentration) and the bags were squeezed gently to mix the dye and to check for leaks.

After 24 h incubation, the bags were removed and the individuals marked with flagging tape. Stained individuals were harvested after 7–8 d, brought to the laboratory, and placed in a 5–10% bleach solution for 5–10 min to remove natural pigment coloration. Subsequent analysis enumerated the number of stained (old growth) and unstained (new growth) segments per axis, wet weight of stained and unstained segments, number of *Ericthonius brasiliensis* (amphipod) habitations (Sotka et al., 1999), and the number of fish bites per individual. Additionally, stained and unstained segments were dried at 65 °C until constant weight was achieved, and dry weights recorded.

Growth rates of *H. tuna* were calculated as: 1. the number of new segments produced $\cdot \text{d}^{-1}$, 2. the amount of biomass (g dry weight) produced $\cdot \text{d}^{-1}$, 3. the percent increase in segment number $\cdot \text{d}^{-1}$ (new segments/total segments $^{-1}$), and 4. the per-

cent increase in weight $\cdot d^{-1}$ (dry weight of new segments/total dry weight $^{-1}$).

CaCO₃ production rates

For 1997 and 2000 data, the mean percentage of CaCO₃ $\cdot individual^{-1}$ was used to determine the mean g CaCO₃ present $\cdot segment^{-1}$, and the mean g CaCO₃ $\cdot individual^{-1}$. Using mean growth rates (number of new segments $\cdot individual^{-1} \cdot d^{-1}$), g CaCO₃ $\cdot individual^{-1} \cdot y^{-1}$ was determined. When combined with the mean number of individuals $\cdot m^{-2}$, yearly estimates of CaCO₃ production $\cdot site^{-1}$ were made.

Survivorship

Survivorship was estimated by attaching a 4 cm embossed tag at the base of each individual with a cable tie ($n = 100\text{--}200$). All plants were located within 10×10 m plots at both sites during each of several sampling periods from June 1997 to August 1998. Different plots were used for each sampling period. Because it was hypothesized that single-axis individuals may be younger than multi-axial plants, data on single- and multi-axial individuals were recorded separately. Ample dive time at the shallow site and saturation diving at the deep site insured that all areas were searched thoroughly for tagged individuals on subsequent site visits.

Tag-associated decrease in fitness (loss of axes because of tag presence) and the fate of tags on plants after axis loss were examined for 100 multi-axial individuals from each site in September 2000. Axes were removed from tagged plants, leaving behind basal portions (ca. 3 segments) with 3, 2, 1, or no upright axes (25 plants $\cdot category^{-1}$). Tagged individuals were observed daily for 10 d to monitor tag loss and/or plant death.

Re-growth/recruitment quadrats

A total of 16 permanent 0.25 m² quadrats were established at each study site between September 1997 and 1999 to monitor re-growth and recruitment. Within each quadrat, all individuals of *H. tuna* were counted and those with <4 segments removed. Remaining plants were tagged (see survivorship above) and trimmed so that only 3 basal segments remained. Biomass removed from each quadrat was dried at 65 °C until constant weight was achieved. Subsequent re-growth and recruitment were evaluated from 9

September 1997 through 3 July 1998 (284 d) and 8 July 1998 through 25 August 1998 (48 d). Measurements at each re-visit included: 1. number of tagged individuals remaining (survivorship), 2. number of new upright axes (discrete photosynthetic axes were considered new recruits), and 3. net growth of tagged plants as determined by re-trimming individuals and collecting biomass for dry weight analysis. In order to determine rate of recovery after trimming and sampling-induced mortality, 15 'wounding' control quadrats were created at Shallow Conch during September 1999. All plants in each quadrat had upright axes removed, and the time interval for resumption of growth or plant death was monitored daily for 6 d.

Reproduction

Reproductively mature individuals of *H. tuna* were monitored at both sites on 16 September 1997, 29 August and 13 September 2000. In 1997, fifteen 0.25 m² quadrats were placed haphazardly at each site to ascertain density of reproductively mature individuals (# reproductive individuals $\cdot total \# individuals^{-1}$). In August 2000, the number of axes, number of segments per plant, and height of entire plant were measured for reproductive individuals at both sites.

Data analysis

All data were analyzed using Minitab 12 for Windows. One-way analysis of variance (ANOVA) was used to test for site differences within sampling dates for percentage of reproductive plants. Two-way ANOVAs were used to test for site, date, and interactive effects with all other data sets. Log transformations were used when necessary to satisfy the assumption of equal variance. Tukey's multiple comparisons were used to determine differences between levels of factors when significant. For alizarin analysis, each plant was regarded as an independent sample. For methods using quadrats (e.g. re-growth/recruitment), each quadrat was regarded as an independent sample.

Results

Density

Densities of *Halimeda tuna* were over 4 \times higher ($p < 0.001$) at Shallow Conch than at Pinnacle for

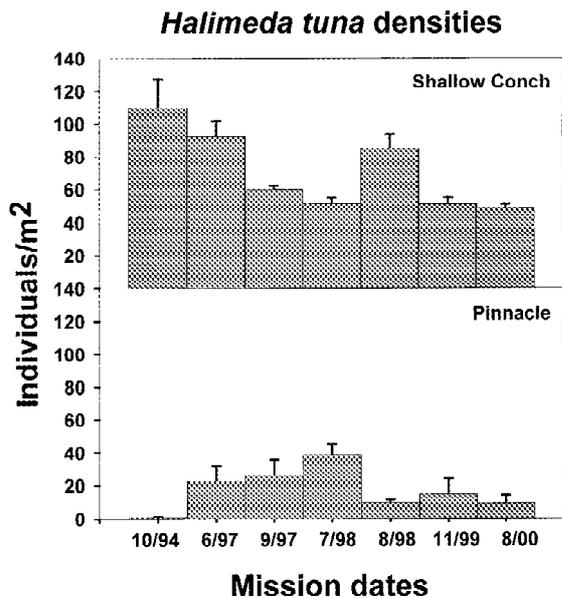


Figure 3. Densities of *Halimeda tuna* at Shallow Conch and Pinnacle.

all sampling periods (Fig. 3, Appendix A). Because of the topographical heterogeneity at both sites, *H. tuna* often appeared clumped with many thalli clustered in areas containing hard, calcified substrate, while sand patches and areas containing rubble were devoid of thalli. The highest number of *H. tuna* individuals enumerated was $224 \cdot \text{m}^{-2}$ at Shallow Conch, and $132 \cdot \text{m}^{-2}$ at Pinnacle, with an average of 16.40 thalli $\cdot \text{m}^{-2}$ at Pinnacle and 70.61 thalli $\cdot \text{m}^{-2}$ at Shallow Conch. Significant changes in the density of individuals occurred at each site between sampling periods (Appendix A), although no distinctive trends were observed.

Growth

During each 7–8 d period, the total number of new segments $\cdot \text{individual}^{-1}$ produced ranged from 0 to 146. Plants at the deeper Pinnacle site grew significantly faster than plants at the Shallow Conch site (Fig. 4, Appendix A). On average, individuals at 20 m produced $1.7\times$ more segments $\cdot \text{d}^{-1}$ and $1.7\times$ more g biomass $\cdot \text{d}^{-1}$ than individuals from 5 m (Fig. 4, Appendix A). Similarly, daily percent increase of segments was $1.5\times$ greater at Pinnacle than Shallow Conch (Fig. 4, Appendix A). Although daily percent increase in biomass did not differ significantly between sites, significant differences between dates of sampling were present (Fig. 4, Appendix A). Sig-

