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VARIABILITY IN THE ECOPHYSIOLOGY OF *HALIMEDA* SPP. (CHLOROPHYTA, BRYOPSIDALES) ON CONCH REEF, FLORIDA KEYS, USA[1]

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The photosynthetic performance, pigmentation, and growth of a *Halimeda* community were studied over a depth gradient on Conch Reef, Florida Keys, USA during summer–fall periods of 5 consecutive years. The physiology and growth of *H. tuna* (Ellis & Solander) Lamouroux and *H. opuntia* (L.) Lamouroux on this algal dominated reef were highly variable. Maximum rate of net photosynthesis (P_{max}), respiration rate, and quantum efficiency (α) did not differ between populations of either species at 7 versus 21 m, even though the 21-m site received a 66% lower photon flux density (PFD). Physiological parameters, as well as levels of photosynthetic pigments, varied temporally. P_{max}, saturation irradiance, compensation irradiance, and growth were greatest in summer months, whereas α, chl *a*, chl *b*, and carotenoid concentrations were elevated each fall. *Halimeda tuna* growth rates were higher at 7 m compared with 21 m for only two of five growth trials. This may have arisen from variability in light and nutrient availability. Individuals growing at 7 m received a 29% greater PFD in August 2001 than in 1999. In August 1999 and 2001 seawater temperatures were uniform over the 14-m gradient, whereas in August 2000 cold water regularly intruded upon the 21-m but not the 7-m site. These results illustrate the potentially dynamic relationship between nutrients, irradiance, and algal productivity. This suggests the necessity of long-term monitoring over spatial and temporal gradients to accurately characterize factors that impact productivity.

Key index words: **chl fluorescence; coral reefs; *Halimeda opuntia*; *Halimeda tuna*; macroalgal productivity; photosynthesis**

Abbreviations: **α, quantum efficiency; β, slope of photoinhibition; dw, dry weight; ETR_{max}, maximum rate of electron transport; fw, fresh weight; I_β, photoinhibitory irradiance; I_c, compensation irradiance; I_k, saturation irradiance; PFD, photon flux density; P_{max}, maximum rate of net photosynthesis; R_d, respiration rate in dark; RLC, rapid light curve**

Members of the genus *Halimeda* (Chlorophyta, Bryopsidales) are important in many tropical and subtropical waters around the globe as primary producers and as stabilizers of and producers of reef sediments (Drew 1983, Flügel 1988, Hine et al. 1988, Johns and Moore 1988, Pizzimenti and Silva 1997, Hillis 2001, Walters et al. 2002). In the Florida Keys reef tract, for example, *Halimeda* annually contributes more to reef sediments than either corals or coralline algae (Wiman and McKendree 1975). Individuals of *Halimeda* have pigmented calcified segments that are joined at noncalcified nodes (genicula). New terminal segments, added sequentially, become calcified within 36 h, and colorless uncalcified rhizoids anchor the plant either on rock or in the sand (Hillis-Colinvaux 1972). Calcium carbonate contribution to reef sediments is the result of branch abandonment, herbivory, and holocarpic reproduction (Drew and Abel 1988, Schupp and Paul 1994, Littler and Littler 1999). After 3 or more years of growth, holocarpic reproduction occurs, whereby gametangia appear overnight on segment tips (Hillis-Colinvaux 1972). On the day after the appearance of gametangia, the entire protoplas-

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mic contents of the segments are explosively released as gametes, with death and disintegration of the parent rapidly following (Hillis-Colinvaux 1972, Drew and Abel 1988, Clifton 1997, Hay 1997, Clifton and Clifton 1999).

Although *Halimeda* is an integral component of many reef ecosystems, the increase in the cover of this genus along with other macroalgae may be indicators of coral reef degradation (Shulman and Robertson 1996). The shift from coral- to macroalgal-dominated reefs around the world and specifically in the Caribbean Sea has been attributed to removal of top-down control with the die-off of the herbivorous urchin, *Diadema antillarum* (Hughes et al. 1987), and bottom-up control in the form of anthropogenic nutrient input (Lapointe 1997, Miller et al. 1999). On coral reefs in the Florida Keys, macroalgal cover was found to be 58% during the summer of 1998 (Lirman and Biber 2000). Two genera, *Halimeda* and *Dictyota*, together comprised 77%–99% of the macroalgal biomass sampled over the course of 1998 on the Upper Florida Keys reef tract (Lirman and Biber 2000). Given the prevalence of this genus at tropical latitudes, it is remarkable that so little is known of the physiological ecology of *Halimeda*.

To examine the physiological ecology of *Halimeda* populations, research was conducted on Conch Reef, Florida Keys, USA from 1997 to 2001. Conch Reef, a research-only reef in Florida Keys National Marine Sanctuary, offers research opportunities with reduced anthropogenic effects and a diverse flora and fauna (Bach 1979, Coyer 1995, Overholtzer and Motta 1999, Sotka et al. 1999). Past research has revealed a typical tropical reef ecosystem with limited nutrient availability (Miller et al. 1999); however, episodic internal waves deliver cold nutrient-rich water onto the reef (Leichter et al. 1996, Leichter and Miller 1999). The two study sites used in this research, a deep reef slope and a shallow back reef region, were chosen as two diverse reef habitats within a short distance (approximately 0.7 km) of each other. The close proximity of these two distinct sites on Conch Reef allowed robust comparisons of the physiological responses to different environmental factors that change with depth, such as irradiance quantity and frequency of internal tidal bores, while minimizing potential genetic differences.

Our objective was to determine the extent to which physiological and environmental variability occurs over time and space, thereby impacting the growth and subsequent fitness of *H. tuna* (Ellis & Solander) Lamouroux and *H. opuntia* (L.) Lamouroux. Specifically, we addressed the following questions: How variable is seawater temperature and irradiance over depth and time on Conch Reef? Does photosynthetic performance, pigmentation, and growth change with increased depth for *Halimeda*? How does photosynthetic performance, pigmentation, and growth vary within and between months for *Halimeda*? Do the two dominant species of *Halimeda* on Conch Reef respond similarly to environmental variations over space and time? Are changes in *in situ* growth rates of *Halimeda* related to shifts in photosynthetic performance?

METHODS AND MATERIALS

Study area. All surveys and experiments were conducted on Conch Reef (24 57′ 00′′ N, 80 27′ 13′′ W) at 21- and 7-m field sites from June 1997 to August 2001.

In situ *photon flux density and temperature.* During August 1999, November 1999, August 2000, September 2000, and August 2001 instantaneous photon flux density (PFD) from 400 to 700 nm was measured and recorded every 5 min at both 21- and 7-m sites using LiCor 4π underwater quantum sensors attached to Licor LI-1400 dataloggers (Lincoln, NE, USA). Dataloggers and quantum sensors were deployed continuously throughout the dates indicated (Fig. 1) on the benthos through the use of custom underwater housings (The Sexton Company, Salem, OR, USA). Seawater temperature was recorded at 6-min intervals at 7 and 21 m with the use of StowAway XTI temperature loggers (Onset, Bourne, MA, USA) during August 1999, 2000, and 2001 (Fig. 2).

