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Short communication

The photosynthetic light response of *Halophila stipulacea* growing along a depth gradient in the Gulf of Aqaba, the Red Sea

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Abstract

Photosynthetic responses to irradiance were measured on the seagrass *Halophila stipulacea* growing along an extensive depth gradient (7–30 m) in the Gulf of Aqaba on three occasions between January and August 2000. Plant samples were collected for morphological and anatomical (chloroplast clumping) characterizations of the leaves and analysis of carbon and nitrogen content. The highest electron transport rates, calculated firstly from the quantum yield and irradiance and then after accounting for the proportion of incident irradiance absorbed, were found in plants at the upper depth limit of 7 m. At this depth, electron transport rates were highest in summer ($35 \mu\text{mol electrons m}^{-2} \text{s}^{-1}$) compared to spring and winter ($20 \mu\text{mol electrons m}^{-2} \text{s}^{-1}$). Relative rates of electron transport at 30 m were 60% lower than those at 7 m in winter and spring and 80% lower in summer. The irradiance at the onset of light saturation was also highest in the shallow growing plants, indicative of successful adaptations to high irradiance. Chloroplast clumping in leaves in situ in shallow water reduced the amount of light absorbed to 55% compared to 85% at 30 m. Despite adequate light for photosynthesis, there was evidence for lower biomass at sampling depths of 7 and 17 m compared to 24 and 30 m. The potential for shallow plants to photosynthesise at high irradiances correlated with their ability to clump chloroplasts, suggesting that factors other than high irradiances are likely to limit growth at shallow depths. Low tissue nitrogen content ($<1.8\%$) indicates that nutrient availability also needs to be considered when determining constraints on growth of *H. stipulacea* in shallow water in the study region.

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1. Introduction

The distribution and extent of seagrasses at a given location is determined by one or a combination of factors, which include exposure, sediment stability (Ward et al., 1984), nutrient availability (Short, 1987; Hemminga et al., 1999) and water clarity (Duarte, 1991). The availability of light sets the maximum depth limit of seagrasses at between 11 and 20% on average, of surface irradiance (Duarte, 1991). In the Red Sea, where water clarity is high, the seagrass, *Halophila stipulacea* has been described as generally having a wide ecological range, growing from the intertidal to depths of greater than 50–70 m (Lipkin, 1979; Hulings, 1979; Beer and Waisel, 1982). In the relatively sheltered Gulf of Aqaba, *H. stipulacea* has been proven to grow on the range of sediment types found in the area, from fine sand/silt (125–500 μm) through to coral rubble and sand (larger than 1 mm; Angel et al., 1995). Hulings (1979) reported high densities of *H. stipulacea* in lagoons at depths of 1–2 m on the Jordanian coast of the Gulf, suggesting that *H. stipulacea* can not only utilize the low irradiances at 50 m, but can also flourish at the high irradiances that prevail in the lagoons. Earlier photosynthetic work also supports the ability of *H. stipulacea* to photosynthesise effectively under high-irradiance conditions. Using oxygen exchange techniques, Drew (1979) showed that *H. stipulacea* collected from a depth of 2 m exhibited higher rates of photosynthesis than plants collected from 18 m. Under certain conditions, however, at an irradiance of greater than 900 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, the shallower plants showed some evidence of photoinhibition. Clumping of the chloroplasts within the leaf appeared to alleviate this photoinhibition with no change to photosynthetic rates (Drew, 1979).

H. stipulacea is an integral component of the coral reef ecosystem in the Gulf of Aqaba (Edwards and Head, 1987), and at the time of this study (during 2000), was the most commonly occurring seagrass taxon on the western shore between Eilat and Taba. It was found in monospecific beds amongst coral heads extending to depths of greater than 40 m, beyond SCUBA diving limits. Despite the many references to *H. stipulacea* growth extending into shallow waters (e.g. Edwards and Head, 1987), we could rarely find it in water depths shallower than 7 m in the northwestern part of the Gulf in 2000. The only places where it did occur (sparsely) in shallower depths were at very localised sheltered areas (e.g. behind breakwaters). Anecdotal evidence suggests that although *H. stipulacea* has been recorded at shallower depths in this region, the current situation has persisted for a number of years. Lipkin (1979) described beds of *H. stipulacea* as undergoing periodic “denudations” in the shallow waters of the Sinai region south of Eilat, and the absence of *H. stipulacea* in shallow waters may simply be a matter of insufficient time having lapsed for plants to recover following such events. Nevertheless, the potential constraints on re-establishment are not well understood.