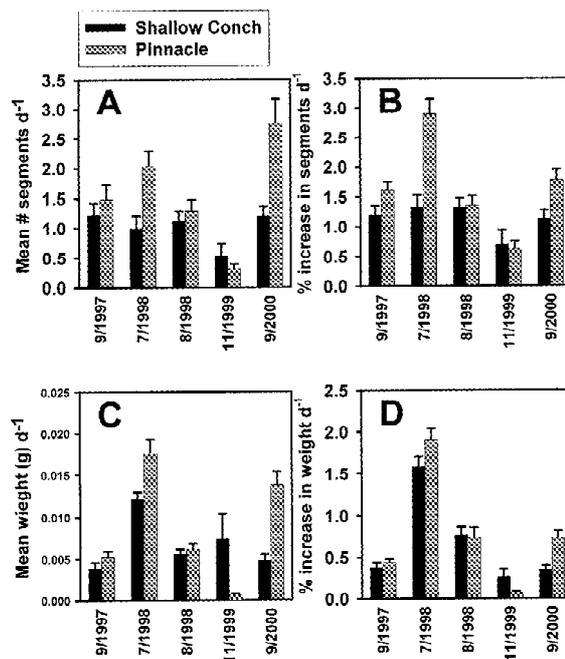


Figure 4. Growth rates of *Halimeda tuna* at two depths on Conch Reef, Florida Keys. (A) mean number of new segments $\cdot \text{d}^{-1}$, (B) percent increase in segments $\cdot \text{d}^{-1}$, (C) mean weight (g) of new biomass $\cdot \text{d}^{-1}$, (D) percent increase in weight $\cdot \text{d}^{-1}$. Error bars represent standard error of means. $n = 48$ (Shallow Conch 1997), 92 (Pinnacle 1997), 55 (Shallow Conch, July 1998), 51 (Pinnacle, July 1998), 64 (Shallow Conch, August 1998), 53 (Pinnacle, August 1998), 14 (Shallow Conch 1999), 35 (Pinnacle 1999), 62 (Shallow Conch 2000), and 47 (Pinnacle 2000).

nificant interactions for all measures of growth were detected for all of the tests, with plants at Shallow Conch growing fairly consistently from year to year, and plants at Pinnacle growing much more in some years than others (Fig. 4, Appendix A). An extreme reduction in growth in 1999 coincided with Hurricane Irene (13–19 October).

No correlation was found between the number or weight of stained (old) segments $\cdot \text{individual}^{-1}$ and the number or weight of new, unstained segments, indicating that plant size was not a good predictor of growth (data not shown). Similarly, regions of new growth on an individual plant could not be predetermined. New segments were often produced terminally on existing axes, but could also appear as new axes from the base of the plant. In some individuals, the majority of growth occurred on the largest axes, while in others all new growth occurred on the smallest axes.

Because of the short growth interval examined in the alizarin staining trials, little or no loss of plant tissue was observed. Therefore, gross growth rates

Table 2. Percent survivorship of plants, net growth, and new upright axes of *Halimeda tuna* at two depths on Conch Reef, Florida Keys (n = number of $1/4$ m² quadrats)

		Growth period	
		9 September 1997– 3 July 1997 284 d	8 July 1998– 25 August 1998 48 d
Percent Survivorship (SE, n)	Shallow Conch	34.0 (6.7, 6)	57.4 (9.5, 10)
	Pinnacle	28.1 (14.3, 6)	63.8 (5.5, 10)
Net growth (dry weight · plant · day ⁻¹ ; SE, n)	Shallow Conch	0.012 (0.003)	0.006 (0.001)
	Pinnacle	0.025 (0.017)	0.007 (0.001)
Upright axes (SE, n)	Shallow Conch	21.3 (4.6, 6)	6.7 (2.2, 10)
	Pinnacle	13.2 (2.1, 6)	17.3 (5.2, 10)
Dry weight of upright axes (g) (SE, n)	Shallow Conch	2.562 (0.228, 6)	1.057 (0.531, 10)
	Pinnacle	3.400 (1.410, 6)	2.701 (0.582, 10)

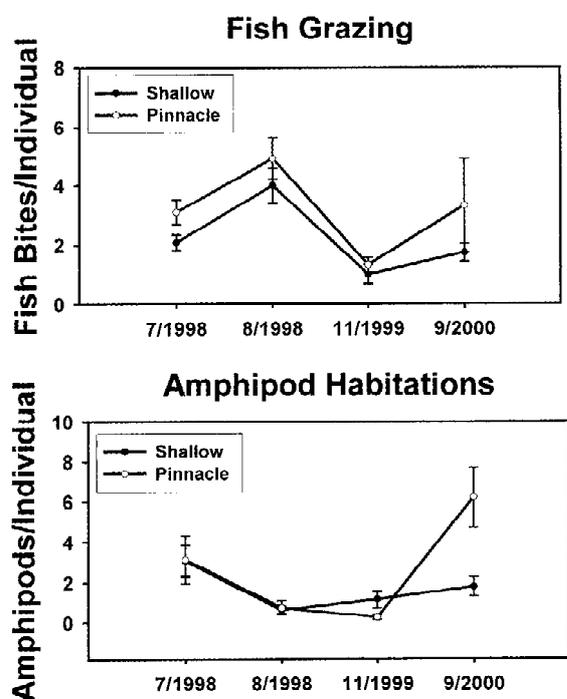


Figure 5. Fish herbivory and amphipod habitation density comparisons of two depths on Conch Reef, Florida Keys. (A) Mean number of fish bites · *Halimeda tuna* plant⁻¹. (B) Mean number of amphipod habitations *Halimeda tuna* plant⁻¹. Error bars represent standard error of means. n = 55 (Shallow Conch, July 1998), 51 (Pinnacle, July 1998), 64 (Shallow Conch, August 1998), 53 (Pinnacle, August 1998), 14 (Shallow Conch 1999), 35 (Pinnacle 1999), 62 (Shallow Conch 2000), and 47 (Pinnacle 2000).

are represented. Growth rates determined using dry weight data from the re-growth and recruitment quadrats represent net growth (new growth minus loss of tissue from storm activity, grazing, etc.). Despite this difference, gross and net growth rates were similar (Fig. 4, Appendix A). Net growth rates of populations as determined by weight · individual⁻¹ · d⁻¹ in the re-growth/recruitment were not significantly different between sites or sampling periods (Table 2, Appendix A).

Fish bites and amphipod habitations · plant⁻¹ were determined from the alizarin stained plants to assess differences between sites. The number of fish bites · plant⁻¹ did not vary significantly between Shallow Conch and Pinnacle, with an overall mean of 2.3 bites · plant⁻¹ (Fig. 5, Appendix A). Similarly, no significant difference in the number of amphipod habitations at each site was present (overall mean = 1.73 habitations · plant⁻¹). However, the number of both fish bites and amphipod habitations did differ significantly between sampling periods (Fig. 5, Appendix A).

Morphometrics and calcification

Segments of *H. tuna* from deep locales had a recognizably different phenotype from individuals located at Shallow Conch, although all remained within the described range of the species. Mature segments from Pinnacle were over 1.1 × wider ($p < 0.001$) and 1.2 × longer ($p < 0.001$) than their shallower counterparts (Table 3), although segment thickness remained relatively constant ($p = 0.138$). Thalli from deeper locales were over 1.1 × taller ($p < 0.001$) and contained

Table 3. Average segment sizes for the largest and smallest segments from three populations of *Halimeda tuna* ($n = 25$, * = significant difference between Shallow Conch and Pinnacle)

	Largest segment			Smallest segment		
	Length (SE, maximum)	Width (SE, maximum)	Thickness (SE, maximum)	Length (SE, maximum)	Width (SE, maximum)	Thickness (SE, maximum)
Shallow Conch	8.22 (0.40, 12)*	12.68 (0.42, 19)*	0.74 (0.05, 1.25)	1.95 (0.22, 5)	1.95 (0.21, 4.5)	0.35 (0.07, 1.5)
Pinnacle	10.02 (0.26, 12)	14.64 (0.42, 19)	0.75 (0.04, 1)	2.60 (0.18, 4)	2.68 (0.27, 6)	0.32 (0.07, 1.5)

Table 4. Segment weight

		9/1997	7/1998	8/1998	9/2000
Mean weight of new segments (g) (SE, $n =$ thalli)	Shallow	0.0030	0.0194	0.0107	0.0035
	Conch	(0.0003, 48)	(0.0027, 55)	(0.0021, 64)	(0.0003, 62)
	Pinnacle	0.0034 (0.0003, 92)	0.0129 (0.0017, 48)	0.0069 (0.0011, 53)	0.0057 (0.0004, 47)
Mean weight of old segments (g) (SE, $n =$ thalli)	Shallow	0.0132	0.0176	0.0129	0.0154
	Conch	(0.0006, 48)	(0.0010, 55)	(0.0007, 64)	(0.0007, 62)
	Pinnacle	0.0167 (0.0012, 92)	0.0193 (0.0006, 48)	0.0157 (0.0028, 53)	0.0154 (0.0007, 47)