Ecophysiology of Halimeda tuna *and* Halimeda opuntia. *Photosynthetic performance:* The field setting at Conch Reef provides a natural experiment into the impact of the irradiances changes with depth on *H. tuna* and *H. opuntia* physiology. *In situ* photosynthetic performance was evaluated using a Diving PAM (pulse amplitude modulation fluorometer) (Walz) to obtain rapid light curves (RLCs) from *Halimeda* spp. samples from 7 (*H. tuna* only) and 21 m (*H. tuna* and *H. opuntia*) during August 2000. Separate surveys were conducted to facilitate statistical comparisons of *H. tuna* between depths and *H. tuna* and *H. opuntia* within a depth. The RLCs were obtained with 10-s illumination times with eight increasing intensities from 0 to 750 μmol photons·m^{-2}·s^{-1}. A flexible fiberoptic probe was used for sample illumination and fluorescence collection. The free end of the fiberoptic probe was mounted to one half of a magnetic leaf clip. When surveying samples, the leaf clip was gently pressed against the samples, thus darkening it as well as ensuring a fixed distance of 5 mm between the probe and the surface of algal thalli. The physiological parameters, saturation irradiance (I_k), quantum efficiency (α), maximum electron transport rate (ETR_{max}), slope of photoinhibition (β), and photoinhibitory irradiance (I_β), were obtained via nonlinear regression using the hyperbolic tangent model of Jassby and Platt (1976) (see below) or Platt et al. (1980): $ETR = ETR_{max} (1 - e(-\alpha I/ETR_{max})) \times e(-\beta I/ETR_{max})$, $I_\beta = ETR_{max}/\beta$. Regressions from Platt et al. (1980) were used if photoinhibition was evident in the response curve.

Photosynthetic performance of *H. tuna* and *H. opuntia* was assessed in the laboratory by measuring the rate of O_2 evolution in response to increasing PFD (Beach et al. 1995). For laboratory-based measures, specimens of each species of *Halimeda* were collected from 7- and 21-m sites in June and September 1997, July 1998, and August and November 1999 and evaluated for photosynthetic performance. Ten to 20 specimens of each species from each depth were assayed. Samples were collected from Conch Reef, stored in seawater-filled coolers, and transferred to the laboratory within 6 h. In the laboratory, two to three segment pieces from individuals were excised at genicula in seawater using a razor blade. Segments were consistently sampled from a basipetal region, two segments behind the apical-most fully expanded segment. Segments were only excised after a regreening period of at least 2 h in a flow-through seawater system. During this period chloroplasts migrated back to the surface of utricles. After excision samples were monitored for 60 s. Samples that closed the excision wound by halting the flow of cytoplasm out of the cell were used in subsequent photosynthetic and pigmentation analyses. Samples were then placed in mesh bags in a flow-through seawater system to allow for wound recovery for 24 h. In pretreatment trials, respiration rates of excised samples of *Halimeda* were elevated for 12 h before returning to baseline levels (data not shown).

Net photosynthesis was measured as oxygen exchange using a water-jacketed Clark-type oxygen electrode (Rank Brothers, Cambridge, UK, following Beach et al. 1995). Temperatures were maintained at sample collection temperature of ±1° C via a Neslab RTE-110 (Neslab, Portsmouth, NH, USA) temperature-controlled water bath. Illumination was provided by a Kodak Ektagraphic III E slide projector (Kodak, Rochester, NY, USA) with a 300-W tungsten halogen lamp. The PFDs were varied from 0 to 2000 μmol photons·m^{-2}·s^{-1} with neutral density filters calibrated with a Hansitech Quantitherm sensor (Norfolk, England). To minimize carbon limitation, filtered seawater was augmented to 20 mM $NaHCO_3^-$ above ambient levels. The physiological parameters, respiration rate in dark (R_d), compensation irradiance (I_c), I_k, α, and net maximum photosynthetic rate (P_{max}), were obtained via nonlinear regression using the hyperbolic tangent model of Jassby and Platt (1976): $P = P_{max}\tanh(\alpha I/P_{max}) + R_d$, $I_c = R_d/\alpha$, $I_k = P_{max}/\alpha$. In November 1999 trials, only saturating PFD and darkness were used, thereby only generating values for P_{max} and R_d, respectively.

Pigmentation: Chl *a*, chl *b*, and total carotenoid concentrations were determined spectrophotometrically using *N,N*-dimethylformamide extraction (Moran and Porath 1980). Calculations of chl *a* and *b* concentrations were based on the extinction coefficients of Porra et al. (1989). An estimate of relative carotenoid content was determined by the use of equations suggested by Henley and Dunton (1995). All pigment contents were normalized to tissue fresh weight (fw).

Reciprocal transplants with Halimeda tuna. In September 1998, the physiology and pigmentation of *Halimeda* thalli over depth were explored further in a manipulative experiment. Ten individuals of *H. tuna* were harvested from each depth (7 and 21 m) and transplanted to their reciprocal depth (between-depth transplants). Transplants were intertwined within the strands of polypropylene rope that had been secured to weighted polyvinyl chloride frames 1 m^2 placed in sandy areas at appropriate experimental depths. Controls for manipulation consisted of 10 individuals of *H. tuna* intertwined within polypropylene rope and attached to a polyvinyl chloride frame at the depth the algae were harvested (within-depth transplants). As an additional control, 10 individuals of *H. tuna* at each depth were tagged and left in place on the benthos and were only harvested just before physiological evaluation (unmanipulated). Individuals were left in place for 10 days and then transported to the laboratory for evaluation of P_{max}, R_d, and pigmentation.

Halimeda tuna *growth.* Alazarin Red-S histochemically stains calcium carbonate of many organisms, including *Halimeda* (Multer 1988). In September 1997, July 1998, August 1999, November 1999, and August 2001 (21-m population only) individuals of *H. tuna* at 7- and 21-m sites were covered with a 4-L plastic bag *in situ.* Bags were secured to the base of the alga's primary axis with a rubber band. One milliliter of a 1% dye solution was initially placed in 1.5-mL Eppendorf tubes and was then dispersed in the bags. After 24 h, the bags were removed and the existing segments were stained red.

Fifty to 75 individuals of *H. tuna* were stained at each depth on each date. After beginning the experimental treatments, *H. tuna* individuals were grown in the field for 9 to 14 days before harvesting. After harvesting, individuals were soaked in a 0.5% bleach solution for 10 min, and the number of new segments (white) was recorded and separated from old-growth (red). The fw of the old and new segments was then determined for each sample.

Statistical comparisons. Three-way analyses of variance (ANOVAs) were used to differentiate the effects of depth, sampling date, and species on laboratory-based measures of physiological performance and pigmentation. Two-way ANOVAs were used to differentiate the effects of reciprocal transplants on the physiology and pigmentation of *H. tuna* performed during September 1998. Normality of the residuals was tested with the equivalent of a Shapiro-Wilk test with an α of 0.05. Interaction of factors was considered significant only if at least one of the individual factors were independently significant. Fisher's protected least significant difference (PLSD) post-hoc comparisons were used to detect differences between levels of the factors used in this study, if significant in an ANOVA using an $\alpha < 0.05$. Unpaired *t*-tests were used to compare photosynthetic performance of *H. tuna* over depth (7 vs. 21 m) and *H.* tuna versus *H. opuntia* at the same depth (21 m) as measured by chl fluorescence. Bonferroni corrections were used on all statistical tests where data were used in more than one comparison. Simple linear regression was used to analyze the relationship between mean photosynthetic performance and mean growth rates of *H. tuna* at 7 and 21 m.