The aim of this study was to investigate possible barriers to the rate of significant community extension into shallow waters. In situ fluorescence methods were used to investigate whether varying photosynthetic responses to light were evident which, when considered in context of plant nutrient content and morphology, could indicate limitations for photosynthesis and growth.

2. Methods

2.1. Study site

The waters of the Gulf of Aqaba (29°50' N, 34°92' E) are very clear. Between 1995 and 2000, K_d (photosynthetically active radiation, PAR) over the euphotic zone ranged between a summer minimum of 0.04 m^{-1} and a spring maximum of 0.07 m^{-1} (Stambler, unpublished data). Slightly higher values of $\approx 0.10\text{ m}^{-1}$ have previously been reported over the depth range of *H. stipulacea* (Hulings, 1979; Beer and Waisel, 1982). Water temperature ranges from 21 °C in winter to 26 °C in summer and the tidal range in the region is small (usually <1.0 m). The study site was located on the western shore of the Gulf, just south of the Inter University Institute in Eilat where an extensive seagrass bed (>1 km long) occurs to depths of greater than 40 m. The upper limit of this bed varied from 20 m up to a clear boundary at 7 m in certain places. Only occasional plants of *H. uninervis* were seen at the upper limit of the *H. stipulacea* bed and none were seen at the study site. We chose a site 1 km south of the Institute where *H. stipulacea* grew with a depth range such that we could sample between 7 and 30 m using SCUBA.

2.2. In situ measurements

The effective quantum yield of electron flow through photosystem II (Y) versus irradiance characteristics of plants at 7, 17, 24 and 30 m were measured on three occasions; winter (January), spring (March) and summer (August), 2000. All measurements were made on cloudless days between 10 a.m. and 2 p.m. Rapid light curves (RLCs), using a Diving-pulse amplitude modulated (PAM) fluorometer (Walz, Germany), were generated in situ for a minimum of three replicate leaves at each depth. At greater depths, the number of replicate curves we could conduct was limited by SCUBA diving time limits. The leaves used were from the 2nd or 3rd leaf pair behind the growing tip, chosen to be mature but free of conspicuous epiphytes, and without evidence of senescence. The leaves were held in a leaf clip, which ensured a standard distance of the Diving-PAM fibre from each leaf. Y was first measured after 10 s in darkness and then at eight consecutively increasing irradiances supplied for 10 s each by the actinic light source of the PAM fluorometer. These irradiances were chosen, from initial measurements, to cover a range of non-saturating and saturating irradiances. Before each dive, the light sensor of the PAM was calibrated against a Li-Cor (USA) Li-189 air photosynthetically active radiation sensor. The light sensor of the PAM fluorometer was then positioned in a leaf clip in the same orientation as a leaf, and the incident irradiances (PAR_i) given by the instrument's internal light source during an RLC were recorded for calculations of a relative electron transport rate (RETR) according to:

$$\text{RETR} = Y \text{ PAR}_i \quad (1)$$

This equation yields only relative rates of electron transport since, it does not account for the fraction of irradiance absorbed by the leaf (which also differs between plants of various depths), nor does it allow for possible variations in light absorption between the two photosystems. The ambient incident PAR was also measured by the Diving-PAMs quantum sensor at each measurement depth.

The values of RETR versus PAR_i for this study were fitted to the hyperbolic tangent relationship of Jassby and Platt (1976), using a curve fitting procedure in Statistica (Statsoft, Tulsa, OK, USA); thus $RETR = RETR_{max} \tanh(PAR_i(\alpha/RETR_{max}))$. The equation yielded values for the RETR at light saturation ($RETR_{max}$) and the initial slope of the light limited relationship (α), enabling the calculation of the irradiance at the onset of saturation (E_k , in $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, calculated as $RETR_{max}/\alpha$).