Table 5. Grams CaCO_3 produced $\cdot \text{m}^{-2} \cdot \text{y}^{-1}$

Year	Site	Mean percent $\text{CaCO}_3 \cdot \text{segment}^{-1}$	Mean weight (g) $\text{CaCO}_3 \cdot \text{segment}^{-1}$	Mean g $\text{CaCO}_3 \cdot \text{thallus}^{-1} \cdot \text{d}^{-1}$	Mean new segments $\cdot \text{thallus}^{-1} \cdot \text{d}^{-1}$	Renewal of standing stock (d)	Mean g $\text{CaCO}_3 \cdot \text{thallus}^{-1} \cdot \text{y}^{-1}$	Mean individuals $\cdot \text{m}^{-2}$	Mean g $\text{CaCO}_3 \cdot \text{m}^{-2} \cdot \text{y}^{-1}$
September 1997	Shallow	75.0	0.0038	0.0046	1.21 (0.21)		1.679	60.07 (3.45)	100.86
	Conch								
	Pinnacle	79.0	0.0032	0.0038	1.20 (0.21)		1.387	33.9 (10.4)	47.02
2000	Shallow	75.3	0.0037	0.0044	1.19 (0.17)	22.7	1.6060	48.33 (2.77)	77.62
	Conch								
	Pinnacle	82.9	0.0028	0.0077	2.75 (0.42)	20.2	2.8105	8.81 (5.35)	24.76

significantly more segments per thallus ($p < 0.001$) than individuals located at Shallow Conch (Fig. 6, Appendix A; but see 1999 data). Because the number of axes $\cdot \text{individual}^{-1}$ did not differ significantly between any of the sites, and because the number of segments

$\cdot \text{individual}^{-1}$ were over $1.5\times$ greater at Pinnacle, it follows that the number of segments $\cdot \text{axis}^{-1}$ were also significantly greater ($p < 0.001$) at Pinnacle than at Shallow Conch (Fig. 6).

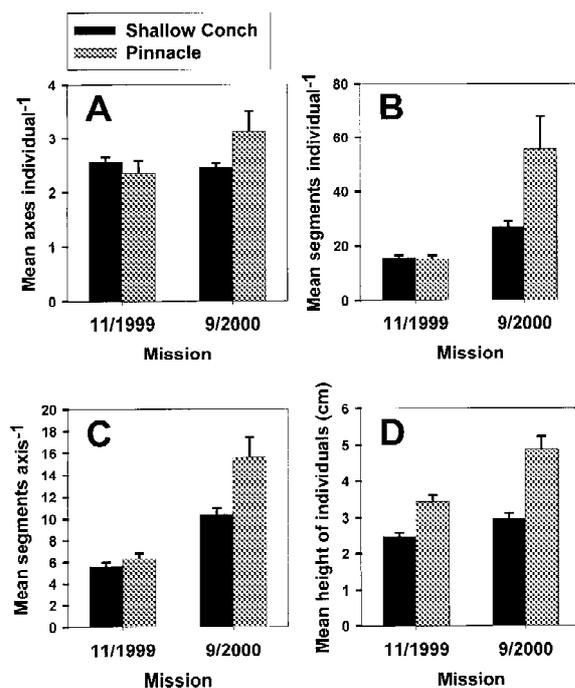


Figure 6. *Halimeda tuna* population characteristics. (A) mean axes individual⁻¹, (B) mean segments · individual⁻¹, (C) mean segments · axis⁻¹, (D) mean height (cm) of thalli. Error bars represent standard error. *N* (number of quadrats sampled) = 28 (Shallow Conch 1999), 13 (Pinnacle 1999), 20 (Shallow Conch 2000), 8 (Pinnacle 2000), 24 (23 m site 1999).

The average weight of existing segments was $1.15\times$ higher at Pinnacle than Shallow Conch ($p = 0.032$; Table 4) and was significantly different between sampling periods. The average weight of new segments did not differ significantly between sites, but did differ between sampling periods (Appendix A). The percentage of $\text{CaCO}_3 \cdot \text{old segment}^{-1}$ was $1.08\times$ greater at Pinnacle than at Shallow Conch ($p < 0.001$), indicating that mature segments from deeper thalli are more heavily calcified than segments from individuals at shallower locales (Table 5, Appendix A).

Reproduction

Halimeda tuna is reported to reproduce during seasons when water temperatures are warmest (Beth, 1962; Drew & Abel, 1988b). During this study, one to two reproductive events were recorded per month during summer and early autumn sampling periods when ambient temperature averaged $\sim 27.5^\circ\text{C}$, and no reproductive events were observed in November

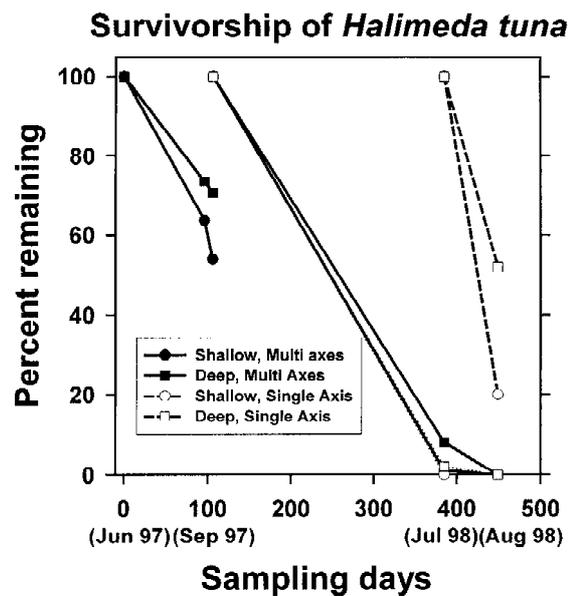


Figure 7. Survivorship of *Halimeda tuna* at two depths on Conch Reef, Florida Keys.

when colder water temperatures ($\sim 25^\circ\text{C}$) and shorter photoperiods prevailed (Table 1).

On 16 September 1997, external compound gametangia began to develop on the terminal side of nodes on some individuals of *H. tuna* at both sites. Haphazard sampling found that the percentage of reproductive individuals did not significantly differ between sites ($p = 0.096$, Table 6). At Pinnacle, reproductive plants averaged 7.0 ± 2.1 axes, and 267.3 ± 46.9 segments · plant⁻¹ ($n = 3$), compared to a mean of 5.6 ± 0.6 axes, and 138.4 ± 26.0 segments · plant⁻¹ ($n = 29$) for Shallow Conch plants.

Survivorship and recruitment

Single- and multi-axis *H. tuna* had similarly short life-spans (Fig. 7). Of the 100 multi-axial individuals tagged at both sites on 13 June 1997, 30–40% fewer plants remained three months later (16 and 26 September 1997). A 40–80% loss of tagged single-axis individuals occurred during one month in summer 1998. Almost all tagged individuals disappeared or lost tags during the winter months as is indicated by few tags being recovered between sampling years (Fig. 7). Removing axes from tagged plants during the 2000 sampling period (a time of no storm activity) did not affect tag survival, as all tagged individuals still possessed tags 10 d later.

Table 6. Percent of reproductive *Halimeda tuna* plants found on 16 September 1997 at two depths on Conch Reef, Florida Keys. Fifteen $1/4$ m² quadrats were sampled at each site

	Mean number total individuals \cdot quadrat ⁻¹ (SE)	Mean number reproductive individuals \cdot quadrat ⁻¹ (SE)	Percent reproductive (SE)
Shallow Conch	51.6 (3.61)	0.87 (0.34)	1.638 (0.54)
Pinnacle	22.6 (4.18)	1.47 (0.46)	5.26 (2.03)

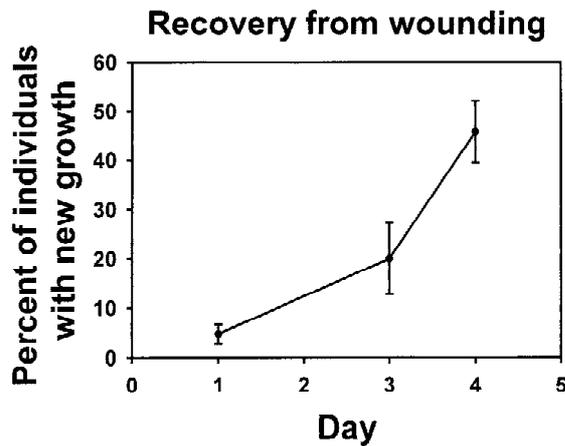


Figure 8. Mean percentages of *Halimeda tuna* plants exhibiting new growth each day after all axes were removed at Shallow Conch. Error bars represent standard error of means. $n = 15$ $1/4$ m² quadrats.