RESULTS

Temperature and irradiance. The 7-m site on average received 3-fold more irradiance than did the 21-m site, regardless of sampling date (Fig. 1). Irradiance levels decreased as expected from August to November, but considerable interannual variation was observed, with August 1999 receiving 27% less PFD·d^{-1} than August 2001 (Fig. 1).

As with the irradiance data, considerable variation was observed in seawater temperature over depth and among years (Fig. 2). In August 1999 and August 2001 water temperatures were similar between 7 and 21 m (Fig. 2, A and C). In August 1999 water temperature was on average 1° C warmer than in 2001. In August 2000 water temperature at 7 m was consistently 30° C with minimal diurnal variations. At 21 m, water temperature varied markedly from 25.6 to 30.2° C over the first 4 days of recording (Fig. 2B). Colder water temperatures were recorded at 21 m on Conch Reef during six discreet time periods with each low temperature separated by approximately 6 h. Water temperatures were similar between depths in August 2000 during the last 4 days of recording (Fig. 2B).

Ecophysiology of Halimeda tuna *and* Halimeda opuntia. In situ *measures via chl fluorescence:* The RLCs of *H. tuna* and *H. opuntia* at 21 m during August 2000 yielded no differences in photosynthetic performance between these species (Table 1). Significant differences in pho-

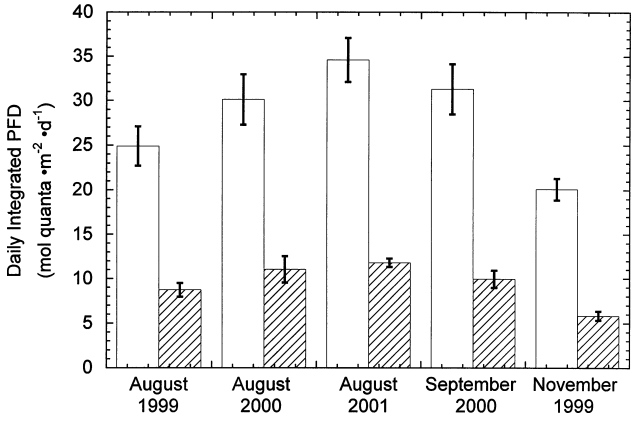

FIG. 1. Mean daily integrated PFD (± SE) at 7- (white bars) and 21-m (hatched bars) sites on Conch Reef, Florida Keys. Photon flux densities collected simultaneously at each depth at 5-min intervals using 4π corrected PAR sensors. Data were collected from 3 to 13 August 1999, 2 to 9 August 2000, 13 to 20 August 2001, 6 to 10 September 2000, and 8 to 17 November 1999.

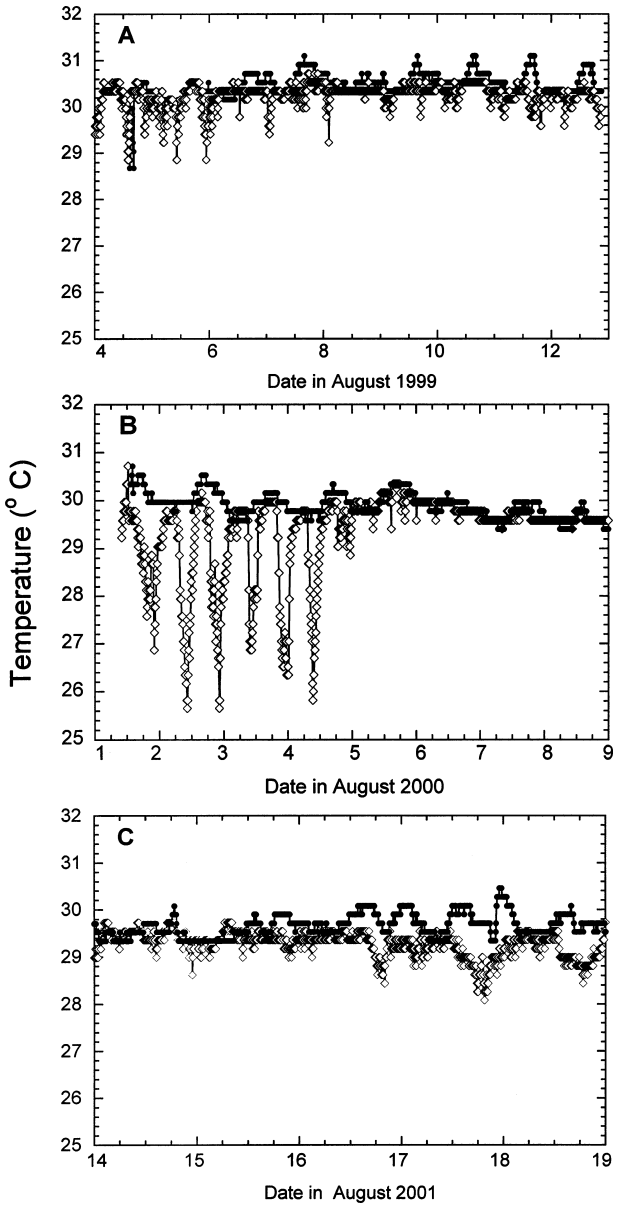

FIG. 2. Seawater temperature at 6-min intervals at 7 (solid circles) and 21 m (open diamonds) on Conch Reef, Florida Keys during dates indicated for August 1999, 2000, and 2001.

tosynthetic performance were observed between *H. tuna* populations at 7 versus 21 m in August 2000 (Table 2). Values of ETR_{max}, I_k, and I_β were significantly higher for individuals of *H. tuna* growing at 7 versus 21 m.

Laboratory measurements with oxygen electrodes: Photosynthesis versus irradiance curves of *H. tuna* and *H. opuntia* from 7 and 21 m sampled during June 1997, July 1998, August 1999, September 1997, and November 1999 yielded significant differences in performances between species, among dates, but not between depths. Overall, P_{max} was higher in *H. opuntia* than in *H. tuna* ($P = 0.002$). Additionally, P_{max} was significantly higher in July 1998 and lower in November 1999 than other sampling dates for both species combined ($P < 0.0001$, Fig. 3). The degree of change in P_{max} over sampling dates differed between species as indicated by the significant interaction between species and date ($P = 0.0001$). In *H. tuna*, P_{max} peaked in July 1998 and decreased on either side of this maximum. In contrast, P_{max} in *H. opuntia* had a less clear trend over time with elevated levels in July 1998 and September 1997. Contrary to the differences detected in P_{max}, R_d did not differ between depth ($P = 0.31$) or species ($P = 0.60$) or among sampling dates ($P = 0.24$). The mean R_d for all samples combined was 5.84 ($\pm$0.18 SE, $n = 336$) μmol O_2 consumed·g(fw)$^{-1}$·min^{-1}. Maximum net photosynthetic rates and R_d were combined into a P:R ratio for each sample. This ratio differed significantly among sample dates ($P < 0.0001$) with the highest ratio of 5.5 ($\pm$0.83 SE) in July 1998 with a decrease on either side (based on month) of this maximum to a minimum of 1.8 ($\pm$0.37 SE) in November 1999.