2.3. Chloroplast clumping

The proportion of incident irradiance absorbed by seagrass leaves has been assessed in other studies by measuring the irradiance incident on the PAM light sensor with and without a seagrass leaf (Beer et al., 1998; Beer and Bjork, 2000). We tested the suitability of this technique for estimating the extent of chloroplast clumping (Drew, 1979) by performing the same measurement on the same leaf over time. Leaves from 9, 24 and 30 m were collected, kept in dim light and returned to shore. Each leaf was placed over the light sensor of the Diving-PAM and ambient PAR recorded with and without the leaf. The leaves were then exposed for 3 h to sunlight at an ambient irradiance of 800–1400 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. At five intervals during this time period, and again after being left in the dark overnight, the proportion of incident light transmitted by the leaf was measured.

The same technique was used in situ to measure the fraction of PAR absorbed by a leaf under ambient irradiance (AF) for at least 10 leaves at depths of 7, 9, 15, 24 and 30 m on two consecutive clear sky days at the same time of day. Using the estimate of AF from the depth which corresponded most closely to in situ Diving-PAM measurements, maximum rates of RETR were re-calculated as absolute values of ETR ($\text{mmol electrons m}^{-2} \text{s}^{-1}$) for comparison with previously published values. This was accomplished by expanding Eq. (1) to:

$$ETR = (Y PAR_i AF) \times 0.5 \quad (2)$$

where multiplying by 0.5 assumes that half the photons absorbed were absorbed by photosystem II.

2.4. Morphology and nutrients

In spring only, five rhizomes were collected from each of the depths at which fluorescence was measured (7, 17, 24 and 30 m) to characterize plant morphology. At each depth, percent cover was estimated within five, 30 cm \times 30 cm quadrants. Rhizomes were collected from the growing tip back to where there were no more leaves. Data from up to the first five internodes only on each rhizome were compared between depths (to reduce variability due to dead and decaying leaves on older parts of the rhizome). The length of each leaf and internode was measured and mean values calculated for each depth. Each rhizome was then separated into leaves and the remaining below ground parts. These were dried and weighed in order to calculate above to below ground ratios of biomass. Dried samples from each depth were combined and ground and three replicate subsamples were taken for analysis of carbon and nitrogen content using a Perkin-Elmer C:H:N analyser. Comparisons between

growth parameters were made using ANOVA with a post-hoc Tukeys test in Statistica (Stasoft, Tulsa, OK).

3. Results

3.1. *In situ* electron transport

Curves of RETR generated using the RLC function of the PAM exhibited saturating relationships with increasing irradiance over the range of irradiances used. On all occasions, RETR_{max} was lowest for the plants growing in the lower light environment at 24 and 30 m (Fig. 1A) and highest for the shallowest growing plants at 7 m. The greatest difference between sampling times was seen at 7 m where RETR varied from approximately 80 in winter and spring to 125 in summer equivalent to ETR values of 20 and 35 $\mu\text{mol electrons m}^{-2} \text{s}^{-1}$. At 24 and 30 m RETR was saturated between 30 and 80 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ on all occasions (Fig. 1B). E_k was progressively higher with decreasing depth and at 7 and 17 m increased from winter to summer.

3.2. Chloroplast movement and morphology

Percent cover was on average 50% at all sampling depths; however, leaf morphology differed markedly. The shortest leaves were found in shallow water (Table 1), and leaves at both 7 and 17 m were both significantly shorter than leaves at 30 m ($P < 0.05$). Internode length was variable over a small range from 7 to 11 mm and above to below ground ratio for biomass was not significantly different between depths.

After 36 h in the dim light of the laboratory (approximately 40 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) leaves from 9, 24 and 30 m absorbed on average 70% of incident irradiance (Fig. 2A). When exposed to high irradiance, the leaves of all plants showed a decrease in the amount of absorbed light with time to between 58 and 62% after 3 h exposure. The response to high light was evident within 10 min of exposure (Fig. 2A) and the pale appearance of the leaves attested to chloroplast clumping. Leaves from all depths showed a return to an absorbance of close to 70% following overnight dark treatment (last group of data in Fig. 2A).

In situ measurements showed that plants at 30 m were absorbing a greater proportion of incident irradiance (approximately 85%) than those at shallower depths (approximately 50%) (Fig. 2B).

