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Studies of Marine Macroalgae: Saline Desert Water Cultivation and Effects of Environmental Stress on Proximate Composition

A Final Subcontract Report

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FOREWORD

This is the annual technical report submitted by the Harbor Branch project entitled, "Studies of Marine Macroalgae: Saline Desert Water Cultivation and Effects of Environmental Stress on Proximate Composition." This work was supported through SERI subcontract XK-4-04077-1 as part of the Aquatic Species Program. The SERI Aquatic Species Program is funded through the Biomass Energy Technology Division of the U.S. Department of Energy.

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Aquatic Species Program

Approved for

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Summary

Studies were conducted under two tasks during this contract year. In task 1, photosynthesis and growth of the macroalgae *Gracilaria tikvahiae* and *Ulva lactuca* were evaluated in artificial media similar in composition to that of saline New Mexico underground aquifers. In task 2, the effects of five environmental parameters (light, temperature, salinity, nitrogen and phosphorus) on the proximate composition of *Gracilaria*, *Ulva* and the attached macroalga *Caulerpa prolifera* were examined. A summary of our results follows.

1) Both type IM and type IIM saline desert media supported rapid short-term photosynthesis by several macroalgae, including *Enteromorpha* sp., *Agardhiella* sp., *Ulva* sp., and *Acanthophora* sp. The type I and type II media differ primarily in their Ca and HCO_3 contents, although both are more alkaline than seawater.

2) Although the type I and type II media supported short-term photosynthesis by *Gracilaria*, growth of this alga during week-long experiments in both media was poor. In contrast, *Ulva* grew well in the IM medium during one- to two-week experiments, with the resulting thalli similar in proximate composition to those cultured in ocean water.

3) Rapid *Ulva* growth (i.e., equivalent to that in seawater) could not be maintained in the IM medium for longer than two weeks. A precipitate formed in the IM medium during these growth studies, making it difficult to determine (based on original ionic composition) whether the poor growth was a result of an ion deficiency or a general ion imbalance.

4) Under "standard" culture conditions, the protein contents (as % of volatile solids) of *Gracilaria*, *Ulva*, and *Caulerpa* were roughly equal, averaging 13.2, 10.2, and 14.4%, respectively. The protein content of the three macroalgae increased with increasing nitrogen availability in the growth medium. The protein content of *Ulva* also increased under low- and high-temperature cultivation.

5) Average soluble carbohydrate concentrations (as % of VS) decreased in the following order among the three macroalgae: *Gracilaria*, 39.4; *Ulva*, 32.4; and *Caulerpa*, 23.9%. Carbohydrate levels in *Gracilaria* were increased by cultivation under conditions of high salinity, low temperature, and low nitrogen and phosphorus availability. Low-nitrogen and low-temperature cultivation also resulted in increased tissue carbohydrate concentrations in *Ulva* and *Caulerpa*.

6) The average lipid content (as % of VS) of *Caulerpa* (10.4%) was higher than that of *Gracilaria* (5.9%) or *Ulva* (3.8%). Thallus lipid concentrations in *Caulerpa* were sharply affected by culture tank light intensity, with levels of this constituent increasing threefold from high- to low-irradiance conditions. Lipid levels in the three macroalgae were not increased by cultivation under low-nitrogen conditions.

7) The production of carbohydrate, protein, and lipid by an alga depends on both dry matter production and the percent composition of these constituents

in the thallus. For *Gracilaria* and *Ulva*, dry matter production was found to be the dominant factor influencing production of these storage products, with relative composition of secondary importance. Our data suggest that it may be possible to maximize production of useful proximate constituents by cultivating the algae under optimum conditions for growth, and then holding the resulting biomass under the environmental conditions which favor tissue accumulation of the desired storage products.

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Introduction

Studies conducted in recent years have shown that saline waters of southwest (U.S.) desert lakes and underground aquifers may be utilized as an alternative medium to seawater for land-based production of microalgae (Thomas et al., 1984). Such desert waters may also be a promising, underutilized resource for macroalgae production, as they occur in regions of high solar radiation inputs where land costs are low. Currently, the purchase of coastal land represents up to 30% of the total fixed cost of a land-based macroalgae biomass farm, depending on factors such as location and pond construction methods (Ryther et al., 1984).

The desert waters under consideration for algae production exhibit a wide salinity range and a diverse ionic composition (Thomas et al., 1984). One goal of this study is to determine whether these saline desert waters provide a suitable medium for cultivation of marine macroalgae, in particular *Gracilaria tikvahiae* and *Ulva lactuca*, two species for which high, sustained yields have been attained in seawater culture.

Macroalgae research conducted during the past seven years at the Harbor Branch Foundation has emphasized the development of culture techniques which both maximize organic matter yields and minimize the energy costs associated with cultivation. For purposes of energy production, however, it is the concentration of storage products such as carbohydrates (for methane, alcohol) and lipids (for hydrocarbons), rather than total organic matter content, which determines the value of the algal biomass produced. For example, Habig et al.(1984) found that methane yields (per g volatile solids) from the seaweed *Ulva* sp. increased 1.5 times in response to a doubling in thallus carbohydrate content. Prior studies with micro- and macroalgae have shown that the synthesis of certain storage products can be enhanced by subjecting algae to environmental stresses. Nitrogen deprivation is one such stress which commonly induces carbohydrate accumulation in macroalgae (Craigie, 1974). However, the effect of other environmental conditions on the synthesis of macroalgal storage products is not well known. The second task addressed in the present contract is to determine how certain environmental stresses (e.g., nitrogen, salinity, and temperature) affect the proximate composition of several marine macroalgae.

Task 1. Photosynthesis, growth and composition of marine macroalgae in saline desert waters.

Methods

Most of the algal photosynthesis and growth studies for this task were conducted on a light table located in a temperature controlled room (22° ± 2° C).This table provided 28 stirrer stations that each contained a recessed alnico magnet which was rotated at 60 rpm by belt driven pulleys. Light was provided by six 96" cool-white fluorescent lamps positioned as 3 banks of two lamps each. The maximum irradiance at the midpoint of each stirrer station was 2.7 X 10^{16} quanta $cm^{-2}sec^{-1}$, or approximately 120 watts m^{-2}.

Measurements of growth and proximate composition of macroalgae in the saline desert waters were also conducted in 13 L aquaria at the same constant temperature room. Each aquarium was provided with gentle aeration, which kept the algae continuously in suspension. Light was provided from two fluorescent lamps at a radiant flux density of approximately 72 watts m^{-2}. Water temperature in each aquarium was maintained at 22° ± 2°C. Detailed methods of the experiments conducted for this task follow.

A. Photosynthesis of macroalgae in saline desert water media

Experiment A1

Net oxygen production of *Gracilaria tikvahiae* and *Ulva lactuca* was measured in media simulating the waters of Black and Harper Lakes, two California desert lakes of widely different ionic composition. Black Lake is characterized by high pH (10.1), moderate salinity (21 ppt), low concentrations of Ca and Mg (relative to seawater), and very high levels of inorganic carbon (Table 1). In contrast, Harper Lake has a pH of 8.6, salinity of 14 ppt, and low levels of Mg, K, and inorganic carbon (Table 1). Analyses of the lake waters were provided by Dr. W. H. Thomas.

For this experiment, *Ulva* and *Gracilaria* thalli (100-300 mg wet weight) were incubated in 300-ml glass bottles containing either ocean water (diluted to 25 ppt with deionized water), Black Lake, or Harper Lake media. Triplicate bottles were established for each treatment. The ocean water had a pH of 8.0, while the pH levels of the Harper and Black Lake media were adjusted with CO_2 to 7.7 and 7.8, respectively. Although nutrients were not added to the media, the algae were well-nourished by a high estuarine water flow and a pulse-feeding of nitrogen immediately prior to their use in the experiment. Photosynthesis incubations were conducted on the light table for four hours, with initial (0-hour), 2-hour, and 4-hour oxygen readings taken with a YSI model 51 B oxygen meter. Seaweed samples were strained from the bottles at the end of the experiment, rinsed in fresh water, dried (70 C for 48 hr), and weighed.

Experiment A2

Studies during the remainder of the contract were conducted with the type I and type II media, solutions which are typical of saline New Mexico aquifer waters. Formulae (Table 2) for these water types were provided by SERI. The monovalent/divalent cation ratios of these media differ, although both are more alkaline than seawater (Table 2).

Table 1. Ionic composition (major cations and anions) of Black Lake, Harper Lake and seawater (35 ppt).

	Seawater[1]	Black Lake[2]	Harper Lake[2]
	------Concentration ($mg\ L^{-1}$)------		
Ca	412	4	435
Mg	1294	4	70
Na	10733	8800	6080
K	399	825	48
CO_3	-	5580	60
HCO_3	142	2526	61
SO_4	2712	3200	2800
Cl	19344	3200	7725
TDS	35000	35000	35000

1 Riley and Chester (1971)
2 Thomas, et al. (1984)

Table 2. Ionic composition (major cations and anions) of seawater (15 ppt) and two saline media typical of New Mexico aquifers.

	Seawater (at 15 ppt)	I M[1]	II M[1]
	------Concentration ($mg\ l^{-1}$)------		
Ca	177	678	89
Mg	555	303	392
Na	4600	3552	4646
K	171	278	194
HCO_3	61	867	2100
SO_4	1162	2973	805
Cl	8290	6349	6775
TDS	15000	15000	15000

[1] Ionic composition provided by Solar Energy Research Institute.

In this experiment, net photosynthesis of four common macroalgae (Ulva lactuca, Entermorpha sp., Acanthophora sp., and Agardhiella sp.) was examined in four media: ocean, artificial ocean, and I and IIM saline desert water media. The artificial seawater was utilized in addition to ocean water as a control since nutrients were not added to any of the media during these short (2-4 hr) incubations. This seawater medium was formulated with deionized water and the following salts, providing an ionic composition equal to that of 15 ppt seawater (Table 2): (in mg L^{-1}); KCl, 327; $CaCl_2 \cdot 2H_2O$, 648; $NaHCO_3$, 84; $MgCl_2 \cdot 6H_2O$, 4658; Na_2SO_4, 1711; and NaCl, 10185. The salinity of all media (including ocean water) was adjusted to ca. 15 ppt. Sections of thalli (discs for Ulva) approximately 100-300 mg in wet weight were stocked into duplicate 300-ml glass bottles of each medium. Initial oxygen levels in the bottles were lowered by bubbling N_2 prior to their placement on the light table. The plants were incubated for 4 hours, with oxygen levels and seaweed dry weights determined as previously described.

A second photosynthesis run was conducted using thalli/discs which were soaked in the four media for 1.5 days prior to the incubation, to determine the effect of "preconditioning" on oxygen production by these algae.