Individuals of *H. tuna* trimmed to their three basal segments recovered quickly and began to grow within days of wounding (Fig. 8). The percent survivorship of trimmed plants did not differ significantly between sites (Table 2, Appendix A), however the rate of tagged plant loss was more rapid (25% loss \cdot month⁻¹) during the 48 day period than during the 284 day period (7.2% loss \cdot month⁻¹).

The number of new upright axes did not significantly differ between sites or periods (Table 2, Appendix A), although a lower mean number of upright axes at Shallow Conch suggests that fewer new plants develop at this site (Table 2), a trend that requires further monitoring to be confirmed. No significant difference in weight of new upright axes was found between sites of sampling periods, although the average weight of new upright axes was higher among those that had longer growth periods (Table 2, Appendix A).

Discussion

Reef systems are heavily influenced by a suite of physical factors that divide a superficially homogeneous ecosystem into many disjointed microcosms. For instance, prevailing currents or internal waves may prevent dissolved nutrients from reaching certain sites within a reef system (Leichter et al., 1996, 1999), or the quality or quantity of light received at two sites of slightly different depth may favor entirely different algal communities. This study underscores that, within a reef system, environmental differences caused by abiotic factors (e.g. light, dissolved nutrients) between sites dramatically affect some biological processes, while other biological processes within the same organism remain static. Thus, we see significant differences in growth of *H. tuna* occurring at geographically close, but environmentally distinct, sites; while reproductive events in *H. tuna* remain constant across the entire reef system.

Density

Since the die-back of the herbivorous sea urchin, *Diadema antillarum*, during a two year period between 1983 and 1984, a significant increase in algal biomass, particularly *Halimeda* spp. and *Dictyota* spp., has been observed in reef systems throughout the Caribbean (Hughes et al., 1987; Shulman & Robertson, 1996; Lirman & Biber, 2000). Because percent cover data for Conch Reef prior to 1994 are lacking, it is unknown how much algal cover has increased in this particular area as a result of the *Diadema* decline. At our study sites, densities of *H. tuna* did not steadily increase on a yearly basis, but fluctuated because of environmental and seasonal parameters. For instance, as *Dictyota* spp. attach to and overgrow individuals of *H. tuna*, potentially reducing light and nutrient availability (Beach et al. in review), densities of *H. tuna* decline (Fig. 3; Vroom et al., in review). Up to 80% of single-axis individuals died as *Dictyota* spp. bloomed during

the summer of 1998 (Fig. 7). Relatively fast growth rates of *Halimeda* spp. allowed population numbers to rebound once *Dictyota* spp. population numbers dropped (Fig. 3, Williams, 1988).

Growth

A study of growth on *H. incrassata* and *H. monile* (Multer, 1988) described four growth phases in *Halimeda* based on staining characteristics and segment morphology. Applying this scheme, internodal regions that actively absorbed stain were classified as active adult segments, and are the regions where most new segment production occurs. The basal unstained portion of the plant contains 'early-' and 'late-senescent' segments. Early senescent segments are capable of producing new segments, particularly under stress (Multer, 1988), and are responsible for new axis production. The late senescent segments form the thick, often encrusted and epiphytized stump of the plant.

Average growth rates of *H. tuna* at Shallow Conch and Pinnacle were highest under summer conditions of longest photoperiods and highest temperatures (Fig. 4), agreeing with previous growth studies on *Halimeda* (Table 7; Bach, 1979; Wefer, 1980; Multer, 1988; Payri, 1988; Ballesteros, 1991; Garrigue, 1991). Significant differences in growth between sampling periods are correlated with prevailing environmental conditions. During the 1999 sampling period, growth at both sites was negatively impacted in part by Hurricane Irene (Fig. 4) when wave heights of up to 7 m exposed portions of Shallow Conch to air and turbulent water motion as wave troughs passed through the area (O. Rutten, pers. comm.). Resultant sand scour, turbulent water conditions, a decline in water temperature, and decreasing photoperiods synergistically contributed to substantially lower growth rates of individuals from both study sites in November 1999 (Fig. 4).

Our finding of significantly higher growth rates for *H. tuna* at the deeper Pinnacle site was unexpected, and potentially was due to increased nutrient availability at depth, and photoinhibition of plants at the Shallow Conch site. A study testing the effects of nutrients on growth in *H. tuna* at Conch Reef (Smith et al., in review) show that plants respond positively to elevated nutrient concentrations. The tidal bores commonly observed at the Pinnacle site (Leichter et al., 1996, 1999) are a likely mechanism for increased nutrient availability leading to increased growth of Pin-

nacle plants, a scenario also found on the Great Barrier Reef (Wolanski et al., 1988). Studies of photosynthesis from both sites reveal that plants from Shallow Conch are photoinhibited for part of the day during summer months, leading to less energy for growth in these populations (Beach et al., in press). Amphipod infestations (Sotka et al., 1999) and epiphytization, two other mechanisms hypothesized to explain growth differences between Shallow Conch and Pinnacle, were equivalent between sites (Appendix A) and not likely causes for observed differences.

Halimeda tuna morphometrics

Halimeda tuna is characterized by holdfasts less than 1 cm long that commonly attach to hard substrate (Hillis-Colinvaux, 1980). Therefore, the distribution of individuals was restricted to hard-bottom areas. Individuals at both sites had an average of about 2.5 axes \cdot thallus⁻¹ (Figs 2 and 6), suggesting that the basic body plan of this alga does not vary between sites or growth seasons.

Population densities and morphologies of individual thalli were affected by a number of physical factors. At Shallow Conch, thalli were significantly shorter with fewer, smaller, and less heavily calcified segments (Fig. 6, Tables 3 and 5, Appendix A), than at Pinnacle. Because *Halimeda* populations from shallow depths are light saturated at irradiances below the average photon flux densities present on sunny days (Littler et al., 1988), smaller segment size may help alleviate photoinhibition (Beach et al. in press). Alternatively, because nutrient pulses from breaking internal waves do not commonly reach back reef regions at Conch Reef (Leichter et al., 1996), low nutrient availability may result in P and P and N starved individuals (Littler et al., 1988) that are only able to produce small segments (Smith et al., in review).

Individuals from Pinnacle had broad segments with more surface area than their shallow counterparts (Table 3; Hillis-Colinvaux, 1980), a feature that is probably essential in order to capture limited photosynthetically active radiation (PAR) at depth (Beach et al. in press). Because large segment sizes presumably cost more to produce, the environment at Pinnacle supports fewer, larger individuals (Figs 3 and 6) than at Shallow Conch. Although still to be tested, breaking internal waves along the reef slope at Conch Reef may increase nutrient availability at Pinnacle (Leichter et al., 1996, 1998; Leichter & Miller, 1999) and provide more of the elements essential for larger segment size.