Table 1

Leaf (petiole not included) and internode length, and above to below ground biomass ratio for *Halophila stipulacea* from four depths in spring 2000

Depth (m)	Leaf length (mm)	<i>n</i>	Internode length (mm)	<i>n</i>	Above:below ground biomass ratio (<i>n</i> = 5)
7	29.9 (0.9)	48	10.6 (0.9)	23	2.50 (0.3)
17	34.9 (2.7)	36	6.8 (0.9)	20	1.98 (0.3)
24	40.7 (1.7)	48	9.2 (1.0)	24	1.56 (0.3)
30	44.3 (1.5)	50	11.2 (1.2)	25	2.15 (0.5)

Data are mean values with standard errors shown in brackets.

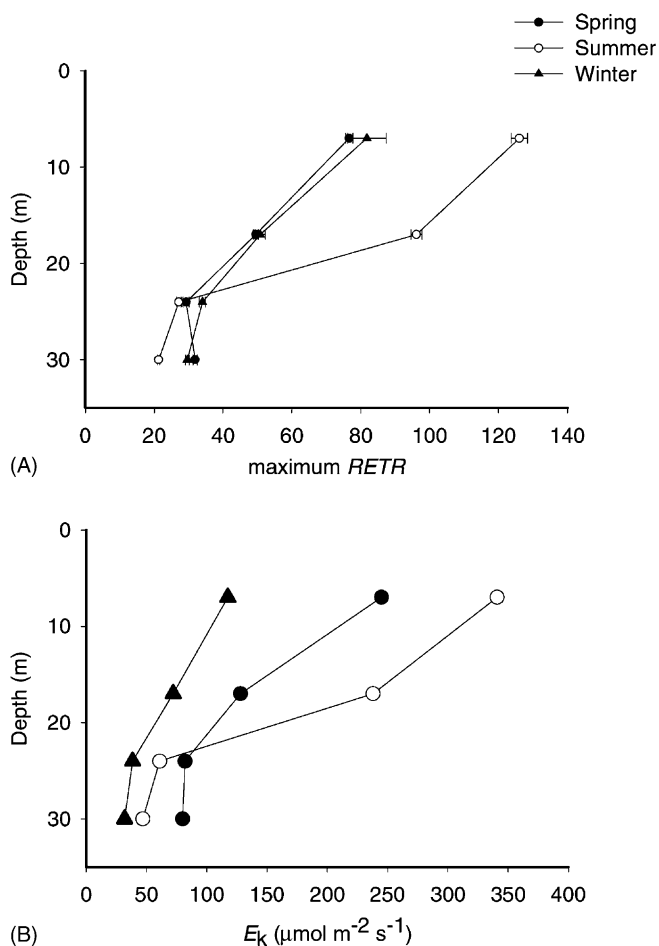


Fig. 1. (A) Maximum relative electron transport rate ($RETR_{\max}$), and (B) irradiance at onset of light saturation (E_k) of *Halophila stipulacea* over the depth profile. Data were collected in spring (closed circles), summer (open circles) and winter (closed triangles), 2000. Bars are the standard error of the $RETR_{\max}$ parameter calculated using an iterative fitting procedure in Statistica.

3.3. Carbon and nitrogen content

Carbon content of both leaves and rhizomes of plants from all four collection depths ranged from 20 to 27% DW (Fig. 3A). Carbon content (C) was less variable than nitrogen (N) which in leaves ranged from 1.34 (17 m) to 1.67% (7 m) and in roots/rhizomes ranged from 0.71 (7 m) to 1.62% (24 m) (Fig. 3B). The C:N ratio in the leaves ranged from 14 to 17 for all depths but was higher in the rhizomes and covered a greater range from 17 to 30. Lower nitrogen concentrations in the roots and rhizomes relative to the leaves, of plants at depths shallower than 24 m, were the determining factor for a higher C:N ratio.