Alpha (α) was significantly different over sampling dates ($P < 0.0001$, Fig. 4) and was lowest in June 1997 and July 1998 and increased to the highest level in September 1997. No significant differences were observed in α over depth ($P = 0.51$) or between species ($P = 0.69$). Values for I_k were significantly elevated during the July 1998 sampling period compared with June 1997, August 1999, and September 1997 ($P < 0.0001$, Fig. 5). Values for I_c were similarly elevated in July 1998 compared with August 1999 and September 1997 ($P = 0.017$, Fig. 5).

Photosynthetic pigments: Total pigment extracts from *H. tuna* and *H. opuntia* from 7 and 21 m yielded signif-

TABLE 1. Mean $\pm$ SE physiological parameters for *Halimeda* from 21-m depths in August 2000.

	H. opuntia	*H. tuna*	*P* value
n	10	10	
ETR_{max} (μmol electrons·m^{-2}·s^{-1})	13.9 $\pm$ 1.9	14.7 $\pm$ 1.7	0.82
α (μmol electrons·μmol quanta^{-1})	0.285 $\pm$ 0.018	0.313 $\pm$ 0.016	0.40
β (μmol electrons·μmol quanta^{-1})	$-$0.021 $\pm$ 0.003	$-$0.020 $\pm$ 0.003	0.80
	$n = 2$	$n = 4$	
I_k (μmol quanta·m^{-2}·s^{-1})	53.8 $\pm$ 7.0	49.2 $\pm$ 6.6	0.78
I_β (μmol quanta·m^{-2}·s^{-1})	436.8 $\pm$ 60.8	662.9 $\pm$ 120.3	0.47

Statistical comparisons via Student's *t*-test. Sample size (n) differs for β and I_β because not all samples exhibited photoinhibition.

TABLE 2. Mean ± SE physiological parameters for *Halimeda tuna* from 7- and 21-m depths in August 2000.

	7 m	21 m	*P* value
n	27	10	
ETR_{max} (μmol electrons·m^{-2}·s^{-1})	26.5 ± 1.1	15.9 ± 1.6	<0.0001
α (μmol electrons·μmol quanta^{-1})	0.305 ± 0.013	0.293 ± 0.016	0.73
β (μmol electrons·μmol quanta^{-1})	−0.026 ± 0.002	−0.019 ± 0.003	0.1174
	n = 22	n = 4	
I_k (μmol quanta·m^{-2}·s^{-1})	91.5 ± 6.6	56.3 ± 4.6	0.0002
I_β (μmol quanta·m^{-2}·s^{-1})	1058.3 ± 47.6	573.9 ± 188.2	0.0072

Statistical comparisons via Student's *t*-test. Sample size (n) differs for β and I_β because not all samples exhibited photoinhibition.

icant differences in pigment concentrations between species, among dates, and between depths. Chl *a* concentrations were significantly higher in *H. tuna* than in *H. opuntia* ($P < 0.0001$) but not significantly different between depths due to a Bonferroni correction ($P = 0.044$, Fig. 6). Chl *a* concentrations varied significantly over sampling dates in this study ($P = 0.003$). Samples from September 1997 and 1998 had elevated chl *a* levels compared with June 1997, August 1999, and November 1999 (Fig. 6). The extent of changes in chl *a* concentrations over sampling dates was not uniform between species ($P = 0.045$). Chl *a* concentrations in *H. opuntia* were highest in September samples with lower levels on either side of this peak based on months. In *H. tuna*, August 1999 samples had lower chl *a* concentrations than either July 1998 or September 1997 and 1998 (Fig. 6).

Chl *b* concentrations were significantly higher in *H. tuna* compared with *H. opuntia* ($P < 0.0001$) but were not different between sampling depths ($P = 0.053$, Fig. 7). As with chl *a*, chl *b* concentrations varied significantly with sampling date ($P = 0.0002$), and the pattern of variation over date was different between species ($P = 0.032$). Chl *b* concentrations in *H. opuntia* and *H. tuna* were higher in August and September samples with lower levels on either side of this peak based on months. Chl *b* concentrations decreased from the August–September maximum at a faster rate in *H. opuntia* than in *H. tuna* (Fig. 7).

Concentrations of carotenoids were significantly higher in *H. tuna* versus *H. opuntia* ($P < 0.0001$) and in 7- versus 21-m samples ($P < 0.0001$, Fig. 8). Carotenoid concentrations were highly variable over the extent of this study, with concentrations ranging from 0.06 to 0.25 g·g fw^{-1}. Carotenoid concentrations were significantly different based on sampling date ($P = 0.013$), and the pattern of variation in carotenoid concentrations differed between species ($P = 0.019$, Fig. 8).

Halimeda tuna and *H. opuntia* had similar chl *b*:*a* ratios over both 7- and 21-m sampling depths ($P = 0.33$ [species], $P = 0.74$ [depth]). The ratio of chl *b*:*a* varied minimally over time, with a higher ratio in August 1999 compared with all other sampling dates and elevated levels in June 1997 versus July 1998. The carotenoid:chl *a* ratio was highly variable, ranging from 0.34 to 0.92. *Halimeda tuna* had a significantly higher carotenoid:chl *a* ratio than *H. opuntia* ($P = 0.014$).

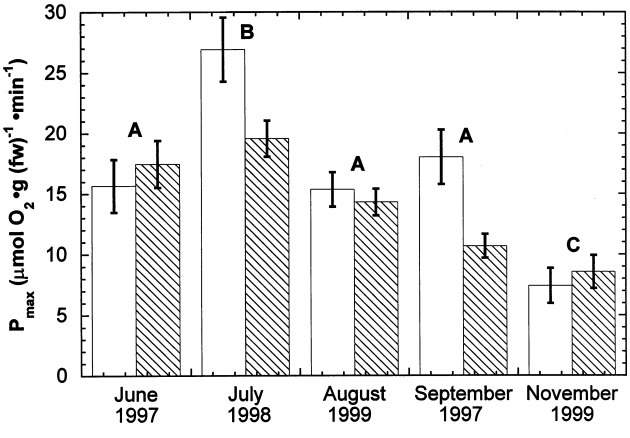

FIG. 3. Mean maximum photosynthetic rate (± SE) for *Halimeda opuntia* (white bars) and *Halimeda tuna* (hatched bars) from 7 and 21 m (combined) over sample dates. Common letters indicate no significant difference among sample dates at $P = 0.05$ as judged by three-way ANOVA and post-hoc tests.

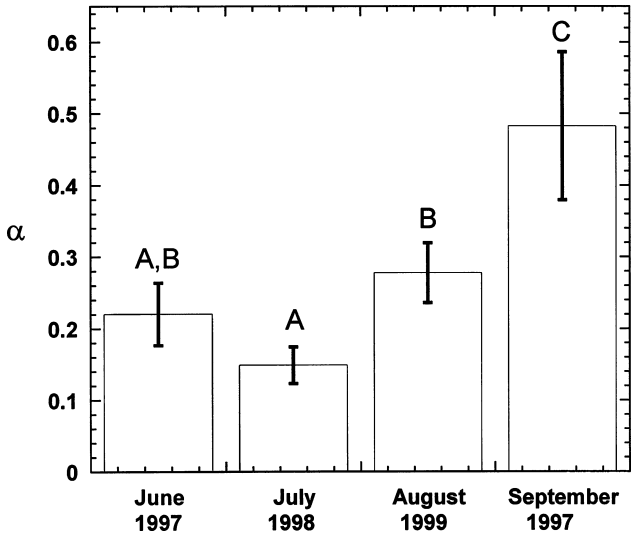

FIG. 4. Mean quantum efficiency (± SE) for *Halimeda opuntia* and *Halimeda tuna* (combined) from 7 and 21 m (combined) over sample dates. Common letters indicate no significant difference among sample dates at $P = 0.05$ as judged by three-way ANOVA and post-hoc tests.