Table 7. Comparative growth data for *Halimeda* species

Species	Location	Depth	Season	Mean standing crop (g dry weight m ⁻²)	Mean new segments · plant ⁻¹ · d ⁻¹	Mean new weight (g) · plant ⁻¹ · d ⁻¹	Mean percent segment increase · plant ⁻¹ · d ⁻¹	Mean percent weight increase · plant ⁻¹ · d ⁻¹	Mean percent CaCO ₃ · thallus ⁻¹ · d ⁻¹	Mean g CaCO ₃ · plant ⁻¹ · d ⁻¹	Mean CaCO ₃ (g) · m ⁻² · y ⁻¹	Standing Stock Replacement (d)	Study
<i>Halimeda tuna</i>	Florida Keys	5 m	June – November	20.09 (2000 data)	0.54 (0.19)–1.21 (0.21)	0.0039 (0.0007)–0.0121 (0.0009)	0.70 (0.24)–1.33 (0.20)	0.26 (0.10)–1.58 (0.12)	24.8 (0.58)	0.0044	77.62	22.7 – 28.6	This study
<i>H. tuna</i>	Florida Keys	21 m	June – November	7.69 (2000 data)	0.31 (0.08)–2.75 (0.42)	0.0006 (0.0002)–0.0176 (0.0017)	0.62 (0.13)–2.90 (0.25)	0.07 (0.02)–1.90 (0.14)	17.1 (0.98)	0.0077	24.76	20.2 – 48.6	This study
<i>H. tuna</i>	Mediterranean	18 m	Year round	–	0.2	–	–	–	45.7 (11.7)	–	314	–	Ballesteros (1991)
<i>H. incrassata</i>	Antigua, West Indies	1 – 2.5 m	April July	–	1.42 – 2.17	0.006 – 0.009	–	–	–	–	60.74 114.31	39	Multer (1988)
<i>H. monile</i>	Florida	0.95 – 3.6 m	Year round	5.8	–	–	–	–	25.57 (4.14)	–	0.1 – 62.3	–	Bach (1979)
<i>H. incrassata</i>	New Caledonia	11 m	Year round	–	0.6 · axis · d ⁻¹	–	–	–	72	–	31.87	–	Garrigue (1991)
<i>H. incrassata</i>	Tahiti	0.5 m	Year round	7.75 (1.50)	1.58 – 6.39	0.0133 – 0.0302	–	–	86.5	0.185	74.5(27.5)	–	Payri (1988)
<i>H. incrassata</i>	Bermuda	1 – 4 m	August – September	6.7 g CaCO ₃	–	–	~3	–	–	–	50	~1 month	Wefer (1980)
<i>H. monile</i>	Florida	1.2 – 3.3 m	Year round	1.5	–	–	–	–	15.99 (3.05)	–	0.1 – 6.9	–	Bach (1979)
<i>H. opuntia</i>	Florida	1.55 – 3.0 m	Year round	1.0	–	–	–	–	–	–	–	–	Bach (1979)
<i>H. opuntia</i>	Puerto Rico	0.7 – 1 m	–	–	–	–	–	–	84.7 (2.05)	–	–	–	Stark et al. (1969)
<i>H. opuntia</i>	Tahiti	0.5 m	Year round	100 (28)	–	–	–	–	–	1.74	1288	–	Payri (1988)
<i>H. discoidea</i>	Puerto Rico	2.7 – 4 m	–	–	–	–	–	–	47.8 (3.65)	–	–	–	Stark et al. (1969)
<i>H. discoidea</i>	New Caledonia	24 m	Year round	–	0.15 (0) · axis · d ⁻¹	–	–	–	65	–	13.11	–	Garrigue (1991)
<i>H. discoidea</i>	Tahiti	0.5 m	Year round	2.20 (0.60)	–	–	–	–	–	0.039	–	–	Payri (1988)

However, episodic increased nutrient supply may not provide enough of a foundation for population densities to reach the high numbers found at shallower sites.

Segments at Pinnacle weigh more than segments at Shallow Conch because they are significantly bigger (Tables 3 and 4), and also contain a higher percentage of calcium carbonate than segments from shallower populations (although within a thallus, percent calcification is dependent upon segment age; Abel & Drew, 1985). Calcification levels reported here (Table 5) are considerably higher than the 45.7% reported for *H. tuna* populations from the Mediterranean (Ballesteros, 1991).

Stark et al. (1969) found higher photosynthetic rates to be linked to higher levels of CaCO₃ precipitation, a phenomenon that might explain the higher percent calcification among deeper Conch Reef *H. tuna*

populations. Past studies have found shallow *H. tuna* populations to be over-saturated with light on sunny days (Littler et al., 1988), making photoinhibition and decreased photosynthetic performance likely (Littler et al., 1988; Franklin et al., 1996; Häder et al., 1996; Beach et al. in press). The increased levels of CaCO₃ produced by plants at depth may further enhance light harvesting capabilities of deep *H. tuna* populations by turning internal siphons into bright-white ‘integrating chambers’ that reflect photons throughout the segment, increasing chances of absorbance by light harvesting complexes. Although some researchers have felt that high levels of calcification might occur because of increased grazing pressure (Paul & Van Alstyne, 1988, 1992), our study does not confirm this hypothesis. Grazing pressures from herbivorous fish did not differ significantly between sites (Fig. 5, Appendix A), and because *Halimeda* is

generally known to possess secondary chemistry to deter herbivores (Paul & Van Alstyne, 1988, 1992), heavier calcification at Pinnacle is probably not an anti-herbivore device.

CaCO₃ production

Population densities of *H. tuna* change in response to environmental parameters (Fig. 3), and growth rates vary with season (Fig. 4). The estimate of CaCO_3 production $\cdot \text{m}^{-2} \cdot \text{y}^{-1}$ presented here is a snapshot of productivity at a given time that can be used for comparative purposes. Because only net growth was studied, calculated numbers may actually under-represent the total amount of CaCO_3 contributed to the ecosystem. Although less heavily calcified than individuals from Pinnacle, the Shallow Conch *H. tuna* population produces more $\text{CaCO}_3 \cdot \text{m}^{-2} \cdot \text{y}^{-1}$ (Table 5) because of the higher number of individuals present in shallow reef settings. Surprisingly, the total $\text{CaCO}_3 \cdot \text{m}^{-2} \cdot \text{y}^{-1}$ produced by Caribbean *H. tuna* populations is lower than Mediterranean populations, despite the fact that Caribbean plants contain higher amounts of CaCO_3 in their thalli. This results from higher numbers of individuals being present in Mediterranean populations. Studies of growth in other *Halimeda* species have determined widely divergent production rates. However, results from the study fall within the same range as other members of the genus (Table 7).

Reproduction

All members of the sub-order Halimedaceae (e.g. *Halimeda*, *Udotea*, *Penicillus*, *Rhipocephalus*, *Rhipidosiphon*) exhibit holocarpic reproduction (Meinesz, 1980a; Vroom et al., 1998) whereby cellular constituents of the entire plant are converted to gametes, leaving only a calcified and empty husk after gamete release (Hillis-Colinvaux, 1972; Clifton, 1997; Hay, 1997; Clifton & Clifton, 1999). Although the trigger for reproduction in *H. tuna* is not well understood (Clifton, 1997; but see Drew & Abel, 1988b), reproductive events are synchronized, with all reproductive individuals in the population releasing gametes simultaneously (Clifton, 1997, Hay, 1997). Individuals of *H. tuna* develop reproductive structures beginning in the evening of 'night 1,' gametangia mature during the following day, and gametes are released slightly before dawn following 'night 2' (ca. 36 h after initiation of reproductive events; Drew & Abel, 1988b; Clifton, 1997).

Reproduction in *H. tuna* occurs when water temperatures are highest (Beth, 1962, Drew & Abel, 1988b). During our study, one to two reproductive events were recorded each month during the summer and early autumn sampling periods, and no reproductive events were observed in November. At peak reproduction, 2–5% (4–10% $\cdot \text{month}^{-1}$) of the population on Conch Reef was reproductive, comparable to studies of Panamanian siphonous algae where 3–5% of the populations under study synchronously reproduced (Clifton, 1997). Generally, it appeared that the largest, and presumably oldest, plants present in the population were the ones to develop gametangia. Three reproductive plants from Pinnacle on 29 August 2000 were nearly twice as long and possessed twofold more axes and eightfold more segments than non-reproductive plants. A pre-dawn release of gametes, as originally described for Panamanian populations (Clifton, 1997), was observed in *H. tuna* both in the field during 2000, as well as in laboratory aquaria where reproductive individuals collected from the field a day earlier had been kept in the dark.

One individual in the laboratory aquarium experiment exhibited all characteristics of a reproductive plant, yet failed to release gametes. Instead of dying, as is the case for individuals that release gametes, the non-gamete releasing individual exhibited pre-reproductive levels of pigmentation and translucent gametangia within a day and remained healthy in following weeks. It is unknown whether gametes in this individual failed to fully develop, were released internally (causing the bleached plant to return to its normal green color), or were reabsorbed. The extent and significance of reproductive reversal among *Halimeda* individuals remains to be determined.