B. Proximate composition and short-term growth of macroalgae in saline desert water media

Experiment B1

Ulva and Gracilaria thalli were cultured in 300-ml glass bottles containing ocean, artificial ocean, and I and IIM saline desert waters. The bottles were placed on the light table, which was adjusted to provide 14/10 hr light /dark photoperiod and intermittant stirring (ca 1 hr/day). The salinity of all media was adjusted to 15 ppt, and a complete nutrient solution (Table 3) was added to each bottle. Specific growth was calculated from the change in wet weight over one week using the expression $u = \log (w_f / w_i) 3.32/t$.

Experiment B2

Gracilaria and Ulva thalli (170-360 mg) were cultured on the light table in 1 L bottles containing ocean, artificial ocean, and IM waters. Salinities of all media were adjusted to 15 ppt, and pH levels to 7.0-8.0. Each medium was also amended with a complete nutrient solution (Table 3). Triplicate bottles were utilized for each treatment. Unlike the preceding experiment, specific growth in this study (doublings day^{-1}) was calculated on a dry weight basis (final dry weight - initial dry weight). Dry matter (70°C for 48 hr) and ash contents (550°C for 4 hr) of the final samples were also measured.

Table 3. Nutrient medium[1] utilized for task I and II experiments.

	Concentration (mg L^{-1})
$NaNO_3$	46.67
Na_2 glycerophosphate	6.67
$FeCl_3$	1.08
Na_2EDTA	5.53
$FeCl_3 \cdot 6H_2O$	0.33
$MnSO_4 \cdot 4H_2O$	1.09
$ZnSO_4 \cdot 7H_2O$	0.15
$CoSO_4 \cdot 7H_2O$	0.03
H_3BO_3	3.80
HCl (3N)	0.81 (ml)
$NaHCO_3$	200.0

[1] Modified from Provasolis' enriched seawater.

Experiment B3

Separate 13 L aquaria were filled with ocean, artificial ocean, and I and IIM saline desert waters. A complete nutrient medium was added to each solution, salinities were adjusted to 15 ppt, and pH levels were normalized to 7.7. Irradiance was provided as previously described. After 7 days, growth and proximate composition of the seaweed in each treatment were determined using methods described in Task 2.

C. Long-term growth of macroalgae in saline desert media

Experiment C1

In order to examine the long-term growth of *Ulva* in the saline IM medium, small discs of algae were placed in 350-ml storage dishes containing ocean, artificial ocean, and IM waters. Light and stirring were provided on a 14:10 hour on:off basis. Plastic screens were placed just above the bottom of each dish to prevent the seaweed from snagging on the magnetic stirrers. Wet weight gains in each treatment were measured weekly, and new discs were re-cut from the thalli. The media were changed and amended with nutrients each week, and the discs restocked. Seaweed wet weights were converted to dry weight values using dry weight:wet weight ratios obtained from the previous experiment.

Experiment C2

This experiment was conducted to determine whether small increments of ocean water (diluted to 15 ppt), when added to the IM medium, would help support *Ulva* growth. *Ulva* discs were placed in 350 ml storage dishes containing the following solutions: ocean water, IM medium, and 1:9, 1:3 and 1:1 mixtures of ocean water and IM water. Growth measurements, media changes, and nutrient additions were conducted weekly.

Results and Discussion

A. Photosynthesis of macroalgae in saline desert waters

Short-term oxygen production by Gracilaria and Ulva in Harper Lake water was equal to or slightly greater than that in ocean water (Fig. 1). In contrast, oxygen production by these two algae in the Black Lake water was poor (Fig. 1). Gracilaria in the latter medium appeared to lose its phycoerythrin pigments during the 4-hour incubation.

It is likely that the low divalent cation levels (probably Ca) were responsible for the poor growth of Gracilaria in the Black Lake water. However, an attempt to stimulate oxygen production by Gracilaria in this medium by the addition of Ca and Mg was unsuccessful. Net photosynthesis was not stimulated by a tenfold increase of Mg in the medium. Additions of Ca to the Black Lake medium resulted in the formation of $CaCO_3$ precipitate. Hardness determinations on the supernatent indicated that none of the added Ca remained in solution. Gracilaria photosynthesis in this "Ca-amended" medium was not significantly higher than that in the standard Black Lake medium.

Following this experiment, studies in this task were conducted with media formulations provided by SERI (Table 2) which are typical of New Mexico saline aquifer waters.

Both the type IM and type IIM media supported rapid photosynthesis by the macroalgae Ulva lactuca, Enteromorpha sp., Acanthophora sp., and Agardhiella sp. (Fig. 2). Net photosynthesis by these algae in the IM medium was generally equal to that in ocean water, while net photosynthesis in the IIM waters was slightly higher, probably due to the high bicarbonate levels (15 times that of full strength seawater) in this water.

Similar trends in net photosynthesis were observed in these media even after the algal thalli were held, or "preconditioned" in the waters for 1.5 days. A slight decrease in oxygen production by Enteromorpha and Ulva in the IIM medium, relative to that in ocean and IM waters, was the only obvious effect of this conditioning period (Fig. 3).

B. Proximate composition and short-term growth of macroalgae in saline desert waters

Although the photosynthesis experiments showed that both of the desert media supported rapid, short-term oxygen evolution by a number of algae, our week-long growth experiments with these solutions indicate that the IIM water is a much poorer growth medium than the IM water. In a comparison of Gracilaria growth in ocean, artificial ocean, IM and IIM waters, the maximum specific growth (on a wet weight basis) occurred in the two ocean water solutions (Fig. 4). Although growth of this alga was not substantially lower in the IM water, the thalli became pale and brittle during the seven day incubation period. In the IIM water, the alga died during the latter part of the seven-day incubation period.

In a companion experiment, Ulva grew at a slightly faster rate in the ocean water than in the artificial ocean water (Fig. 4). However, the most

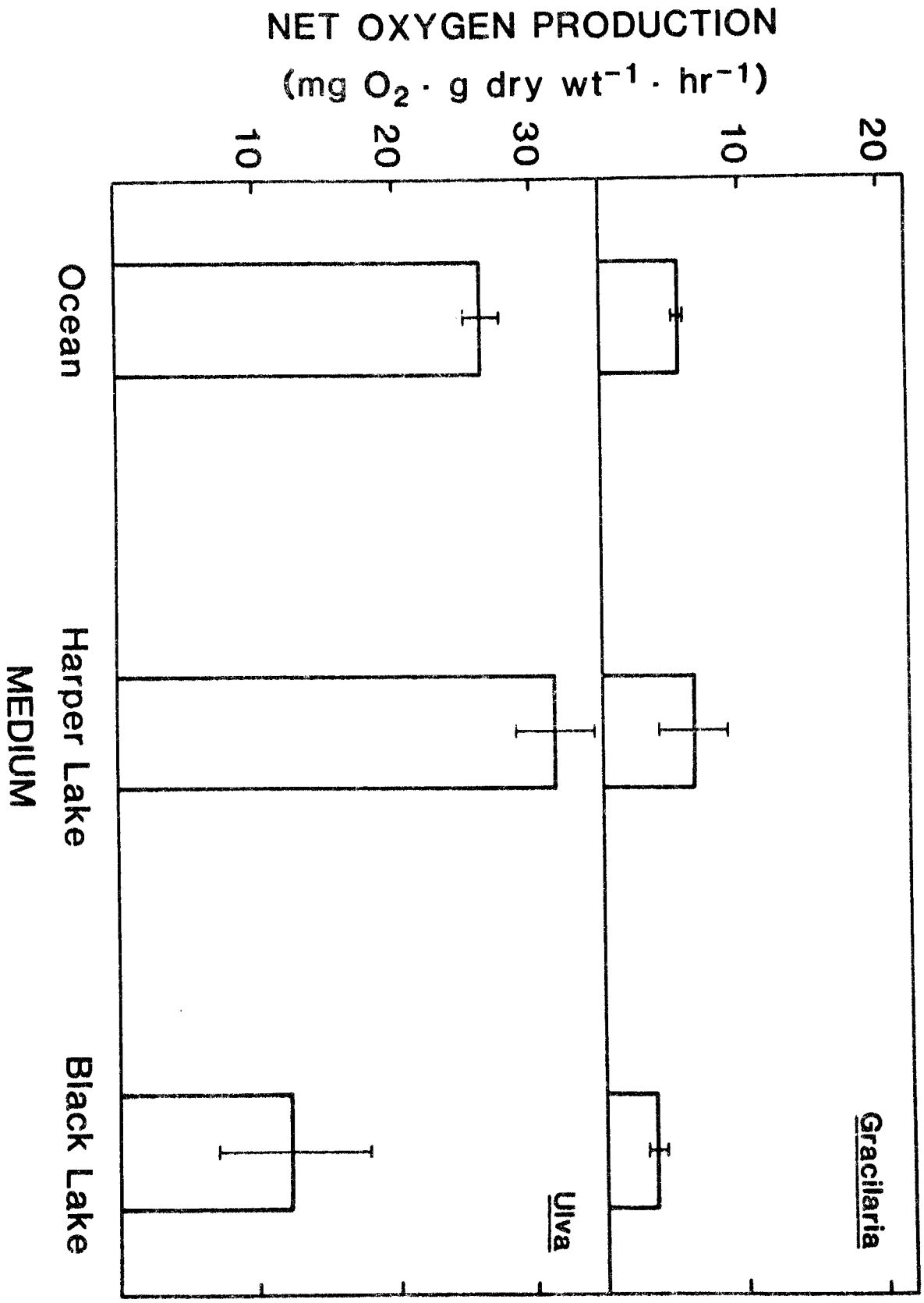

Figure 1. Net photosynthesis of Gracilaria and Ulva in ocean water, and in two artificial saline desert waters. Error bars denote ±1 s.d.