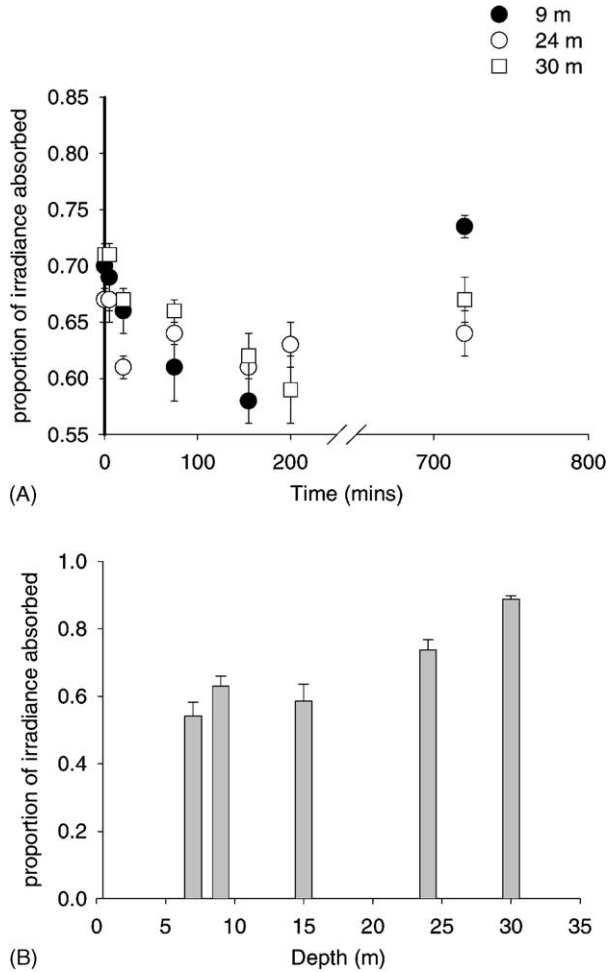


Fig. 2. (A) Percent of incident irradiance absorbed by detached leaves of *Halophila stipulacea* from three depths, exposed to $>800 \mu\text{mol m}^{-2} \text{s}^{-1}$ over a 3 h period and after overnight in darkness (last group of data; bars are standard errors, $n = 4$), and (B) average proportion of ambient incident irradiance absorbed by 10 leaves in situ at five depths; bars are one standard error.

4. Discussion

The in situ measurements made in this study illustrate clear patterns of acclimation to a variable light environment with depth and season in *H. stipulacea* where the highest E_k and maximum RETR were measured in the high irradiance environment of shallow water in the summer.

The parameters estimated from the RLCs provide only a snapshot of the status of light adaptation in ambient conditions at the time of measurement; nonetheless there was a

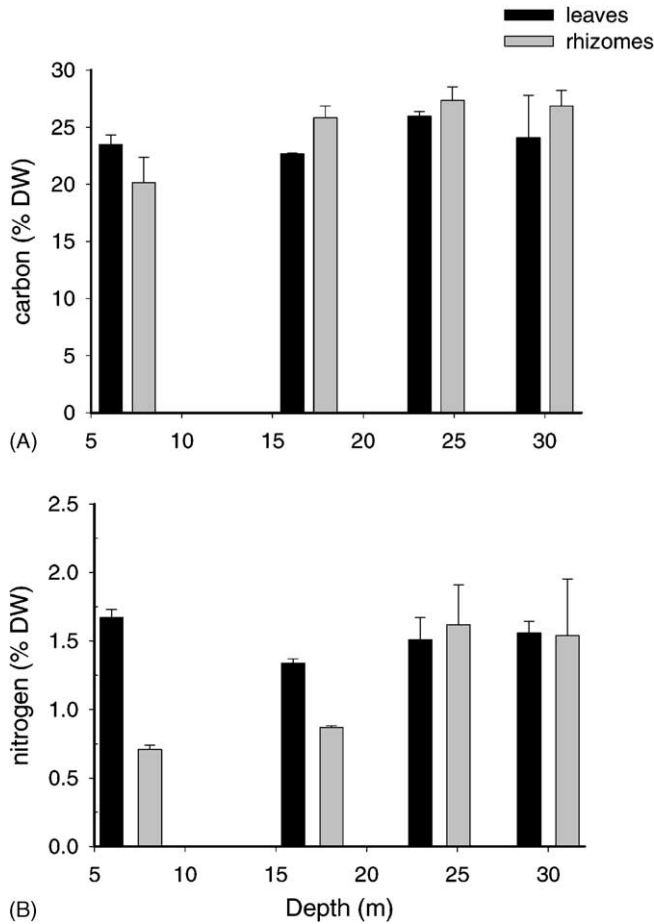


Fig. 3. Percentage of: (A) carbon, and (B) nitrogen by dry weight (DW) in leaves and rhizomes of *Halophila stipulacea* from four depths (7, 17, 24 and 30 m); bars are one standard error.