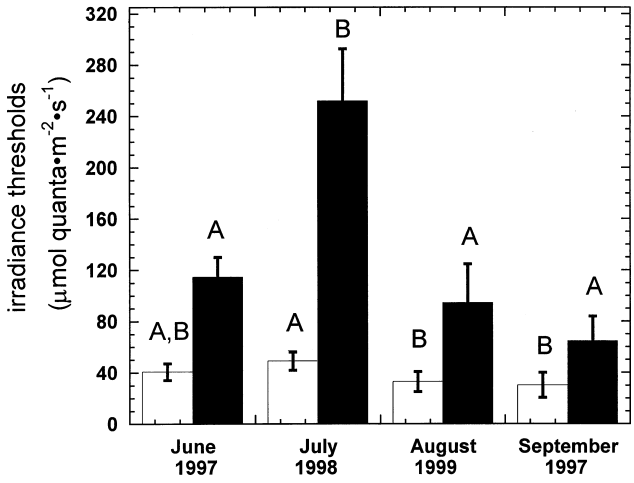

FIG. 5. Mean compensation irradiance (white bars) and saturation irradiances (black bars) (± SE) for *Halimeda opuntia* and *Halimeda tuna* (combined) from 7 and 21 m (combined) over sample dates. Common letters indicate no significant difference among sample dates at $P = 0.05$ as judged by three-way ANOVAs and post-hoc tests conducted separately on I_c and I_k.

This ratio did not differ between sampling depths for both species but did vary significantly among all sampling dates except July 1998 and November 1999 ($P < 0.0001$).

Reciprocal transplant experiment with Halimeda tuna. Transplantation of *H. tuna* in September 1998 between depths had a significant impact on P_{max} ($P = 0.0005$, Fig. 9). Individuals from 7 m did not respond to the transplant in the same manner as individuals from 21 m as indicated by the significant interaction between treatment and depth ($P < 0.0001$). Reciprocal transplantation had no significant effect on R_d ($P = 0.49$), chl *a* ($P = 0.06$), chl *b* ($P = 0.07$), carotenoid ($P = 0.008$, only manipulation effect) concentrations, or pigment ratios (chl *b*:*a*, $P = 0.35$; carotenoid:chl *a*, $P = 0.04$, only manipulation effect). Values of P_{max} of *H. tuna* at 21 m were on average 38% higher for of individuals at 7 m during September 1998. When within depth transplants were performed, P_{max} of *H. tuna* was negatively impacted with mean depression of P_{max} by 21%. Transplantation of individuals of *H. tuna* from 21 to 7 m lowered P_{max} by at least 38% when taking into account the within depth depression of P_{max} for 21-m individuals. Movement of individuals from 7- to 21-m depths elevated P_{max} by at least 35% (50% if within depth transplantation is taken into account).

Growth. Growth rates of *H. tuna* varied from a minimum rate of 0.0039 g fw·d^{-1} at 7 m in September 1997 to maximum rate of 0.121 g fw·d^{-1} at the same depth in July 1998. During July 1998 and November 1999, growth rates of *H. tuna* were higher on average at 7 m compared with 21 m. In September 1997 this trend was reversed, with elevated growth rates for the 21-m population. In August 1999, the growth rates of *H. tuna* were approximately equal at 7 and 21 m. Despite the variation in growth rates over depth and sampling date, labora-

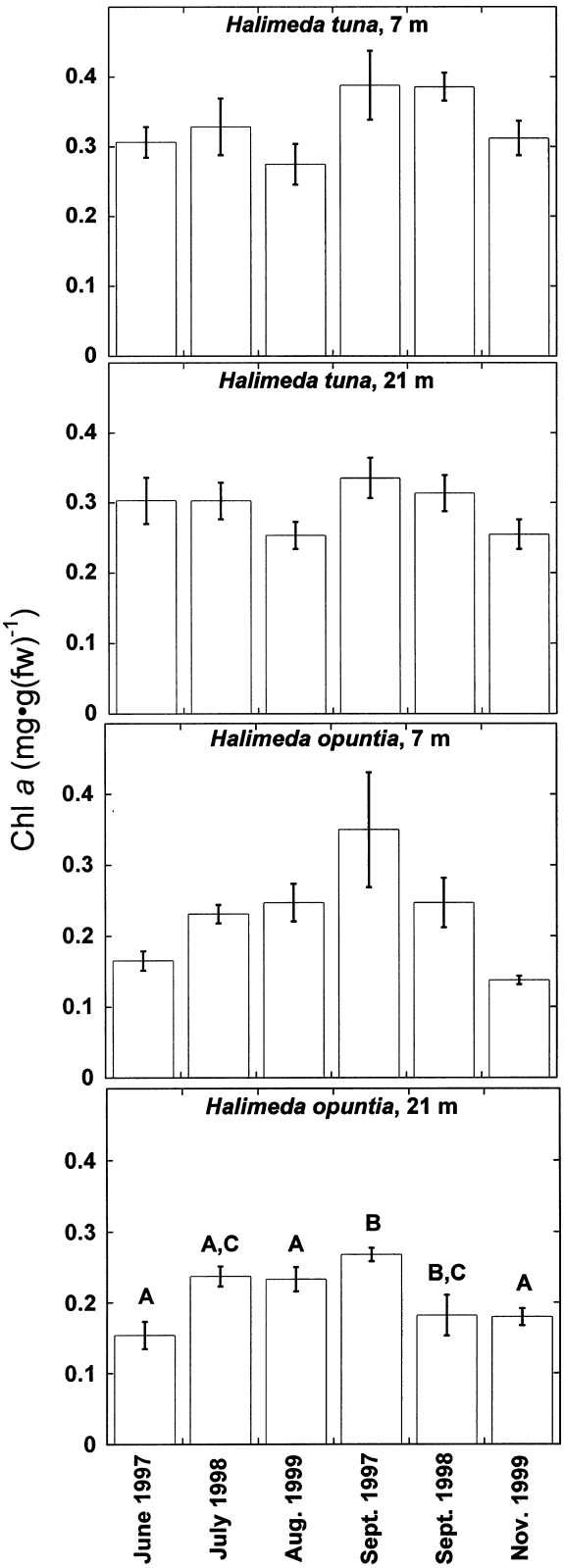

FIG. 6. Mean chl *a* concentrations (± SE) in *Halimeda opuntia* and *Halimeda tuna* from 7 and 21 m on Conch Reef, Florida Keys over time. Common letters over 21-m *H. opuntia* indicate no significant differences among sample dates for all depths and both species at $P = 0.05$ as judged by three-way ANOVA and post-hoc tests.