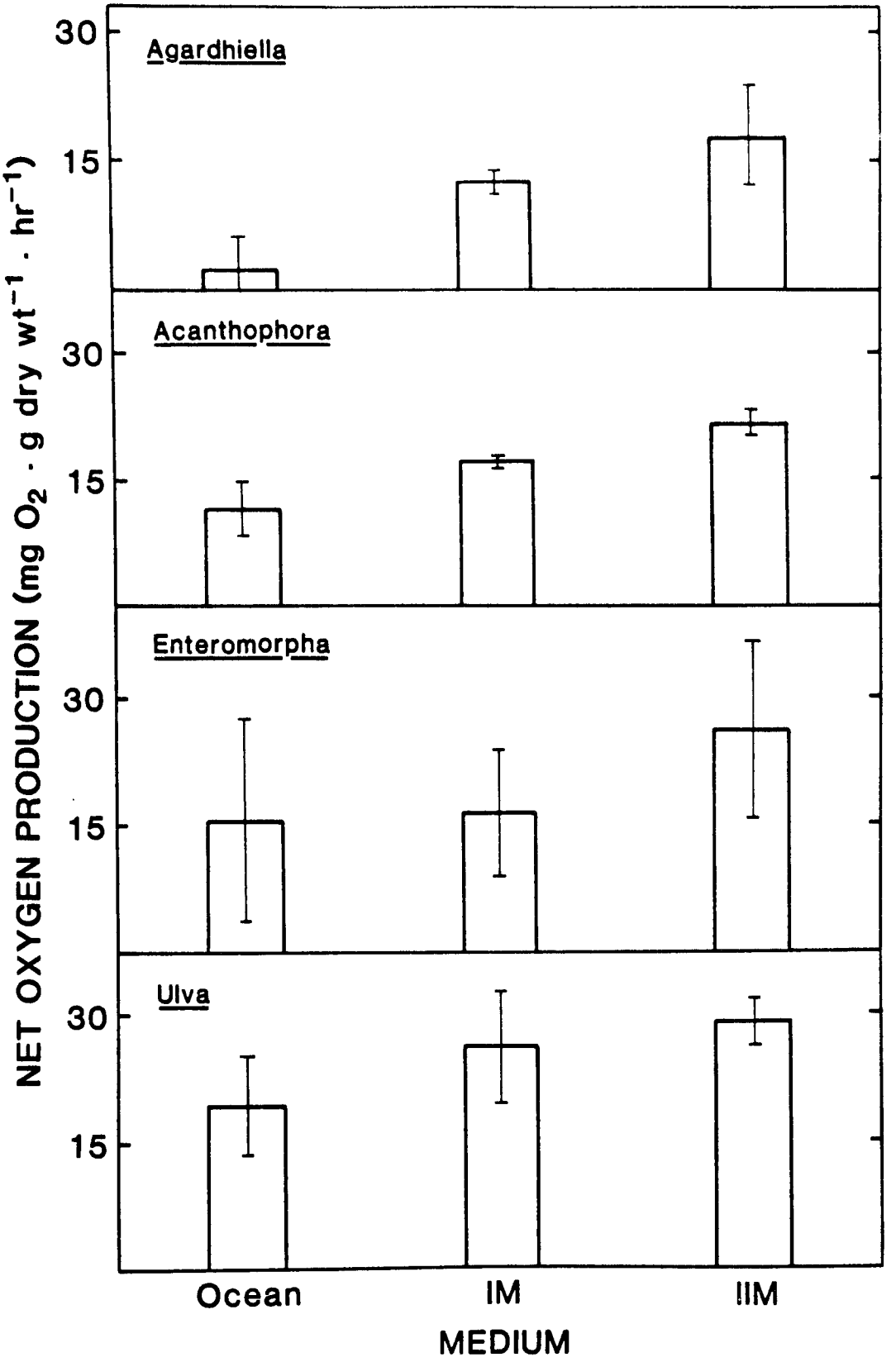

Figure 2. Net photosynthesis of four macroalgae in ocean water, and in the IM and IIM saline desert media. Error bars denote ±1 s.d.

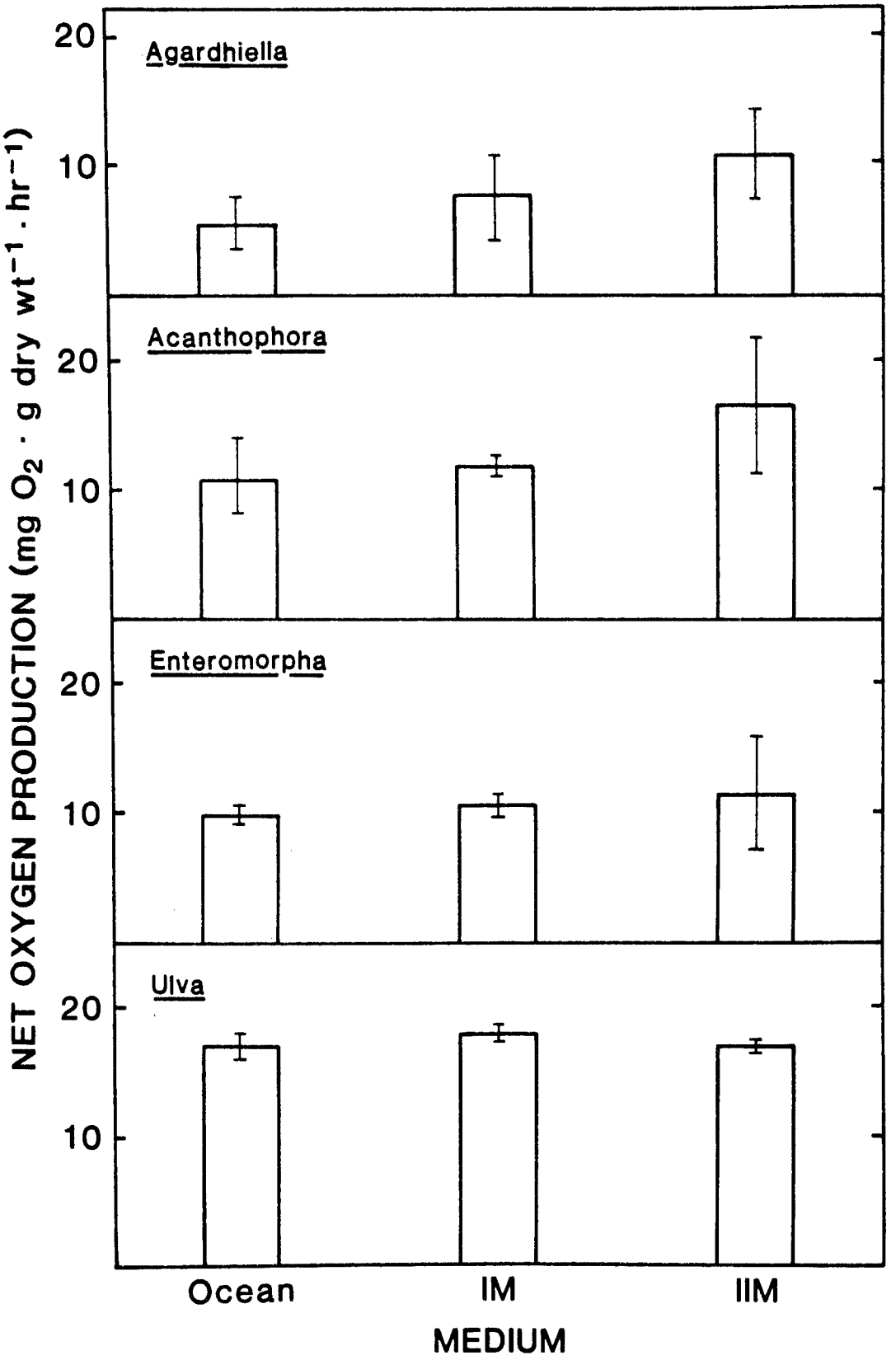

Figure 3. Net photosynthesis of four macroalgae in ocean water, and in the IM and IIM saline media. Algae were held in the respective media for 1.5 days prior to the incubations. Error bars denote ±1 s.d.

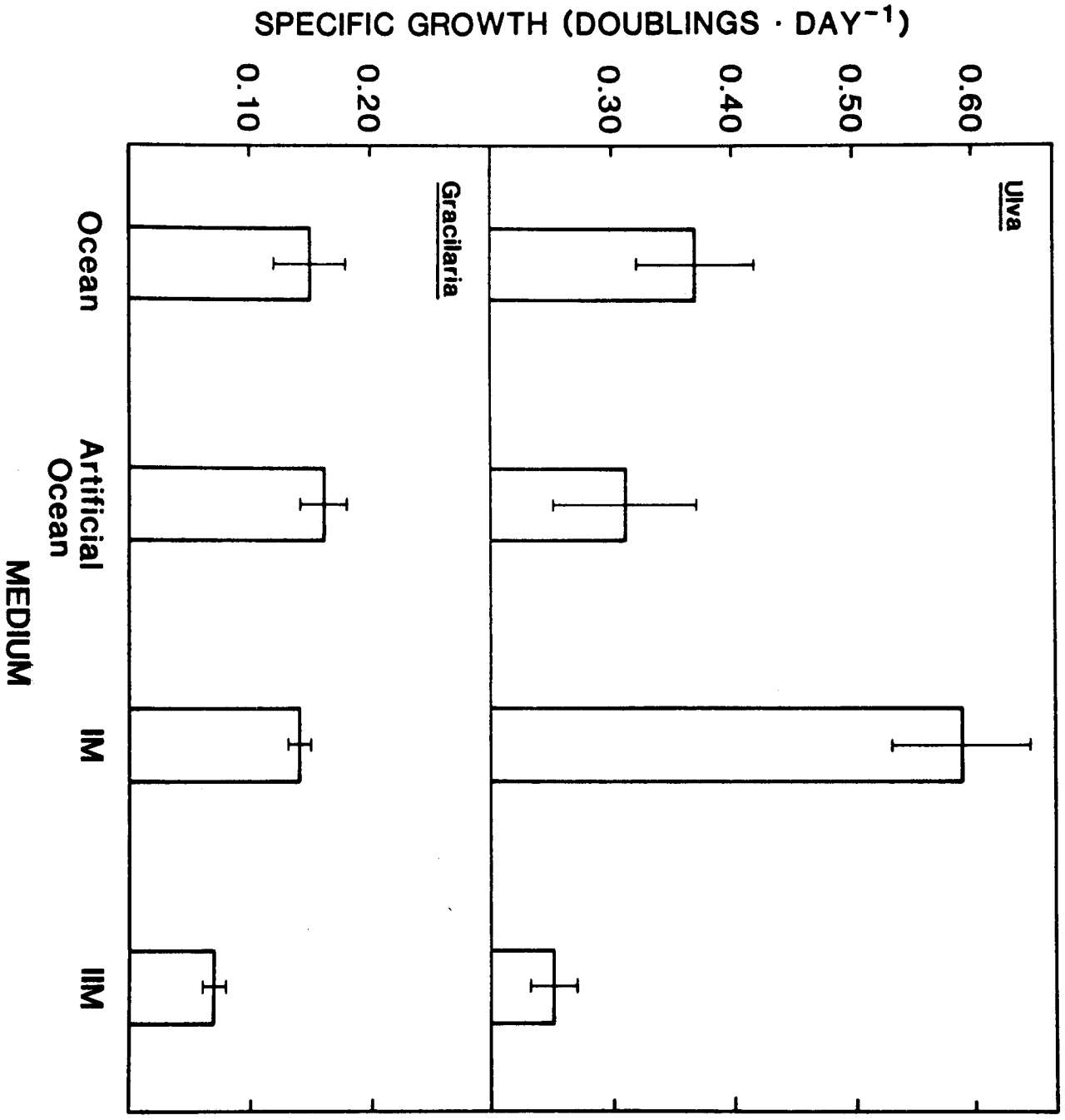

Figure 4. Specific growth of Ulva and Gracilaria during a one week study in ocean water, artificial ocean water, and two saline desert water media.

rapid growth for this species occurred in the IM water, with the alga exhibiting a doubling time of less than one day. Relative to the seaweed in the ocean and artificial ocean water media, however, the Ulva in the IM and IIM waters became pale and soft textured during the incubation period. Ulva growth in the latter treatment (IIM) was relatively poor (Fig. 4).