Survivorship, re-growth, and recruitment

The extent and bias of tag loss on estimates of survival could not be reliably estimated. Over-wintering and turbulence-resistant stumps of *H. tuna* have been reported from Conch Reef (Alina Szmant, personal communication), and in *H. incrassata* from St. Croix, U.S. Virgin Islands after upright portions were grazed (Williams, 1988). It is likely that tags can be lost as turbulent environmental conditions rip upright axes from perennial bases, thereby providing unrealistically low estimates of life span. To test the importance of tag loss, we placed tags around the base of individuals prior to removing upright axes. Unfortunately, the test occurred during the calmest conditions experi-

enced during our 7-year study and the 100% recovery of tagged individuals probably does not reflect field conditions encountered during most of the year.

Despite the possibility of tag loss resulting in an artificially low estimate of *H. tuna* survivorship, data from other experiments suggests that our estimates are realistic. The re-growth/recruitment study showed that 25–80% of all single- and multi-axis individuals were lost in July–August 1998, probably from high levels of epiphytism by species of the brown alga, *Dictyota* (Beach et al., in review, Vroom et al., in review). The lower rates of plant loss (7–13% · month⁻¹) during other sampling periods when severe environmental factors (such as *Dictyota* spp. blooms) were not present were comparable to loss rates due to reproduction (4–10% · month⁻¹).

The unique unicellular structure of the Bryopsidales presents a potential disadvantage during wounding events (e.g., grazing) because the absence of cellular cross-walls means that large amounts of cellular constituents could be expelled when the cell is ruptured (Vroom & Smith, in press). The wound response consists of carbohydrate-based wound plugs forming within seconds of injury, thereby preventing loss of cytoplasm and turgor pressure (Dawes & Rhamstine, 1967; Dawes & Goddard, 1978). Wounding is common in *Halimeda*, as both herbivorous and carnivorous fish graze segments (Sotka et al., 1999, Overholtzer & Motta, 1999), even though the heavily calcified segments provide low food value (Littler & Littler, 1980). As an added defense against grazing (wounding), *Halimeda* contains a suite of inducible secondary metabolites (Paul & Van Alstyne, 1988, 1992). Wounding experiments conducted during our study, and grazing experiments conducted by Bach (1979), showed that *H. tuna* not only survived severe injury (eg. axis loss, but produced new segments within a day of experimental manipulation (Fig. 5; see also Bach, 1979).

Both sexual and asexual reproduction commonly occur in *Halimeda* (Drew & Abel, 1988b; Walters & Smith, 1994). However, the fate and development of *Halimeda* zygotes has not been studied (but see Meinesz, 1972) and the length of time required for an individual to mature to a recognizable morphology is unknown. Settlement of asexual fragments of *Halimeda* produced from fish bites and physical disturbance (Walters & Smith, 1994; Walters et al., 2002), or new plants produced at the end of rhizoidal runners (Meinesz, 1980b, but see Drew & Abel, 1988b), can quickly boost plant numbers without

sexual reproduction. The proportion of individuals produced via each method at Conch Reef method is unknown. Despite the mean number of new upright axes at Shallow Conch being less than half the number at Pinnacle, no significant difference was discernible during sampling periods in this study.

Meinesz (1980b) reports 172 new upright axes · 20 cm² · y⁻¹ (equivalent to 2150 new upright axes · 250 cm² · y⁻¹) from a population of high disturbance 5 m deep in the Mediterranean. The number of new upright axes reported here (17.2–131.5 · 250 cm² · y⁻¹; Table 2) is up to 2 orders of magnitude less. Because these two studies are separated by both location and time, it is impossible to determine why such large differences in recruitment were observed. Millennia of human habitation around the Mediterranean may have increased nutrient concentrations, thus encouraging rapid rhizoidal growth not found in oligotrophic reef environments (Clark, 1979; Bethoux & Copin-Montegut, 1988), or space availability and competition in each environment may differ substantially.

In conclusion, this study documented variation in growth parameters at two geographically close depth gradients on Conch Reef, Florida Keys during multiple growth seasons, and lays the groundwork for future studies of *H. tuna* population dynamics on Conch Reef. Subtle abiotic environmental differences dramatically affected growth, allowing plants from a deep reef slope region to grow significantly faster than plants from a shallow back reef despite lower light intensities. A possible increase in nutrients at depth and photoinhibition of shallow plants are likely responsible for observed differences. Fish grazing, amphipod habitations, and epiphyte loads were comparable at both sites, and not responsible for growth rate differences observed. Despite environmental differences between the sites, sexual reproduction occurred simultaneously across the reef system, demonstrating that the abiotic factors triggering reproduction are different than those affecting growth. Sexual reproduction permanently removed plants from the populations, but only accounted for a fraction of plant loss over time. Survival of individuals of *H. tuna* on Conch Reef is dependent on prevailing environmental conditions. Perennial bases of *H. tuna* persist within the turf community even as upright axes are removed during storms. Biological factors such as sexual reproduction and overgrowth by epiphytic algae are two important factors in permanently removing individuals from the population.

Acknowledgements

We gratefully acknowledge members of 'Team *Halimeda*' without whom this research would not be possible: Giacomo Bernardi, Heidi Borgeas, Elizabeth Calvert, Nicole Crane, Kathryn DeAngelis, Clare Dominik, Deborah Gochfeld, John Heine, Erica Klohn, Brenda Konar, Julie Liss, Kenyon Mobley, David Pence, Ryan Okano, Cassandra Roberts, Diana Steller, Laura Wick, Monica Woo, Jill Zamzow; and the superbly competent staff at the National Undersea Research Center: Greg Buck, Kendall Boykin, Craig Cooper, Kea Foreman, William Fox, Ken Johns, Doug Kessler, Steven Miller, Fuzzy O'Leary, Buddy Person, Otto Rutten, Monica Van Every, David Ward, and the Aquarius support team. This work was funded by National Undersea Research Center, University of North Carolina, Wilmington, grant numbers 9419, 9720, 9814 and 9925 to CMS, LJW and JAC. National Marine Sanctuary Authorization FKNMS-99-065; our thanks to Mr Benjamin Haskell, Science Coordinator, Florida Keys National Marine Sanctuary for his efforts.