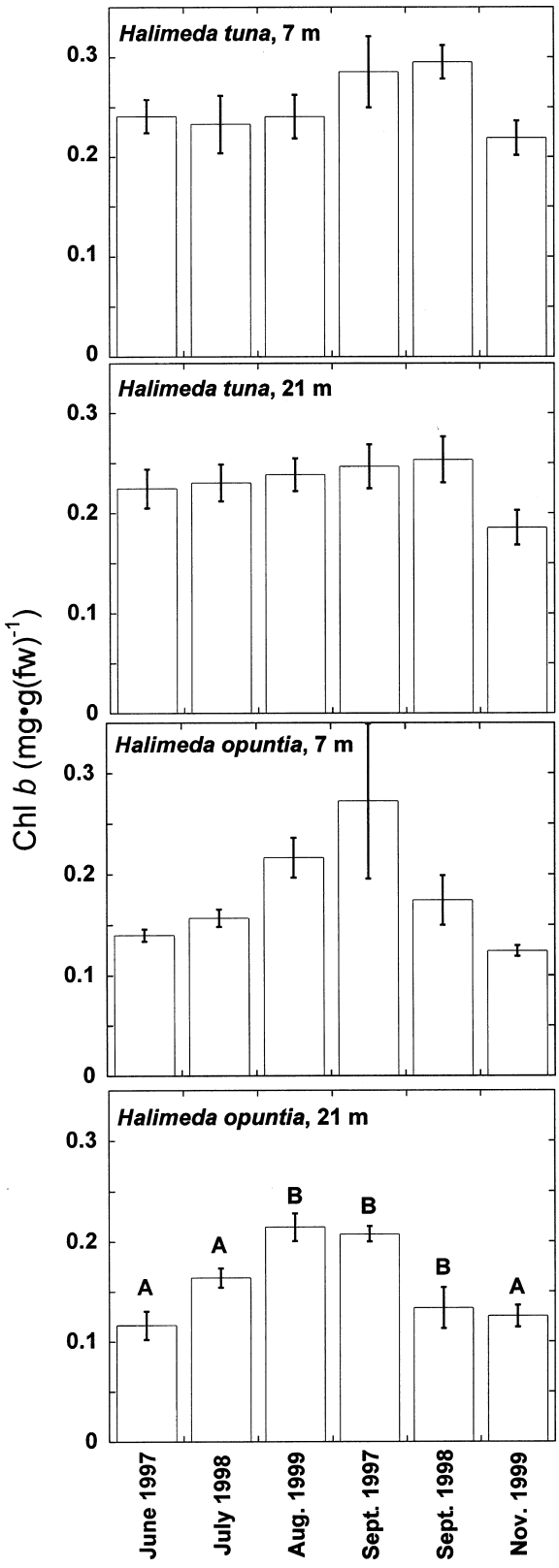

FIG. 7. Mean chl *b* concentrations (± SE) in *Halimeda opuntia* and *Halimeda tuna* from 7 and 21 m on Conch Reef, Florida Keys over time. Common letters over 21-m *H. opuntia* indicate no significant differences among sample dates for all depths and both species at $P = 0.05$ as judged by three-way ANOVA and post-hoc tests.

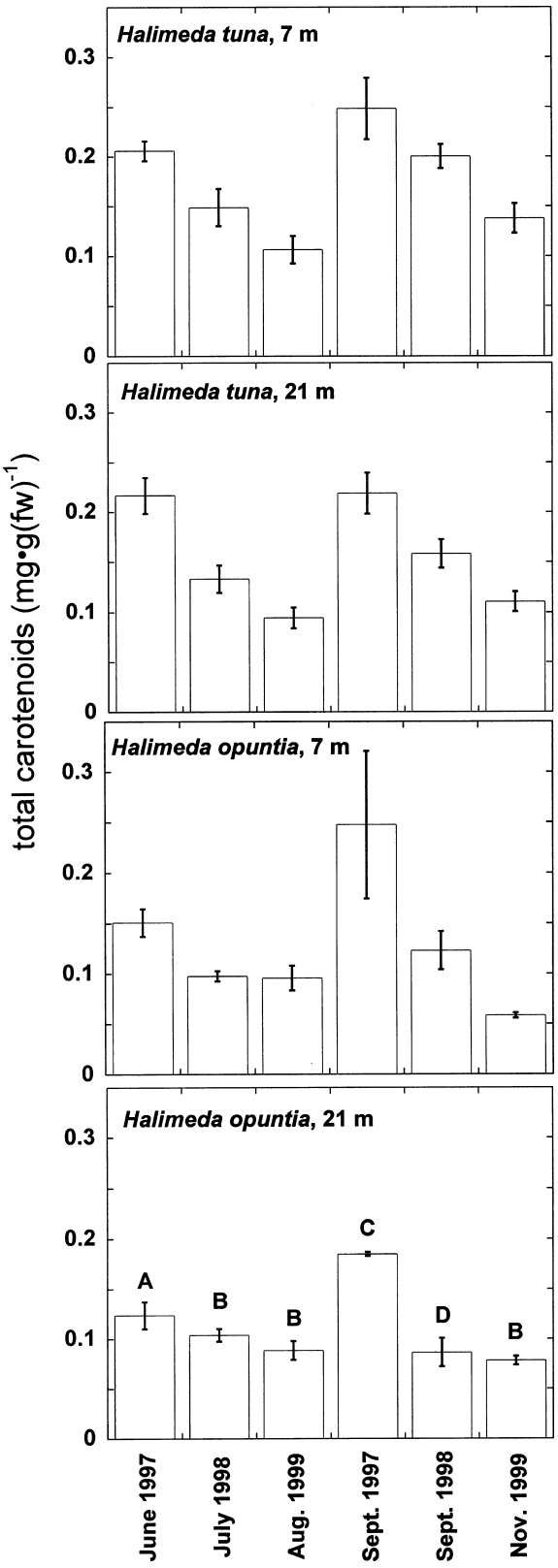

FIG. 8. Mean carotenoid concentrations (± SE) in *Halimeda opuntia* and *Halimeda tuna* from 7 and 21 m on Conch Reef, Florida Keys over time. Common letters over 21-m *H. opuntia* indicate no significant difference among sample dates for all depth and both species at $P = 0.05$ as judged by three-way ANOVA and post-hoc tests.

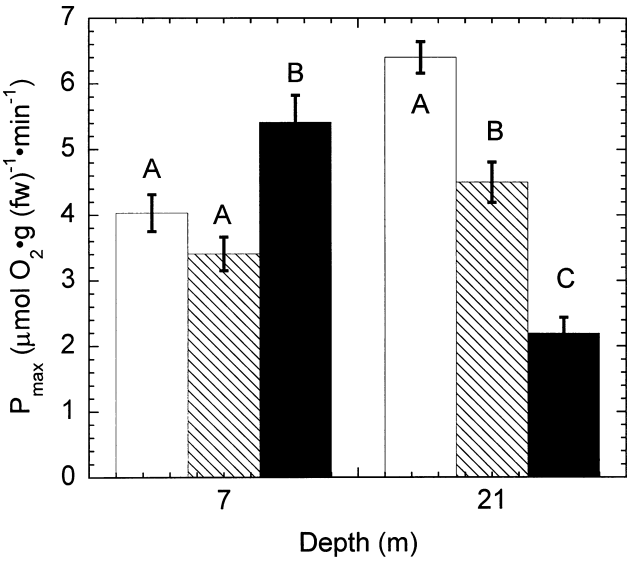

FIG. 9. Maximum mean photosynthetic rate (± SE) for treatments of *Halimeda tuna* in reciprocal transplant experiment at 7 and 21 m. White bars, unmanipulated *H. tuna* (controls); hatched bars, within-site transplants (manipulation controls); black bars, reciprocal depth transplants. Note: Mean (± SE) for each treatment is found above the depth labeled individuals originated from. Common letters indicate no significant difference among treatments within depths at $P = 0.05$ as judged by two-way ANOVA and post-hoc tests.