repeated pattern over the depth profile. At the time that these measurements were made, all plants at 7 and 17 m were exposed to irradiances above E_k ($>240 \mu\text{mol photons m}^{-2} \text{s}^{-1}$). The maximum ETR of $20 \mu\text{mol electrons m}^{-2} \text{s}^{-1}$ at these depths in winter and spring equates to that measured at an irradiance of $206 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ by Beer et al. (1998) but the summer values of $30\text{--}35 \mu\text{mol electrons m}^{-2} \text{s}^{-1}$ are higher. On all occasions when RLCs were measured irradiance was approximately $250 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ at 17 m and approximately $450 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ at 7 m. Thus, in situ irradiance at depths of 7 and 17 m does not explain the higher values of maximum ETR in summer compared to winter and spring. There is insufficient temporal data to be confident of seasonal differences in photosynthetic acclimation although longer daylength and maximum midday irradiance in summer are likely to result in a greater daily irradiance dose. This may however, be offset by a tendency toward lower water clarity during summer (Stambler, unpublished data).

Seasonal and depth dependent patterns related to light may be better reflected in E_k . Previous studies on *H. stipulacea* from the region, using laboratory-based oxygen exchange techniques, reported E_k values ranging from $300 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ for plants from 10 m (Beer and Waisel, 1982) to approximately $100 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ for plants from 2 to 18 m (Drew, 1979). Both values fall within the range measured at depths of 7 and 17 m in this study, but the deeper plants have now been shown to be light saturated at much lower intensities of between 30 and $80 \mu\text{mol m}^{-2} \text{s}^{-1}$.

Higher summer temperatures might be expected to result in higher respiration rates, but in the absence of measurements of whole plant respiration, we are not able to attempt a translation of electron transport rate to growth. Percent cover and above to below ground biomass were similar between all sampling depths. However, the smaller leaves at 7 m and, to a lesser degree, at 17 m, suggests that areal biomass (not measured) tends to increase with depth and that growth limiting factors in shallow water should be considered. In the predominantly carbonate sediments of nutrient-poor, tropical waters, nutrient limitations to growth can occur (Hemminga et al., 1999; Short, 1987), and enhancement of growth of *H. stipulacea* by phosphate enrichment of sediments in laboratory experiments has been shown (Angel et al., 1995). Additional nutrient constraints on this species in the Gulf of Aqaba may be imposed in situ by a lack of nitrogen, as suggested by the depleted nitrogen relative to carbon content in these plants. This is particularly so for the rhizomes where nitrogen contents were less than 1.0% at 7 and 17 m. The nitrogen content of both leaves and rhizomes fell below the median value of 1.8% for 23 seagrass species, which was suggested by Duarte (1992) as indicating strong nutrient limitation. Carbon contents at depths of 17 m and greater were similar to previously recorded values for leaves of *Halophila* spp. (Wahbeh, 1988; Duarte, 1992), tending to be at the lower end of that dataset. Although, there was a tendency toward lower carbon content at the two shallower depths, it was the greater relative depletion of nitrogen which determined the higher C:N ratios in the roots of the shallow plants compared to deeper plants.

Despite the relatively sheltered nature of the Gulf of Aqaba, local observations suggest that extensive areas of shallow seagrass beds in the Gulf can disappear following stochastic events such as covering by filamentous algae in spring, occasional storms and very low tides, as well as more chronic effects such as sediment transport along the shore as a result of construction and other physical disturbances (Wahbeh, 1988). The occurrence of *H. stipulacea* in a small marina near the study site, faster growth found by Angel et al. (1995) in sediments with a higher organic content and observations made by Wahbeh (1984) that *H. stipulacea* tended to be found in more sheltered areas than *H. uninervis*, all suggest that shelter and nutrient availability are important determinants for retention of extensive beds of *H. stipulacea* over time.

The in situ observations made during this study complement laboratory measurements made by Angel et al. (1995) to suggest that a combination of factors currently constrain the rate of re-establishment of *H. stipulacea* in depths shallower than 7 m. There do not appear to be high-light constraints on photosynthesis, suggesting that there are other limitations to the growth potential which might include nutrient supply and chronic and acute physical disturbances. Although nitrogen deficiency appears to be one limiting factor, multi-factor experiments are necessary to elucidate these limiting factors further.

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