Because specific growth rates of Ulva and Gracilaria in this experiment were calculated based on changes in wet weight biomass, a followup experiment was conducted to determine whether the recorded growth rates (particularly that of Ulva in the IM medium) represented a true increase in dry matter, or merely an increase in thallus water (or ash) content.

On a dry weight basis, the specific growth rates of Gracilaria in the ocean and artificial ocean waters were found to be equal, at 0.1 doublings day^{-1} (Fig. 5). Although a gain in Gracilaria wet weight was observed in the IM medium, the thallus dry matter content dropped 50% (initial % dry matter was 12.3), so net specific growth on a dry weight basis was zero (Fig. 5). There were no differences between media in ash content, although it is notable that this parameter decreased sharply (initial % ash was 44.7) after Gracilaria was transferred from high (32 ppt in the outdoor holding tanks) to low salinities (15 ppt for the experimental media) (Fig. 5).

In contrast to Gracilaria, Ulva grew at a slower rate in the artificial ocean medium than in the ocean water (Fig. 5). The reason for this reduced growth, which was also observed in the preceding experiment (Fig. 4), is unknown. The weight increase of Ulva in the IM medium was 40% higher than that in ocean water (Fig. 5). However, because of the high ash content of Ulva in the IM medium (Fig. 5), the difference between the organic matter increase in ocean water and that in the IM medium on a volatile solids basis is narrower.

Because Ulva grew more rapidly in the artificial desert media than did Gracilaria, the former species was selected for an experiment to determine the effect of the saline desert waters on macroalgal proximate composition. This study was conducted in 13-L aquaria with an algal stocking rate of 40 g, so that plant samples of an adequate size for tissue analyses could be retrieved at the end of the study. During this one-week incubation, productivity (on a volatile solids basis) of Ulva decreased among treatments in the following order: ocean, artificial ocean, IM and IIM (Fig. 6). The following effects of water type on composition were also observed (Fig. 6):

1) Ulva grown in the IM water had elevated dry matter and ash contents (relative to ocean water).
2) The carbohydrate composition of the slow-growing Ulva in the IIM water was low.
3) The protein content of Ulva was equal among all water types.

These data show that over a short period of cultivation (one week), the IM medium supports moderate Ulva growth, and the seaweed produced is not atypical in composition.

C. Long-term growth of macroalgae in saline desert media.

Additional studies were conducted to determine how long the IM and IIM media could support continuous Ulva growth. Ulva discs were cultured in the two desert media and in control solutions of ocean and artificial ocean

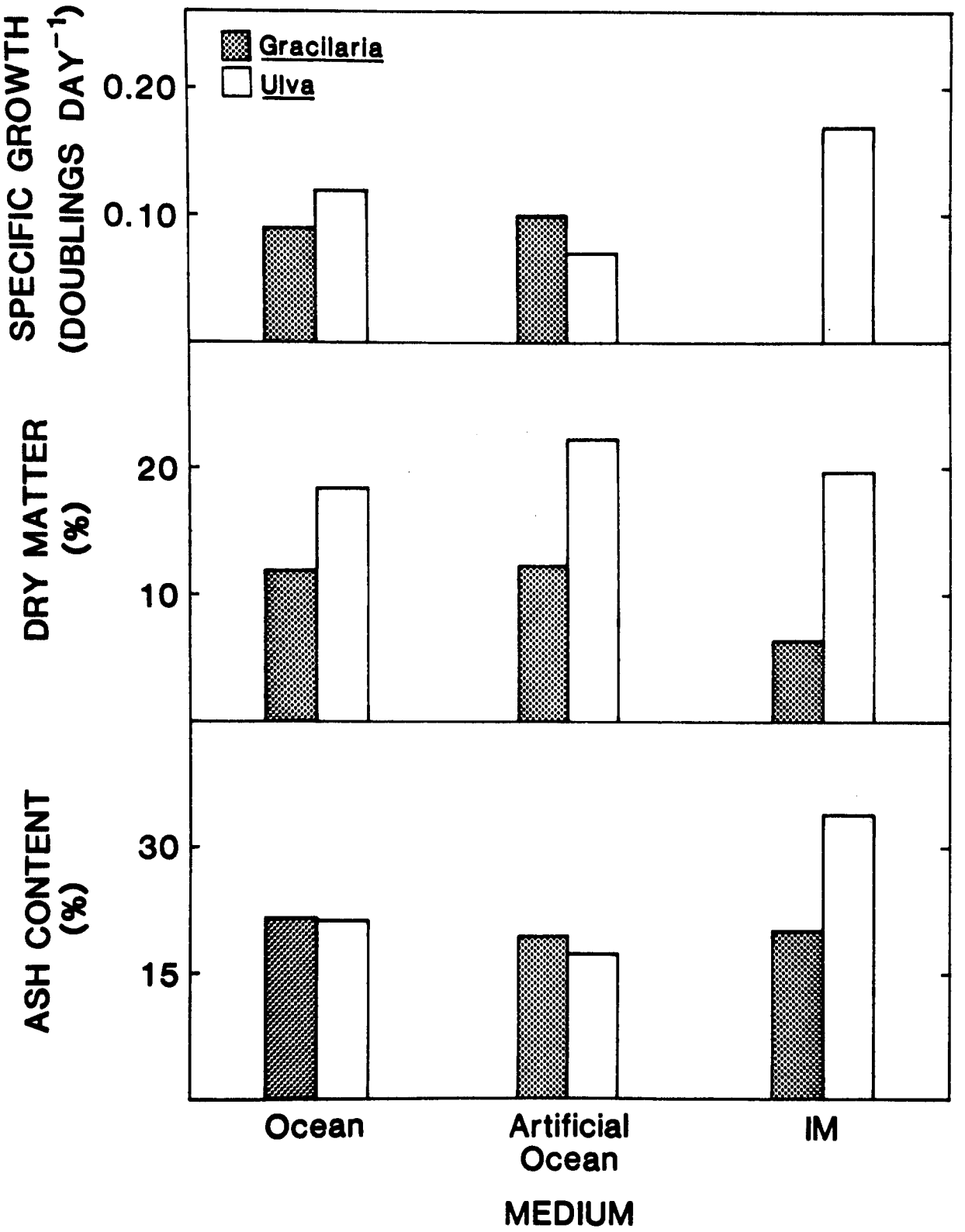

Figure 5. Specific growth, dry matter content, and ash content of Gracilaria and Ulva cultured for one week in ocean water and the artificial ocean and IM media.

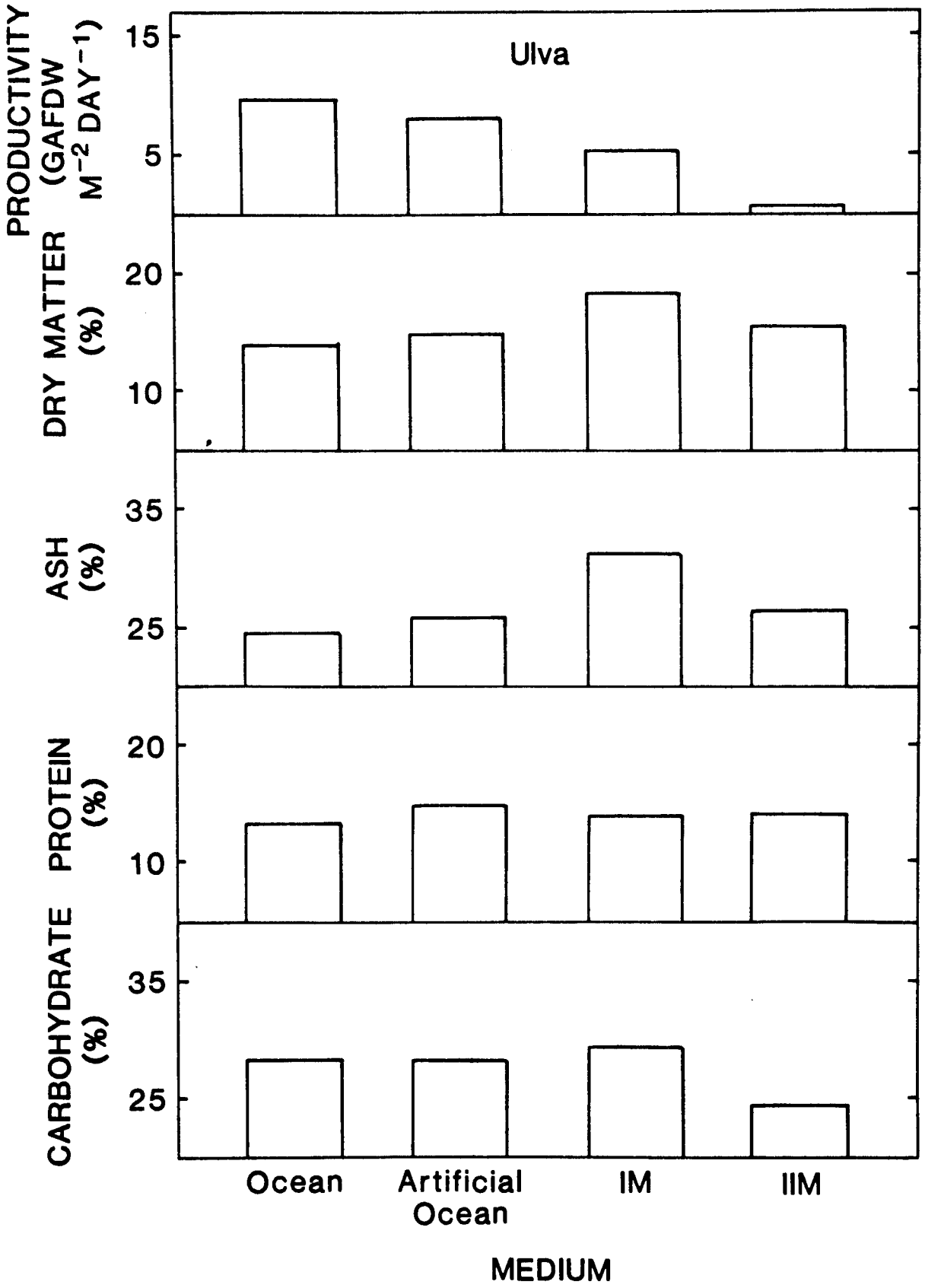

Figure 6. Productivity and proximate composition of Ulva cultured in ocean water and in three artificial media.

water. As expected, growth in the IIM medium was poor, with the thalli deteriorating within the first week of the experiment. In the ocean and artificial ocean waters, growth was relatively constant both between treatments and over time (from weeks one through three). In the IM medium, Ulva growth was equal to that in the ocean waters in weeks one and two, but then dropped sharply during week three (Fig. 7). The Ulva in this treatment fragmented when new discs were cut at the end of week three, so the experiment was terminated.