References

- Bach, S. D., 1979. Standing crop, growth and production of calcareous Siphonales (Chlorophyta) in a south Florida lagoon. *Bull. mar. Sci.* 29: 191–201.
- Ballesteros, E., 1991. Seasonality of growth and production of a deep-water population of *Halimeda tuna* (Chlorophyceae, Caulerpaceae) in the North-western Mediterranean. *Bot. Mar.* 34: 291–310.
- Beach, K. S., L. J. Walters, P. S. Vroom, C. M. Smith, J. A. Coyer & C. L. Hunter, (in press). Variability in the ecophysiology of *Halimeda* spp. on Conch Reef, Florida Keys. *J. Phycol.*
- Beach, K. S., L. J. Walters, C. M. Smith, J. A. Coyer & P. S. Vroom (in review). *Dictyota* spp. bloom and its impact on *Halimeda tuna* at Conch Reef, Florida Keys. *J. exp. mar. Biol. Ecol.*
- Benoît, H. P. & P. Pepin, 1999. Individual variability in growth rate and the timing of metamorphosis in yellowtail flounder *Pleuronectes ferrugineus*. *Mar. Ecol. Prog. Ser.* 184: 231–244.
- Beth, K., 1962. Reproductive phases in populations of *Halimeda tuna* in the Bay of Naples. *Stn. Zool. Napoli Publ.* 32: 515–534.
- Bethoux, J. P. & G. Copin-Montegut, 1988. Phosphorous and nitrogen in the Mediterranean Sea: specificities and forecasting. *Oceanol. Act.* 9: 75–78.
- Blair, S. M. & J. N. Norris, 1988. The deep-water species of *Halimeda* Lamouroux (Halimedeaceae, Chlorophyta) from San Salvador Island, Bahamas: species composition, distribution and depth records. *Coral Reefs* 6: 227–236.
- Borowitzka, M. A. & A. W. D. Larkum, 1976. Calcification in the green alga *Halimeda* II. The exchange of Ca²⁺ and the occurrence of age gradients in calcification and photosynthesis. *J. exp. Bot.* 27: 864–878.
- Borowitzka, M. A. & A. W. D. Larkum, 1976. Calcification in the green alga *Halimeda* III. The sources of inorganic carbon for photosynthesis and calcification and a model of the mechanism of calcification. *J. exp. Bot.* 27: 879–893.
- Borowitzka, M. A. & A. W. D. Larkum, 1976. Calcification in the green alga *Halimeda* IV. The action of metabolic inhibitors on photosynthesis and calcification. *J. exp. Bot.* 27: 894–907.
- Borowitzka, M. A. & A. W. D. Larkum, 1977. Calcification in the green alga *Halimeda*. I. An ultrastructure study of plant development. *J. Phycol.* 13: 6–16.
- Clark, R. B., 1979. The health of the Mediterranean. *Mar. Pollut. Bull.* 10: 277–278.
- Clifton, K. E., 1997. Mass spawning by green algae on coral reefs. *Science* 275: 1116–1118.
- Clifton, K. E. & L. M. Clifton, 1999. The phenology of sexual reproduction by green algae (Bryopsidales) on Caribbean coral reefs. *J. Phycol.* 35: 24–34.
- Coyer, J. A., 1995. Use of a rock as an anvil for breaking scallops by the Yellowhead Wrasse, *Halichoeres garnoti* (Labridae). *Bull. mar. Sci.* 57: 548–549.
- Dawes, D. J. & R. H. Goddard, 1978. Chemical composition of the wound plug and entire plants for species of the coenocytic green alga, *Caulerpa*. *J. exp. mar. Biol. Ecol.* 35: 259–263.
- Dawes, C. J. & E. L. Rhamstine, 1967. An ultrastructural study of the giant green algal coenocyte, *Caulerpa prolifera*. *J. Phycol.* 3: 117–126.
- Dodge, R. E., 1984. Coral calcification rates by the buoyant weight technique: effects of Alizarin staining. *J. exp. mar. Biol. Ecol.* 75: 217–232.
- Drew, E. A. & K. M. Abel, 1988a. Studies on *Halimeda*: I. The distribution and species composition of *Halimeda* meadows throughout the Great Barrier Reef province. *Coral Reefs* 6: 195–205.
- Drew, E. A. & K. M. Abel, 1988b. Studies on *Halimeda*: II. Reproduction, particularly the seasonality of gametangia formation, in a number of species from the Great Barrier Reef province. *Coral Reefs* 6: 207–218.
- Flügel, E., 1988. *Halimeda*: paleontological record and palaeoenvironmental significance. *Coral Reefs* 6: 123–130.
- Freile, D., J. D. Milliman & L. Hillis, 1995. Leeward bank margin *Halimeda* meadows and draperies and their sedimentary importance on the western Great Bahama Bank slope. *Coral Reefs* 14: 27–33.
- Garrigue, C., 1991. Biomass and production of two *Halimeda* species in the Southwest New Caledonian lagoon. *Oceanol. Acta* 14: 581–588.
- Häder, D. P., M. Porst, H. Herrmann, J. Schäfer & R. Santas, 1996. Photoinhibition in the Mediterranean green alga *Halimeda tuna* Ellis et Sol measured *in situ*. *Photochem. Photobiol.* 64: 428–434.
- Hay, M., 1997. Synchronous spawning: when timing is everything. *Science* 275: 1080–1081.
- Hillis, L. W., 2001. The calcareous reef alga *Halimeda* (Chlorophyta, Bryopsidales): a cretaceous genus that diversified in the Cenozoic. *Palaeogeogr. Palaeoclimat. Palaeoecol.* 166: 89–100.
- Hillis-Colinvaux, L., 1972. Reproduction in the calcareous green algae of coral reefs. *J. mar. biol. Ass. India* 14: 328–334.
- Hine, A. C., P. Hallock, M. W. Harris, H. T. Mullins, D. F. Belknap & W. C. Jaap, 1988. *Halimeda* bioherms along an open seaway: Miskito Channel, Nicaraguan Rise, SW Caribbean Sea. *Coral Reefs* 6: 173–178.
- Hughes, T. P., D. C. Reed & M. J. Boyle, 1987. Herbivory on coral reefs: community structure following mass mortalities of sea urchins. *J. exp. mar. Biol. Ecol.* 113: 39–59.

- Johns, H. D. & C. H. Moore, 1988. Reef to basin sediment transport using *Halimeda* as a sediment tracer, Grand Cayman Island, West Indies. *Coral Reefs* 6: 187–193.
- Lapointe, B. E., 1997. Nutrient thresholds for bottom-up control of macroalgal blooms on coral reefs in Jamaica and southeast Florida. *Limnol. Oceanogr.* 42: 1119–1131.
- Leichter, J. J. & S. L. Miller, 1999. Predicting high-frequency upwelling: spatial and temporal patterns of temperature anomalies on a Florida coral reef. *Cont. Shelf Res.* 19: 911–928.
- Leichter, J. J., G. Shellenbarger, S. J. Genovese & S. R. Wing, 1998. Breaking internal waves on a Florida (U.S.A.) coral reef: a plankton pump at work? *Mar. Ecol. Prog. Ser.* 166: 83–97.
- Leichter, J. J., S. R. Wing, S. L. Miller & M. W. Denny, 1996. Pulsed delivery of subthermocline water to Conch Reef (Florida Keys) by internal tidal bores. *Limnol. Oceanogr.* 41: 1490–1501.
- Lirman, D. & P. Biber, 2000. Seasonal dynamics of macroalgal communities of the northern Florida reef tract. *Bot. Mar.* 43: 305–314.
- Littler, M. M. & D. S. Littler, 1980. The evolution of plant form and survival strategies in benthic marine macroalgae: field and laboratory tests of a functional form model. *Am. Nat.* 116: 25–44.
- Littler, M. M., D. S. Littler, S. M. Blair & J. N. Norris, 1985. Deepest known plant life discovered on an uncharted seamount. *Science* 22: 57–59.
- Littler, M. M., D. S. Littler, S. M. Blair & J. N. Norris, 1986. Deep-water plant communities from an uncharted seamount off San Salvador Island, Bahamas: distribution, abundance, and primary productivity. *Deep Sea Res.* 33: 881–892.
- Meinesz, A., 1972. Sur le cycle de l'*Halimeda tuna* (Ellis et Solander) Lamouroux (Udotéacée, Caulerpale). *Compt Rendu Hebd. Acad. Sc. (Paris)* 275: 1363–1365.
- Meinesz, A., 1980a. Connaissances actuelles et contribution à l'étude de la reproduction et du cycles des Udotéacées (Caulerpales, Chlorophytes). *Phycologia* 19: 110–138.
- Meinesz, A., 1980b. Contribution à l'étude des Caulerpales (Chlorophytes). PhD thesis. Université de Nice.
- Miller, M. W., M. E. Hay, S. L. Miller, D. Malone, E. E. Sotka & A. M. Szmant, 1999. Effects of nutrients versus herbivores on reef algae: a new method for manipulating nutrients on coral reefs. *Limnol. Oceanogr.* 44: 1847–1861.
- Multer, H. G., 1988. Growth rate, ultrastructure and sediment contribution of *Halimeda incrassata* and *Halimeda monile*, Nonsuch and Falmouth Bays, Antigua, WI. *Coral Reefs* 6: 179–186.
- Overholtzer, K. L. & P. J. Motta, 1999. Comparative resource use by juvenile parrotfishes in the Florida Keys. *Mar. Ecol. Prog. Ser.* 177: 177–187.
- Paul, V. J. & K. L. Van Alstyne, 1988. Chemical defense and chemical variation in some tropical Pacific species of *Halimeda* (Halimedaceae: Chlorophyta). *Coral Reefs* 6: 263–269.
- Paul, V. J. & K. L. Van Alstyne, 1992. Activation of chemical defenses in the tropical green algae *Halimeda* spp. *J. exp. mar. Biol. Ecol.* 160: 191–203.
- Payri, C. E., 1988. *Halimeda* contribution to organic and inorganic production in a Tahitian reef system. *Coral Reefs* 6: 251–262.
- Pizzimenti, P. B. & A. J. Silva, 1997. Stress-strain behavior of surficial carbonate sediments from Key West, Florida, U.S.A. *Mar. Georesources Geotechnol.* 15: 335–362.
- Rao, V. P., M. Veerayya, R. R. Nair, P. A. Dupeuble & M. Lamboy, 1994. Late quaternary *Halimeda* bioherms and aragonitic faecal pellet-dominated sediments on the carbonate platform of the western continental shelf of India. *Mar. Geol.* 121: 293–315.
- Shinn, E. A., B. H. Lidz, J. L. Kindinger, J. H. Hudson & R. B. Halley, 1989. Reefs of Florida and the Dry Tortugas: a guide to the modern carbonate environments of the Florida Keys and the Dry Tortugas. U.S. Geological Survey, St Petersburg, FL.
- Shulman, M. J. & D. R. Robertson, 1996. Changes in the coral reefs of San Blas, Caribbean Panama: 1983–1990. *Coral Reefs* 15: 231–236.
- Smith J. E., C. M. Smith, P. S. Vroom, K. S. Beach & S. L. Miller, (in review). Nutrient dynamics of *Halimeda tuna* on Conch Reef: possible influence of internal tides? *Limnol. Oceanogr.*
- Sotka, E. E., M. E. Hay & J. D. Thomas, 1999. Host-plant specialization by a non-herbivorous amphipod: advantages for the amphipod and costs for the seaweed. *Oecologia* 118: 471–482.
- Szmant, A. M. & A. Forrester, 1996. Sediment and water column nitrogen and phosphorus distribution patterns in the Florida Keys, U.S.A. *Coral Reefs* 15: 21–41.
- Vroom, P. S. & C. M. Smith, 2001. The challenge of siphonous green algae. *Am. Sci.* 89: 524–531.
- Vroom, P. S. & C. M. Smith, in press. Life without cells. *Biologist.*
- Vroom, P. S., C. M. Smith & S. C. Keeley, 1998. Cladistics of the Bryopsidales: a preliminary analysis. *J. Phycol.* 34: 351–360.
- Vroom, Peter S., Linda J. Walters, Kevin S. Beach, James A. Coyer, Jennifer E. Smith, Marie-Josée Abgrall, Dorothy Byron, Kathryn L. DeAngelis, Brenda Konar, Julie Liss, Ryan Okano, Cassandra Roberts, Laura Wick, Monica Woo, Steven Miller & Celia M. Smith (in review) Hurricane induced propagation and rapid re-growth of the weedy brown alga *Dictyota* in the Florida Keys. *Bot. Mar.*
- Walters, L. J. & C. M. Smith, 1994. Rapid rhizoid production in *Halimeda discoidea* Decaisne (Chlorophyta, Caulerpales) fragments: a mechanism for survival after separation from adult plants. *J. exp. mar. Biol. Ecol.* 175: 105–120.
- Walters, L. J., C. M. Smith, J. A. Coyer, C. L. Hunter, K. S. Beach & P. S. Vroom, 2002. Asexual propagation in the coral reef macroalga *Halimeda* (Chlorophyta, Bryopsidales): Production, dispersal and attachment of small fragments. *J. exp. mar. Biol. Ecol.* 278: 47–65.
- Wefer, G., 1980. Carbonate production by algae *Halimeda*, *Penicillus*, and *Padina*. *Nature* 285: 323–325.
- Wilbur, K. M., L. Hillis-Colinvaux & N. Watabe, 1969. Electron microscope study of calcification in the alga *Halimeda* (order Siphonales). *Phycologia* 8: 27–35.
- Williams, S. L., 1988. Disturbance and recovery of a deep-water Caribbean seagrass bed. *Mar. Ecol. Prog. Ser.* 42: 63–71.
- Wolanski, E., E. Drew, K. M. Abel & J. O'Brien, 1988. Tidal jets, nutrient upwelling and their influence on the productivity of the alga *Halimeda* in the Ribbon Reefs, Great Barrier Reef. *Estuar. coast. shelf Sci.* 26: 169–201.