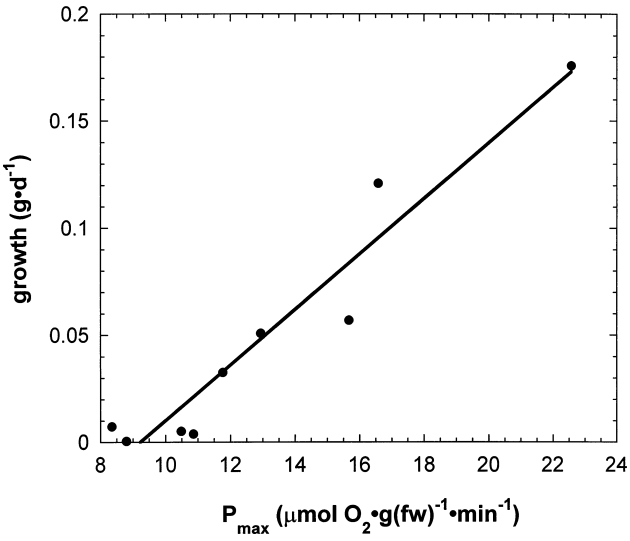

FIG. 10. Regression relationship of P_{max} and growth rate of *Halimeda tuna* sampled from 7 and 21 m on Conch Reef, Florida Keys in September 1997, July 1998, August 1999, November 1999, and August 2001. Growth ($g \cdot d^{-1}$) = $0.013 \times P_{max} - 0.12$ ($R^2 = 0.93$).

tory-based measures of P_{max} served as a good predictor of *in situ* growth (Fig. 10).

DISCUSSION

Photosynthetic performance and growth of *Halimeda* spp. on Conch Reef were highly variable over the spatial and temporal scales of this study. Examination of trends from one sampling interval (August 1999) led to the conclusion that *H. tuna* exhibits a typical sun- to shade-type photoacclimation over depth. This was evident as elevated ETR_{max}, and I_k coupled with decreased susceptibility to photoinhibition at 7 versus 21 m. These trends are similar to findings for *H. tuna* in the Mediterranean Sea (Häder et al. 1996). Productivity and photosynthetic performance data from other sampling intervals led to very different conclusions. In September 1997, growth rates at 21 m exceeded those of 7-m populations. In the following year, P_{max} was 38% higher for individuals of *H. tuna* at 21 versus 7 m. Elevated productivity with increased depth is likely due to photoinhibitory irradiances negatively impacting productivity at shallow depths and/or increased frequency of nutrient enrichment concomitant with cold water intrusions at 21 versus 7 m (Wolanski et al. 1988, Häder et al. 1996, Leichter and Miller 1999). Clearly, in light of the worldwide transition from coral- to algal-dominated reefs, a better understanding of the mesoscale factors that impact algal productivity is necessary. This can only be accomplished with robust sampling over both temporal and spatial scales that are relevant to the population, community, or ecosystem being studied.

Light and temperature. Daily PFD varied within months, between months, and over depth on Conch Reef. Individuals at 7-m depths received 2.7-fold to 3.4-fold more PAR than at 21 m. With a 2-h decreased day length and alterations in the incident angle of the sun, daily PFD decreased from August to November. Within the month of August for 1999 to 2001, daily PFD varied by ± 10 mol photons$\cdot m^{-2} \cdot d^{-1}$ at 7 m. Such variations can substantially impact productivity. Based on *in situ* photosynthetic performance, *H. tuna* in August 2001 at 7 m would have spent 26% of each photoperiod experiencing photoinhibitory PFD versus 15% in August 1999. The impact of photoinhibitory irradiances lessens with increased depth. At 21 m photoinhibitory PFD was only achieved for 0.5 h out of 10 days of sampling in August 1999 and for 0 h during 6 days of sampling in August 2001. Although Häder et al. (1996) demonstrated that deeper growing individuals are more susceptible to reversible photoinhibition when transferred to near-shore tide pools, *H. tuna* from 21 m on Conch Reef rarely experienced photoinhibitory irradiances *in situ*.

Halimeda productivity was also impacted by cold water upwelling events onto the reef in August 2000. Leichter and Miller (1999) demonstrated that at the 21-m site in summer months, upwelling events returned approximately every 14 days, whereas an average of 136 days transpired between upwelling events at the 7-m site. Upwelling events may have impacts beyond providing limiting nutrients to reef primary producers. Upwelling at 21 m in August 2000 corresponded to increased variance in daily PFD at 21 m. Intrusion of

cold, nutrient-rich, plankton-rich water at 21 and not 7 m exposed the 21-m *Halimeda* community to waters of likely reduced PFD and possibly altered spectral qualities (Leichter et al. 1998).

Species-specific differences. From 3 to 4 August 2000, the *in situ* photosynthetic performance of *H. opuntia* and *H. tuna* from 21 m were compared using chl fluorescence. This physiological "snapshot in time" revealed no differences between these two species. In contrast, long-term examination of photosynthetic performance and pigmentation did reveal significant species-specific differences. *Halimeda opuntia* had a higher overall P_{max} than *H. tuna* when determined on a fw basis. Normalization of P_{max} to tissue fw likely diminishes the differences in productivity between these two species. *Halimeda opuntia* has a lower fw-to-dry weight (dw) ratio (2.0) than *H. tuna* (3.1) and has a high percentage of $CaCO_3$ per unit dw (Stark et al. 1969). These results are similar to observations by Stark et al. (1969) for light-induced calcification rates for *H. opuntia* versus *H. discoidea* (similar in overall form to *H. tuna*) and to observations by Payri (1988) where Tahitian populations of *H. opuntia* grew the fastest out of three *Halimeda* spp.

Photosynthetic accessory pigments differed in their concentrations in *H. opuntia* versus *H. tuna*. Chl *a* and *b* and carotenoid concentrations were all higher in *H. tuna* than *H. opuntia*. Differences in chl concentrations may be an artifact of the different fw:dw ratios in these species. In support of this, the ratio of chl *b*:*a* was not different between species in this study. On the other hand, the ratio of carotenoid:chl *a* was elevated in *H. tuna* relative to *H. opuntia* on Conch Reef. Carotenoids found in *Halimeda* predominantly consist of siphonein and siphonoxanthin, which act as light-harvesting instead of photoprotective pigments. Elevated carotenoid concentrations in *H. tuna* did not translate into differences in light-harvesting performance between species because α, I_k, and I_c were similar in *H. tuna* and *H. opuntia*.