Although our experiments show that neither the I nor IIM media will support Ulva growth for longer than a few weeks, the reason for the gradual death of the alga in these media is unknown. Seaweed decline may have resulted from the lack of a particular ion or nutrient, or because of a general ionic imbalance of the medium. Relative to seawater at a salinity of 15 ppt, the IIM formula has an extremely high bicarbonate level, but is low in cations, particularly calcium. The IM formula is also high in bicarbonate and low in Mg, but unlike the IIM water, it has a high Ca concentration. However, because of precipitate formation, the true ionic composition of these media during this experimental work was unknown. The IM medium precipitated immediately upon mixing, whereas a precipitate would not generally form in IIM medium until day 2 or 3 of a growth experiment.

The precipitate which formed immediately in the IM waters was probably $CaCO_3$. Surface seawater is roughly 100-300% oversaturated with respect to both Ca^{++} and $CO_3^{=}$ (Riley and Chester, 1971), and the IM medium provides for even higher levels of both minerals. Attempts were made during media formulation to eliminate precipitation by altering both medium pH (to reduce $CO_3^{=}$ levels) and the order of salt additions (to eliminate heterogeneous substances which could act as nucleation sites [Stumm and Morgan, 1970]), but none of these procedures were successful.

A final experiment was conducted to determine whether various mixtures of ocean water and IM medium would support long-term Ulva growth. Maximum growth rates (doublings day^{-1}) during the two-week study occurred in the 1:1 mixture of ocean water and IM medium (Fig. 8). Growth rates declined among the remaining media in the following order: 1:3; ocean water; 1:9; and finally, IM. The high growth in the 1:1 mixture probably was a result of the high bicarbonate levels provided by the IM medium. During week two, Ulva growth in the IM medium amended with 25% ocean water was substantially higher than that in the IM medium alone (Fig. 8). The relatively large percentage of ocean water required to improve Ulva growth in the artificial solution suggests that the IM water is an unbalanced medium for macroalgae growth.

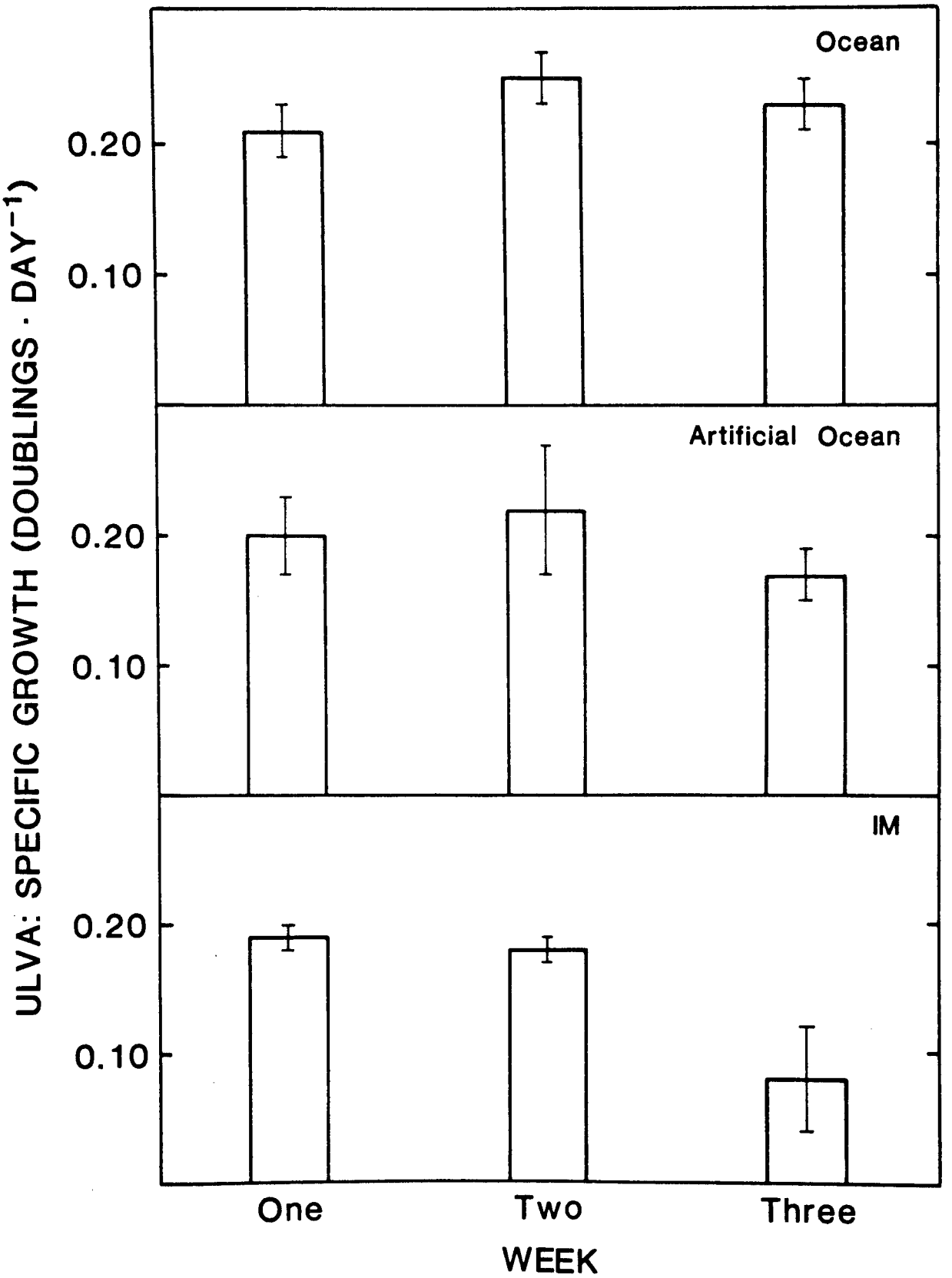

Figure 7. Specific growth of Ulva in ocean water and the artificial ocean and IM media. Each week, thalli were trimmed back to their original size and restocked.

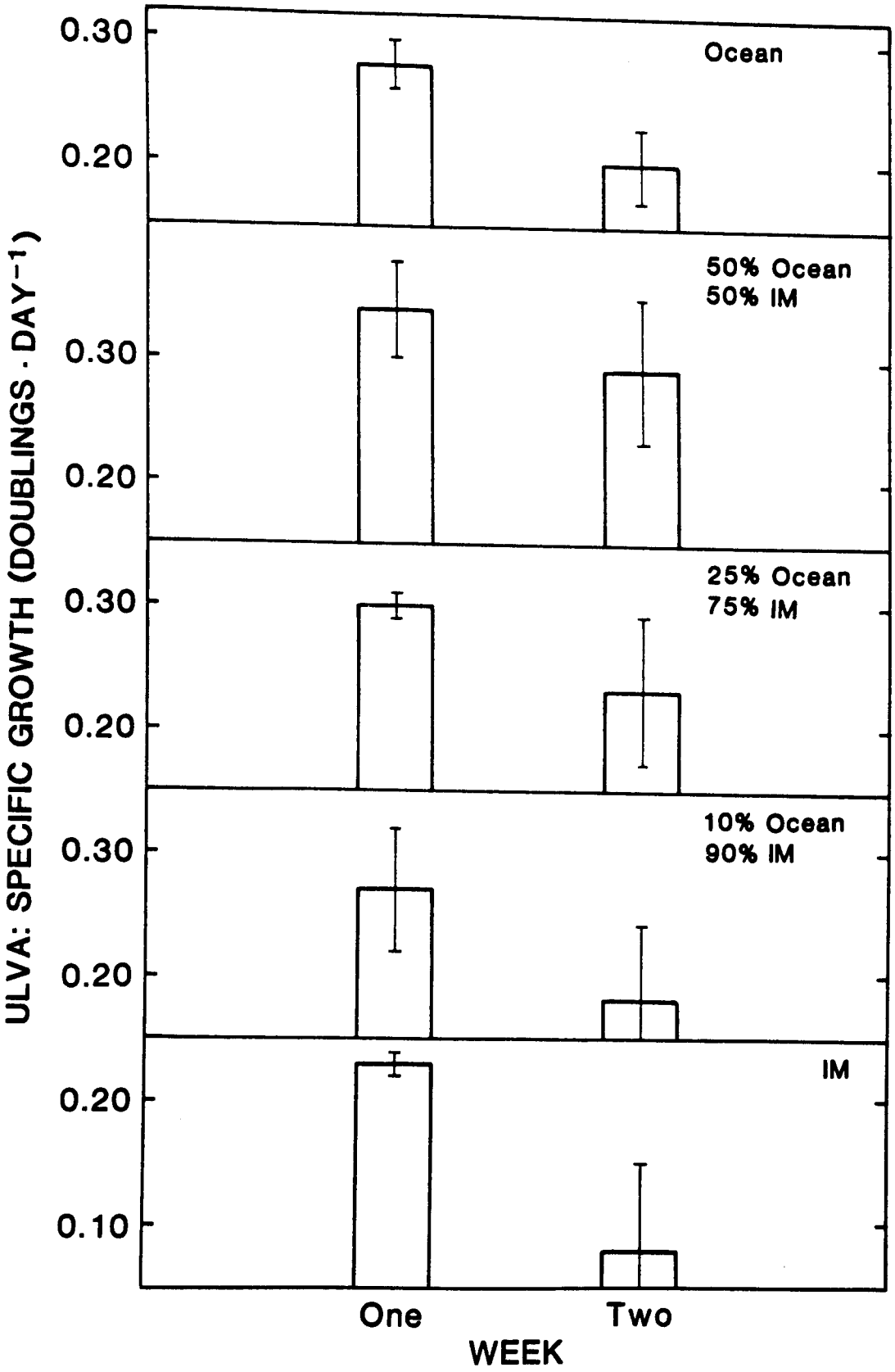

Figure 8. Specific growth of Ulva in ocean water, IM medium, and in three mixtures of these two solutions.

Task 2. Effect of environmental conditions on the proximate composition of marine macroalgae.