Appendix A. Results of two-way ANOVA tests

			<i>F</i> -value	<i>p</i> -value
Number of individuals · m ⁻²	Site	1	219.96	< 0.001
	Mission	6	4.58	< 0.001
	Site*Mission	6	7.75	< 0.001
	Error	326		
Number of axes · thallus ⁻¹	Site	1	2.11	0.151
	Mission	1	3.30	0.074
	Site*Mission	1	4.88	0.031
	Error	63		
Number of segments · thallus ⁻¹	Site	1	42.80	< 0.001
	Mission	1	9.52	0.003
	Site*Mission	1	4.49	0.038
	Error	63		
Mean number of segments · axis ⁻¹	Site	1	71.73	< 0.001
	Mission	1	11.28	0.001
	Site*Mission	1	1.77	0.189
	Error	63		
Mean height of thallus	Site	1	25.77	< 0.001
	Mission	1	58.89	< 0.001
	Site*Mission	1	6.28	0.015
	Error	63		
Survivorship	Site	1	0.00	0.977
	Mission	1	10.02	0.004
	Site*Mission	1	0.43	0.519
	Error	28		
Recruitment	Site	1	0.10	0.758
	Mission	1	1.74	0.198
	Site*Mission	1	5.61	0.025
	Error	27		
Weight of upright axes (g)	Site	1	2.75	0.109
	Mission	1	2.17	0.152
	Site*Mission	1	0.29	0.597
	Error	27		
Growth rate, New segments · d ⁻¹	Site	1	6.17	< 0.001
	Mission	4	10.01	0.002
	Site*Mission	4	3.56	0.007
	Error	511		
Growth rate, New weight · d ⁻¹	Site	1	37.30	< 0.001
	Mission	4	7.49	0.006
	Site*Mission	4	11.28	< 0.001
	Error	511		
Growth rate, Percent increase (segments · d ⁻¹)	Site1	16.53	< 0.001	
	Mission	4	10.80	< 0.001
	Site*Mission	4	5.95	< 0.001
	Error	511		
Growth rate, Percent increase (weight · d ⁻¹)	Site	1	2.85	0.092
	Mission	4	78.77	< 0.001
	Site*Mission	4	2.67	0.032
	Error	509		
Average weight of unstained (new) segments	Site	1	3.73	0.054
	Mission	4	32.54	< 0.001
	Site*Mission	4	3.80	0.010
	Error	428		

Continued on p. 166

			<i>F</i> -value	<i>p</i> -value
Average weight of stained (old) segments	Site	1	4.60	0.033
	Mission	4	3.86	0.010
	Site*Mission	4	0.77	0.511
	Error	508		
Number of fish bites · thallus ⁻¹	Site	1	11.98	< 0.001
	Mission	3	1.96	0.162
	Site*Mission	3	0.88	0.452
	Error	268		
Number of tuna tacos · thallus ⁻¹	Site	1	6.26	< 0.001
	Mission	3	0.02	0.901
	Site*Mission	3	4.13	0.008
	Error	152		
Growth rate, New segments · d ⁻¹	Alizarin staining		<i>F</i> -value	<i>p</i> -value
	Sampling period	4	6.17	< 0.001
	Site	1	10.01	0.002
	Site*Sampling period	4	3.56	0.007
Growth rate, New weight · d ⁻¹	Error	511		
	Sampling period	4	37.30	< 0.001
	Site	1	7.49	0.006
	Site*Sampling period	4	11.28	< 0.001
Growth rate, Percent increase (segments · d ⁻¹)	Error	511		
	Sampling period	4	10.80	< 0.001
	Site	1	16.53	< 0.001
	Site*Sampling period	4	5.95	< 0.001
Growth rate, Percent increase (weight · d ⁻¹)	Error	511		
	Sampling period	4	78.77	< 0.001
	Site	1	2.85	0.092
	Site*Sampling period	4	2.67	0.032
Number of fish bites · plant ⁻¹	Error	509		
	Sampling period	3	11.98	< 0.001
	Site	1	1.96	0.162
	Site*Sampling period	3	0.88	0.452
Number of amphipod habitations · plant ⁻¹	Error	268		
	Sampling period	3	6.26	< 0.001
	Site	1	0.02	0.901
	Site*Sampling period	3	4.13	0.008
Survivorship	Error	152		
	Re-growth/Recruitment Quadrats			
	Site	1	0.00	0.977
	Sampling period	1	10.02	0.004
Net growth rate, New weight · plant · d ⁻¹	Site*Sampling period	1	0.43	0.519
	Error	28		
	Site	1	0.90	0.351
	Sampling period	1	3.15	0.087
Recruitment	Site*Sampling period	1	0.77	0.388
	Error	28		
	Site	1	0.10	0.758
	Sampling period	1	1.74	0.198
Weight of upright axes (g)	Site*Sampling period	1	5.61	0.025
	Error	27		
	Site	1	2.75	0.109
	Sampling period	1	2.17	0.152
	Site*Sampling period	1	0.29	0.597
	Error	27		