Differences over depth. Several studies have provided insight into how depth impacts the physiology of *Halimeda* spp. Häder et al. (1996) demonstrated that both surface and deep populations of *H. tuna* are susceptible to excess PFD, resulting in reversible photoinhibition. *Halimeda tuna* from depth were more strongly photoinhibited than individuals from shallow populations when exposed to surface PFDs. Solazzi and Tolomio (1976) found that chl concentrations increased with increased depth in *H. tuna*. In contrast, Mariani-Colombo et al. (1976) found no relationship between depth and pigment levels over a 15.5-m depth gradient.

Other studies examined how anatomy and morphology are impacted with increased depth. Electron microscope examination of plastids of *H. tuna* from 0.5 and 6 m showed that shallow individuals had a greater number of senescent plastids, an indicator of damage to the photosynthetic apparatus (Mariani-Colombo and Orsenigo 1967). Plastids from 16-m populations were larger and wider on the convex face of *H. tuna* segments, indicative of the capacity for photoacclimation (Mariani-Colombo et al. 1976). Several morphological changes that have the potential to impact light harvesting have been documented to correlate with increased depth in *H. tuna* (Mariani-Colombo and Orsenigo 1967, Mariani-Colombo et al. 1976). Surface area, number of branches, and number of segments all increased with depth, whereas segment and cell wall thicknesses decreased. All these studies provide insight into the effects of depth but do so over very limited temporal scales (i.e. samples from only 1 day or month).

Within discrete sampling intervals (months), the 14-m depth gradient on Conch Reef apparently had significant impacts on the photosynthetic performance of *Halimeda*. In August 2000, *in situ* measures of chl fluorescence revealed that *H. tuna* exhibited a sun to shade acclimation with increased depth. In September 1998 this trend was reversed. *Halimeda tuna* from 21 m had elevated P_{max} when compared with 7 m, and when 7-m individuals were transplanted to 21 m, P_{max} increased significantly. Thus, it is clear that photosynthetic performance of *Halimeda* was more strongly impacted by environmental changes over time than with depth on Conch Reef.

In contrast to the lack of impact of depth on photosynthetic performance, photosynthetic accessory pigment levels were significantly impacted by increased depth. Chl *a* and carotenoid concentrations were significantly higher in *Halimeda* sampled from 7 m when compared with 21-m sites. This finding is in contrast to earlier observations and the expected sun to shade acclimation response with decreased PFD (Solazzi and Tolomio 1976, Boardman 1977). Several factors may contribute to this apparently counter-intuitive trend. As with the apparent differences in pigment levels in *H. tuna* and *H. opuntia*, the fw:dw ratio may change with depth, thereby making this trend an artifact of the normalization. In *H. tuna* no difference in fw:dw ratio was found between depths (Vroom 2001). Photodamage, like that indicated by elevated level of senescent plastids in shallow *H. tuna* (Mariani-Colombo and Orsenigo 1967), may create a pool of nonfunctional pigments. Such pools could lead to elevated levels of extractable pigments but have no impact on photosynthetic performance. This explanation would go far in clarifying the lack of agreement in trends of changes in pigment levels with depth for *H. tuna* if samples recently exposed to photodamaging PFDs were used in one but not another study (Mariani-Colombo and Orsenigo 1967, Solazzi and Tolomio 1976).

Temporal differences. In situ growth rates have been assessed for many populations of *Halimeda* (Drew 1983, Multer 1988, Payri 1988, Ballesteros 1991, Garrigue 1991). Most studies observed that seasonal variability was strongly correlated with changes in water temperature (Wefer 1980, Ballesteros 1991, Garrigue 1991). Conversely, uniform growth rates have been observed over the course of a year in some tropical populations

(Drew 1983). The metabolism and growth of *Halimeda* on Conch Reef was markedly impacted by season and the concomitant shifts in seawater temperature and incident irradiance.

Photosynthetic performance in *H. opuntia* and *H. tuna* was most strongly influenced by season. Both species acclimated to changes in environmental conditions but did so with different kinetics as indicated by the significant species and time interaction for P_{max}, chl *a* and *b*, carotenoid concentrations, and carotenoid:chl *a* ratio. Both irradiance and temperature were likely factors controlling the observed acclimation response. Values for P_{max}, P:R ratios, and growth rates (Vroom 2001) peaked in summer months and declined with the onset of winter. This finding is in support of earlier work that found growth of *Halimeda* spp. ceases at 20° C and increases with increased temperature from this low temperature threshold (Wefer 1980). It is noteworthy that surface seawater temperatures monitored at Molasses Reef C-Man station indicated that the thermal regime at this nearby reef (approximately 5 km) is suitable for continued *Halimeda* growth year round (*www.ndbc.noaa.gov*). Mean low seawater temperature from 1997 to 2001 was 22.4° C, and water temperature dropped below 20° C for a total of 5 h over the course of this 5-year interval.

Increases in α and chl *a* and *b* concentrations of *H. tuna* and *H. opuntia* accompanied decreases in P_{max}, P:R ratio, and growth rates from June to September. Decreased PFD with the progression from summer to fall likely controls this acclimation response, given that mean seawater temperature varied minimally from 28 to 30.5° C from 1997 to 2001 (*www.ndbc.noaa.gov*) during these time periods. Alpha (α) has been shown to vary with growth temperature but not over such a narrow range of temperatures (Davison 1991). Increased pigment levels such as chl *a* and *b* within antennae complexes often lead to concomitant shifts in α (Beach and Smith 1996).

Carotenoid concentrations exhibited the highest level of variability out of the three pigment pools in *Halimeda* spp. A 4-fold shift in carotenoid concentrations was observed over the course of this study. A similar 3-fold shift was observed in the carotenoid:chl *a* ratio (range, 0.3–0.9), whereas the chl *b*:*a* ratio was uniform (range, 0.7–1.0). The variable nature of siphonein and siphonaxanthin concentrations were likely due to short-term photoacclimative changes that dynamically adjust this specific pigment pool to optimize light harvesting. In contrast, chl *a* and *b* levels are likely adjusted to longer term shifts in PFD. This type of photoacclimation strategy may minimize the demand for rapid synthesis of nitrogen-rich chl relative to nitrogen-poor carotenoids.

In conclusion, *Halimeda* photosynthetic performance and pigmentation are altered in response to environmental changes over time and to much lesser degree spatial gradients on Conch Reef in the Florida Keys. Productivity of this calcified macroalga varied between species, with *H. opuntia* exceeding that of *H. tuna* over the 5 years of this study. Growth of *H. tuna*, although highly variable over depth, is tightly coupled to shifts in net P_{max}. Dynamic shifts in growth and photosynthetic performance were impacted by seasonal and daily changes in PFD and seawater temperature. At 7 m daily integrated PFD from the month of August in 1999 through 2001 encompassed the same range (± 10 mol quanta $m^{-2} \cdot d^{-1}$) as the predicted decrease from September to November. The range of temperatures experienced during an upwelling event can encompass the range of mean seawater temperatures over the course of an entire year (Leichter and Miller 1999). The potential for photoinhibition of shallow populations during times of elevated PDF and an increased frequency of inundation with cold nutrient-rich water over deeper populations makes macroalgal productivity in this reef system complex and necessitates research over long temporal and broad spatial scales. Otherwise, conclusions regarding macroalgal productivity based on physiological performance from limited temporal or spatial sampling schemes should be treated with caution.

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