Methods

The effect of the environmental parameters salinity, nitrogen availability, phosphorus availability, water temperature and irradiance on seaweed composition were examined in Task 2. Three macroalgae species were utilized for this study. Gracilaria tikvahiae and Ulva lactuca grow rapidly year-round in central Florida and typically contain moderate to high levels of carbohydrates (Habig et al., 1984). These species were thus chosen as candidates for experiments on increasing the yields of carbohydrates and other storage products. In contrast with the carbohydrate fraction, lipid levels in macroalgae are generally quite low: the average lipid composition of 20 seaweeds examined by Ryther et al. (1984) was only 2-3%. One of the species found to contain highest lipid levels (6%) was Caulerpa prolifera, a green alga common to the Indian River. This species was also utilized for the experiments in this task.

These studies were conducted using eighteen 13 L aquaria held in a temperature-controlled room ($23^{\circ} \pm 2^{\circ}C$). An air wand was placed inside each tank along the back wall to provide continuous mixing of the tank's contents. A styrofoam cover was placed over each aquarium to prevent evaporation. Gracilaria and Ulva were grown loose in the aquaria, whereas Caulerpa was grown attached, or "rooted" in washed quartz sand. The following standard conditions were established and utilized as a control treatment in each experiment.

-Irradiance of 6×10^{15} quanta $cm^{-2} sec^{-1}$ (measured in situ) provided by two fluorescent lamps on a 14:10 hour light:dark photoperiod (ca 72 watts m^{-2}).
-Water temperature of 22 ± 2 C.
-A tank medium consisting of offshore ocean water, diluted to a salinity of 29 ppt with well water.
-A nutrient medium provided at the concentrations listed in Table 3.

The Ulva and Gracilaria utilized for this study were each single clones, maintained in outdoor tanks under a "fixed" cultivation regime. Caulerpa prolifera was obtained on four dates from a natural population near Merritt Island, FL. The plant material was transplanted and held in 700-L outdoor tanks for at least one week prior to its use in the experiments. Detailed methods for the experiments follow.

Salinity

Based on data from prior studies with Gracilaria and Ulva (M. D. Hanisak, Harbor Branch Foundation, personal communication), salinities of 8 and 50 ppt were selected to provide media which would stress, but not kill the three macroalgae during the incubation period. Gracilaria and Ulva were stocked at densities of 50 and 40 g, respectively, into aquaria containing a seawater solution at a salinity of 29 ppt. Triplicate tanks (for each species) with waters of low salinity (8 ppt) were obtained by gradual dilution (over 3 days) with well water. A highly saline medium (50 ppt) was established in another set of replicate tanks by the gradual addition of a balanced salt mixture similar to that described in experiment A2. An

additional group of three tanks for each species was maintained at 29 ppt as a control. Nutrients (Table 3) were added to each tank after final salinity adjustments were made.

On day 11 of the experiment, the algae in the high and moderate salinity tanks were weighed, harvested back to initial stocking weights (to prevent overcrowding), and restocked. Nutrients in the media were replenished at this time with levels 0.6X those shown in Table 3. Ulva and Gracilaria in the low salinity treatment were harvested on this date, but were not restocked because of the poor appearance of the plants. On day 13, the experiment was terminated and the algae (high and medium salinities) were rinsed and weighed. Dry matter production was calculated as the dry weight biomass increase from day 1 to day 13 (or 11).

In the salinity study with Caulerpa, duplicate, rather than triplicate tanks were utilized at each salinity. Salinity adjustments (to high and low levels) were made over a period of 4 days, and the total duration of the study was 14 days. Productivity was not measured because of the plants attached habit.

Nitrogen

Gracilaria and Ulva were cultured in seawater containing high, moderate and low levels of nitrogen. A medium containing the $NaNO_3$ concentrations shown in Table 3 was established as the "moderate" nitrogen level. High nitrogen levels were established in a medium by elevating the preceding $NaNO_3$ concentration eightfold. Nitrogen was omitted from the standard medium to create a solution deficient in nitrogen. Initial nitrogen concentrations in these treatments were 0, 7, and 54 mg L^{-1}, or approximately 0, 19, and 130 mg N per gram of dry seaweed stocked in the tanks.

Three aquaria were utilized for each species under each N-loading treatment. Gracilaria and Ulva were stocked at 50 and 40 g wet weight, respectively. After seven days, the algae were harvested back to their original densities and restocked. The water in the tanks was then changed and the nutrient media were replenished. On day 14 of the experiment, the algae were harvested, weighed, and subsamples were collected for tissue analyses. Water samples were collected from one tank in each treatment, filtered, and analyzed for inorganic N and P. Productivity of the algae under the three nitrogen loading regimes was calculated as the biomass increase between day 7 and day 14.

A similar experiment to determine the effect of nitrogen availability on the proximate composition of Caulerpa prolifera was conducted using only moderate and low nitrogen levels. High nitrogen concentrations were not attempted in the Caulerpa aquaria because of past experience under such conditions of severe epiphyte fouling. Caulerpa was cultured under the low and moderate nitrogen levels for 10 days, with nutrient media replenished on day 5.

Temperature

Gracilaria, *Ulva*, and *Caulerpa* were cultivated under three temperature regimes in the 13-L aquaria. High water temperatures (31-34 C) were maintained in one group of aquaria with 50 watt heaters. In another group of aquaria, low temperatures were maintained by passing chilled water through heat exchangers placed along an inside wall of each tank. Cold water was produced either in an ice bath or by using a commercial chiller (Aqua-chiller).

Experiments with *Gracilaria*, *Ulva*, and *Caulerpa* were conducted separately. *Gracilaria* was stocked (40 g wet weight) into 9 aquaria containing water at the ambient room temperature (22°C). Temperatures were then slowly adjusted to the following levels: high, 34-35°C; moderate, 22-23°C; and low, 8.5-14°C. On day 6 of the experiment, the seaweed was harvested back to its original density, the water in each aquarium was changed, and the nutrient media were replenished. The experiment was terminated on day 10.

Ulva was stocked at 40 g into nine aquaria containing media at a temperature of 22°C. Temperature levels were changed over the course of 2 days to the following ranges: High, 34°C; moderate, 23°C; and low, 10°C. The *Ulva* was harvested and the experiment was terminated on day 7 due to the poor appearance of the seaweed in the high and low temperature treatments.

Caulerpa was stocked at 11 g into aquaria containing media at a temperature of 22°C. Three temperature levels were established in the tanks after 2 days: high, 31-32°C; moderate, 23°C; and low, 10°C. On day 10, the water in each aquarium was changed and nutrient levels were replenished. The experiment was terminated on day 14.

Light

The effects of light intensity on the proximate composition of the three macroalgae were studied in a glass greenhouse. Nine 13-L aquaria were partially submersed in small water baths which received a constant flow of well water (24°C). The resulting diel fluctuations in temperature within each aquarium during the study were slight (23-25.5°C).

All aquaria were covered with a sheet of clear polyethylene (4 mil) to prevent evaporation. Layers of neutral density screening were used to establish three light levels among the tanks. Total insolation was measured with an Epply pyrheliometer, while the extinction of PAR within each tank was determined with a Biospherical Instruments quantum sensor. Seaweeds cultured at the highest irradiance received approximately 150 g cal cm^{-2} day^{-1}, while those in the medium and low light treatments received approximately 0.2 and 0.02 times this amount, respectively.

Experiments on the three macroalgae were conducted at separate times. *Gracilaria* was stocked at 40 g per tank. Supplemental fertilization was provided on days 4 and 8. Water in each aquarium was changed on day 12, at which time nutrient solutions were also replenished. On days 9 and 12, the seaweed was harvested back to original densities and restocked. The

experiment was terminated on day 15, with productivity calculated from biomass changes from days 1 through 15.

Ulva was also stocked at 40 g, and was harvested back and restocked on day 7. Supplemental fertilization was provided on days 4 and 7. The experiment was terminated on day 9, with productivity calculated as the biomass change from days 1-9.

Caulerpa was stocked at a density of 13 g per tank. On day 7, the water in each aquarium was changed and nutrients were replenished. The experiment was terminated on day 13.

Phosphorus

Gracilaria and Ulva were cultured in seawater containing high, moderate and low levels of phosphorus. A medium containing the Na_2 glycerophosphate concentrations shown in Table 3 was established as the "moderate" phosphorus level. High phosphorus levels were established in another medium by elevating the preceding Na_2 glycerophosphate concentration tenfold. For the final treatment, phosphorus was omitted from the standard medium to create a solution deficient in phosphorus. Initial phosphorus concentrations in these media were 0.0, 0.7, and 7.0 mg l^{-1}, or approximately 0.0, 1.9, and 19 mg P per gram of dry seaweed stocked in the tanks.

Three aquaria were utilized for each species under each phosphorus loading treatment. Both seaweed species were stocked at 40 g wet weight per tank. At one-week intervals, the algae were harvested back to their original densities and restocked. The water in the tanks was changed at this time and the nutrient media were replenished. After approximately three weeks, the algae were harvested and weighed, and subsamples were collected for tissue analyses. Water samples were collected from one tank in each treatment, filtered, and analyzed for inorganic N and P. Productivity of the algae under the three phosphorus loading regimes was calculated as the biomass increase between day 14 and day 21.

Seasonal Growth and Composition of Ulva and Gracilaria

Cultures of Ulva and Gracilaria were maintained in duplicate 700-l outdoor tanks during this study to provide a "uniform" stock of plant material for the indoor experiments. These tanks received estuarine water at a flow rate of 4.8 l min^{-1} (10 water exchanges per day) and were aerated continuously. Supplemental nitrogen (NH_4NO_3) was provided on a weekly, pulse-feeding basis. The algae were harvested weekly, weighed, and restocked to their original densities (1.1 and 2.1 kg wet weight m^{-2} for Gracilaria and Ulva, respectively). Triplicate plant samples were collected semi-monthly and dried (70°C for 48 hr) for the determination of percent dry weight. An additional set of three samples was collected monthly, rinsed in deionized water, and dried. These samples were ground to a fine powder and analyzed for ash, protein, soluble carbohydrate and crude fiber content. Inorganic nitrogen and phosphorus concentrations of the influent estuarine water were also measured monthly.

Composition analyses

Algae harvested from the tanks were rinsed in freshwater, blotted dry and weighed. Productivity calculations were based on dry weight biomass changes of the seaweeds (final dry weight - initial dry weight) and thallus ash content. Two subsamples of algae were collected from each tank at the termination of all experiments. The first was lyophilized and stored in a freezer for lipid analyses. The second was placed on a tared square of foil, weighed, dried (70°C for 48 hr) and then reweighed, thus providing a measure of % dry weight. The sample was then ground and stored for later analyses.

The ash content of the oven-dried samples was determined gravimetrically following combustion in a muffle furnace (500°C for 4 hrs). The protein content of ca. 20 mg dried algal samples was determined colorimetrically using a slight modification of the Folin-Phenol method (Lowry et al. 1951). Following the addition of 5 ml of 1 N NaOH to the test tubes containing the samples, the extraction was conducted in a water bath at 50°C for 12 hours, rather than at room temperature for 24 hours.

The carbohydrate content of 10 mg portions of the dried samples was determined colormetrically by the Phenol-Sulfuric Acid method (Dubois et al. 1956). Lipids were extracted from 1-3 g of freeze-dried algal samples by grinding (Virtis tissue homogenizer) in 100 ml of extraction solvent (methanol:methylene chloride:water, 10:5:4 by volume) (Tornabene et al. 1982). The methylene chloride fraction was separated and evaporated just to dryness on a Buchler Evapomix. The remaining "lipid" fraction was measured gravimetrically.

Elemental analyses (N,P) were conducted on plant tissues obtained from the nitrogen and phosphorus studies. Nitrogen composition was measured using a Perkin-Elmer 240A elemental analyzer. Total phosphorus in the algae samples was oxidized by persulfate digestion, and the concentration of the resulting inorganic phosphate was measured colorometrically. Crude fiber (cellulose and lignin) determinations on plants collected from the outdoor, seasonal growth study were conducted according to Van Soest (1963).

Results and Discussion

Salinity

Gracilaria and Ulva exhibited different trends in productivity in response to salinity of the culture medium (Fig. 9). Ulva grew well at both low and moderate salinities, but growth at the high salinity was poor. In contrast, organic matter production by Gracilaria at the low salinity was slight, but then increased with increasing salinity. These data suggest that Gracilaria is an oceanic species, whereas Ulva is more adapted to estuarine conditions.

Although salinity of the culture medium affected the dry matter content (% of wet wt.) of the three macroalgae, the effects were not consistent between species (Fig. 9). The % dry weight of Gracilaria increased with increasing salinity, whereas that of Ulva decreased. Maximum % dry wt. of Caulerpa occurred at the medium salinity level. In contrast, the % ash content of all three macroalgae increased with increasing salinity. This increase was most pronounced in Gracilaria between the salinities of 8 and 29 ppt, where ash content increased from 28 to 48%. Such a high thallus mineral content may be required at high salinities in order to maintain the osmotic balance between the cells and the culture medium.

Little difference was observed between salinities in the composition of protein, carbohydrate, and lipid for each species (Fig. 9). A very gradual increase in protein (from 12.2 to 13.8%) in Gracilaria was observed with increasing salinity. The protein content of Ulva did not change with salinity, whereas the maximum protein content of Caulerpa (11.8%) occurred at the lowest salinity.

Highest soluble carbohydrate levels (60.0%) in Gracilaria were found at a salinity of 50 ppt. Elevated salinities have also been found to stimulate the production of the carbohydrates floridoside and isofloridoside in the red alga Porphyra perforata (Craigie, 1974). In contrast, salinity of the culture medium had little effect on carbohydrate levels in Caulerpa, and carbohydrate levels in Ulva actually declined with increasing salinity.

A slight increase in the lipid composition of Gracilaria and Caulerpa was observed with increasing salinity of the medium. The sharpest increase was observed in Caulerpa, the species with the highest lipid content, where lipid levels increased from 6.3 to 8.2%. Previous investigators have reported an even greater accumulation of lipid-related storage products in microalgae at high salinities. Craigie and McLachlan (1964) found that a hundredfold increase in culture medium NaCl concentrations increased glycerol levels in Dunaliella tertiolecta by 133 times.

The most prominent effect of salinity on relative concentrations of potentially valuable storage products was found in Gracilaria, where the carbohydrate content of thalli cultivated at 50 ppt was 34% higher than that of seaweed cultured at 29 ppt. High salinity also stimulated lipid accumulation in Caulerpa.

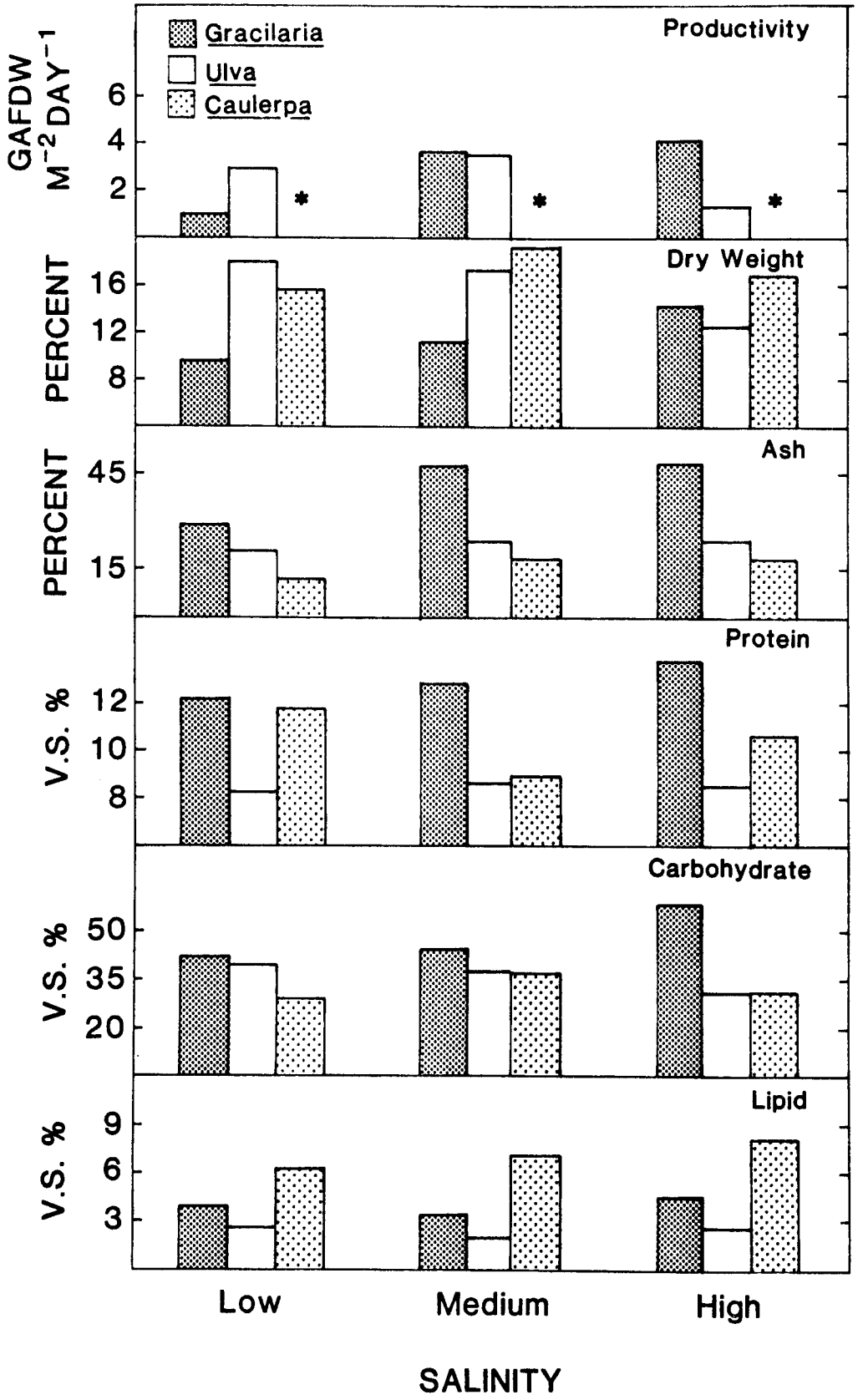

Figure 9. Productivity and proximate composition of three macroalgae cultured at low (ca 8 ppt), medium (29 ppt) and high (50 ppt) salinities. Parameters not measured for Caulerpa are denoted by an asterisk.

Nitrogen

Nitrogen availability in the medium markedly affected the productivity and composition of the three macroalgae (Fig. 10). Productivity of both Ulva and Gracilaria increased with increasing nitrogen level in the medium. This stimulation in organic matter production was greatest between low and moderate nitrogen levels. Only a slight increase in productivity (4% for Gracilaria and 10% for Ulva) was observed between moderate and high nitrogen levels.

The observed trends in dry weight, ash, protein and carbohydrate composition among nitrogen levels were similar for each of the three macroalgae. With decreasing nitrogen availability, % dry weights increased, ash and protein contents decreased, and carbohydrate levels increased. Tissue nitrogen concentrations for Gracilaria were 1.0, 1.8, and 3.1% in the low, moderate, and high nitrogen media at the end of the experiment. The respective tissue nitrogen concentrations for Ulva were 0.7, 2.1, and 2.6% in the three treatments. Amino acid synthesis and, hence, protein production is limited when little nitrogen is available in the medium. Sugars produced in photosynthesis under N-deficient conditions are utilized primarily for the production of structural and nonstructural (soluble) carbohydrates (Bird et al., 1982; Habig et al., 1984). Increased structural carbohydrate levels in the cells may in turn reduce the water content of the plant (Russell, 1971). The high ash content observed in algae cultured at elevated salinities may be related to an increase in mineral (cation) uptake which would accompany increased NO_3 assimilation.

Nitrogen availability in the medium had little effect on the lipid composition of the three macroalgae. The lipid composition of Caulerpa was equal at low and moderate nitrogen levels, whereas the high nitrogen medium produced highest lipid levels in Gracilaria, but lowest lipid levels in Ulva. Prior studies have shown that prolonged N-starvation can stimulate the production of lipids in microalgae (Shifrin and Chisholm, 1981). Carbohydrate synthesis apparently occurs during the initial stages of N-deprivation , whereas after a prolonged period without nitrogen, lipid synthesis dominates. None of the macroalgae in the present study exhibited a substantial increase in lipid concentration after two weeks cultivation in a N deficient medium. Moreover, in a separate experiment with Caulerpa, no lipid accumulation was observed even after one month's incubation in a nitrogen-free medium (Table 4).

Temperature

Maximum growth of Ulva and Gracilaria in this experiment occurred in the media maintained at a moderate (21-24°C) temperature (Fig. 11). However, Gracilaria was more tolerant of the high and low temperature extremes than was Ulva. Growth of Ulva was less than 1 gafdw m^{-2} day^{-1} in both the 8°C and 34°C treatments during the one week study.

The macroalgae cultivated at 8-12°C had the highest % dry weight, while the maximum ash content for all species occurred at 22°C (Fig. 11). It is unknown what effect temperature may have on dry matter content of algae. The reduced ash content of the plants cultured at the temperature extremes may

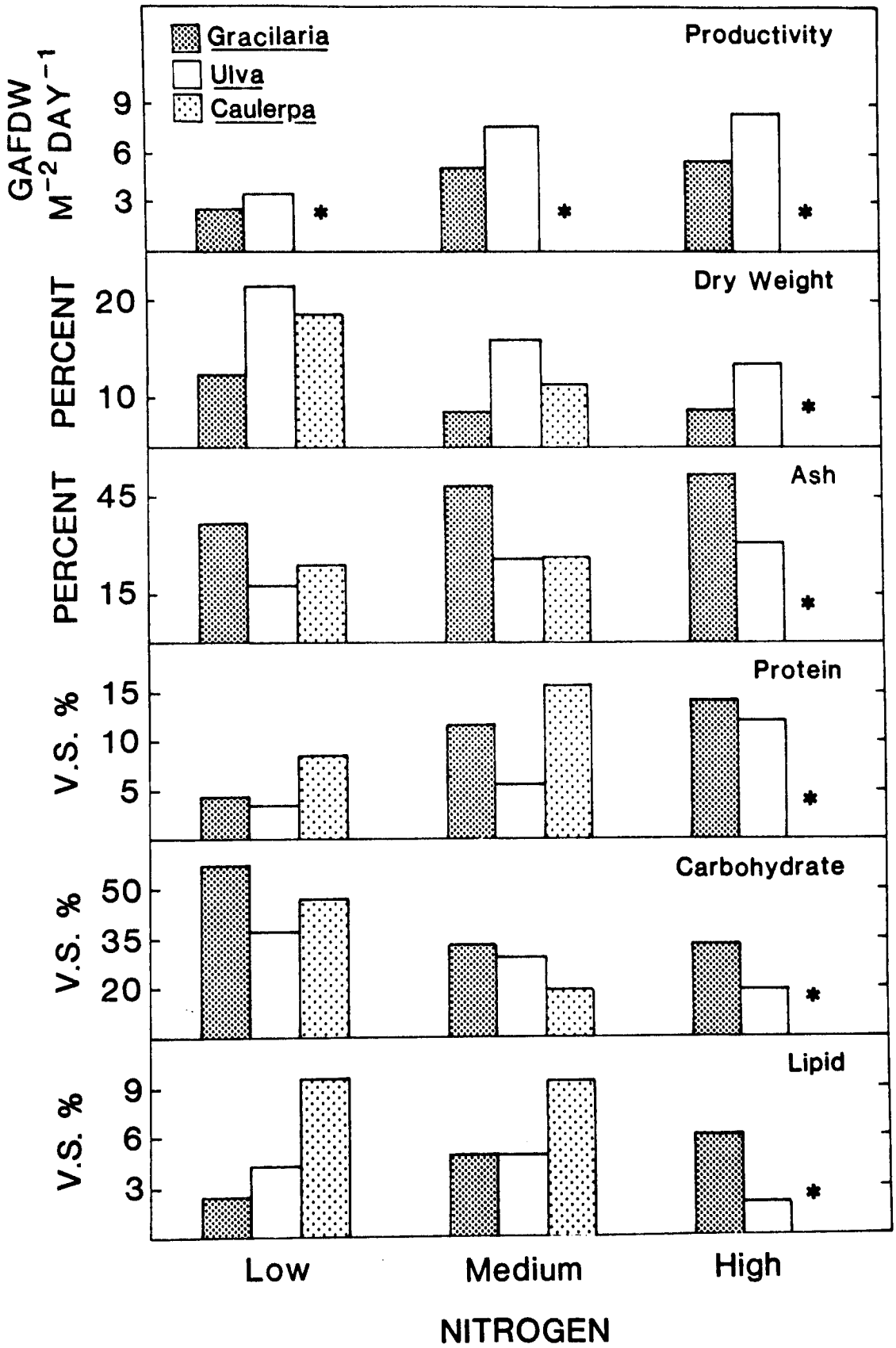

Figure 10. Productivity and proximate composition of three macroalgae cultured at low (0 mg L^{-1}), medium (7 mg L^{-1}), and high (54 mg L^{-1}) nitrogen levels. Parameters not measured for Caulerpa are denoted by an asterisk.

Table 4. Lipid content of *Caulerpa prolifera* cultured in media containing low (0.0 mg L^{-1}) and moderate (7.0 mg L^{-1}) concentrations of nitrogen.

	Low Nitrogen	Moderate Nitrogen
	-----Lipid content[1] (as % of V.S.)-----	
Initial	11.9 (1.0)	11.9 (1.0)
2 weeks	13.1 (0.9)	---
4 weeks	13.7 (2.0)	12.1 (1.6)

1 $\bar{x}$, (s.d.) n=2

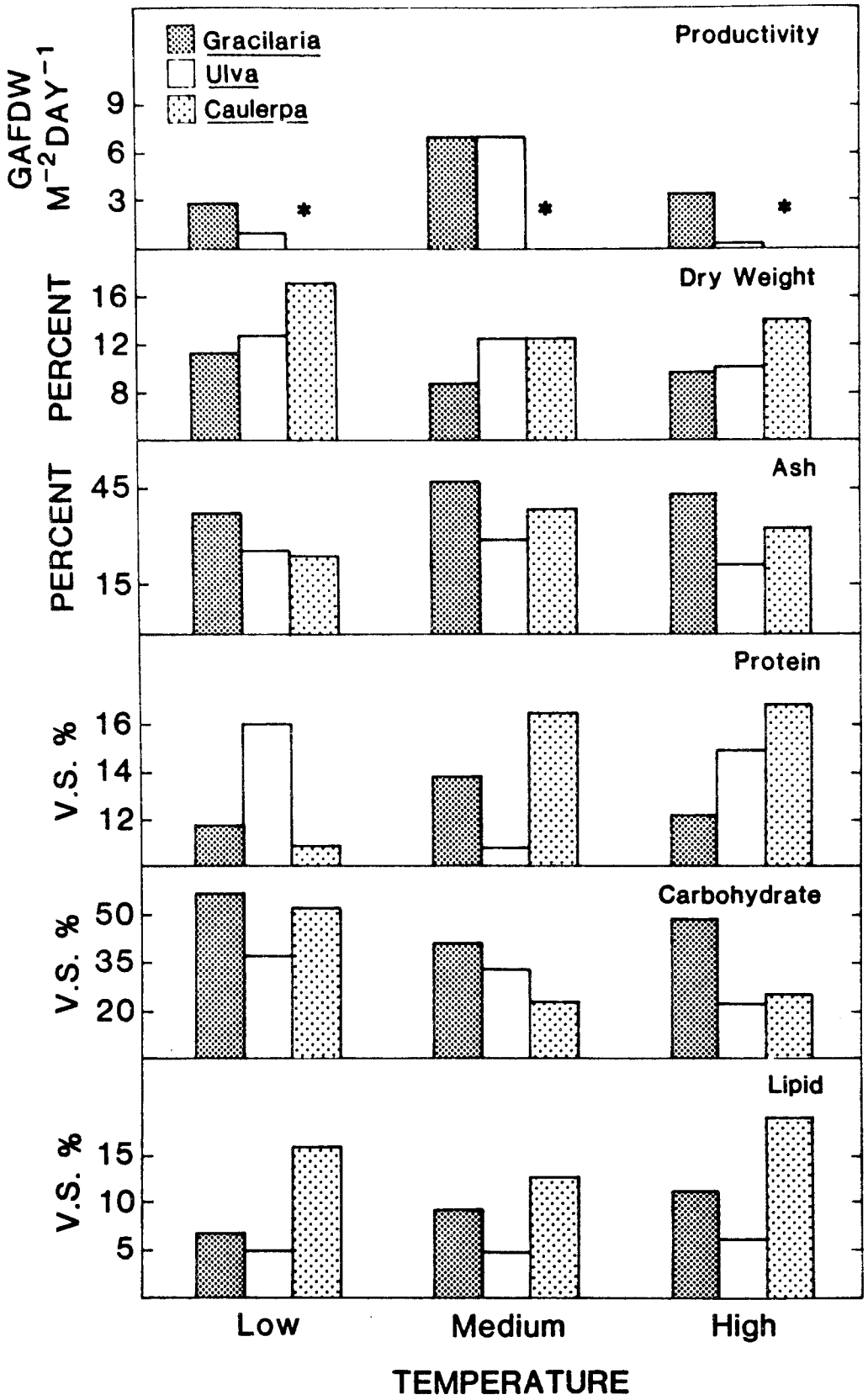

Figure 11. Productivity and proximate composition of three macroalgae cultured at low (ca 10°C), medium (22°C) and high (ca 33°C) water temperatures. Parameters not measured for Caulerpa are denoted by an asterisk.

have resulted from lower rates of nutrient assimilation by the relatively slow-growing plants in these treatments.

Gracilaria and Caulerpa cultivated at the lowest temperature contained the lowest levels of protein (Fig 11). This suggests a low temperature inhibition of either NO_3 uptake or protein synthesis, as the growth of the algae in this treatment was probably not sufficiently rapid to dilute protein reserves. In contrast, however, protein levels of Ulva cultured in the low-temperature (8°C) treatment were quite high.

The carbohydrate content of the three macroalgae was greatest at the lowest cultivation temperatures (Fig. 11). For Caulerpa, carbohydrate levels at 10°C were twice that in thalli cultured at 22°C. The increased levels of carbohydrate of these algae at 10°C may be explained by the differing effect of temperature on photosynthesis and respiration reactions. Q_{10} values for the collective reactions of photosynthesis, some of which are mediated photochemically, range from 1.0 to 2.7. Because the Q_{10} values for respiration are generally higher (2.0-2.5), the catalysis of sugars and carbohydrates for energy may not keep pace with their synthesis under conditions of low temperature. Hence, relative quantities of soluble carbohydrates in the algal cells may increase at low temperatures, particularly when other environmental factors (i.e., light) favor rapid photosynthesis.

Although between treatment differences in lipid composition of these algae were not great, maximum lipid concentrations for all species were found in thalli cultured at 34 C.

Light

Irradiance strongly affected organic matter production by Gracilaria and Ulva (Fig. 12). Productivity of both species was almost negligible at the lowest light level, but then increased sharply as culture tank irradiance was increased. Gracilaria appeared slightly more tolerant of low light levels than Ulva.

Light intensity had little effect on most compositional parameters of the three macroalgae (Fig. 12). Dry weight, ash, and carbohydrate contents were essentially equal among the high, medium, and low light levels. However, the protein composition of Ulva and Caulerpa did vary inversely with irradiance. It is likely that nitrogen uptake by the "high light" algae could not keep up with tissue synthesis, or growth, and thus internal nitrogen pools were diluted. Lapointe (1981) observed this phenomenon in a rapidly growing culture of Gracilaria tikvahiae, even though the thalli were maintained in a medium containing high nitrogen levels.

Irradiance had little effect on the lipid composition of Ulva and Gracilaria (Fig. 12). The highest lipid compostion for Ulva was found at the highest light level, whereas maximum lipid concentrations in Gracilaria occurred at the lowest light intensity. Unlike Gracilaria and Ulva, however, the lipid levels of Caulerpa were sharply affected by irradiance, with lipid concentrations increasing with decreasing culture tank irradiance (Fig. 12). Of the five environmental parameters examined in the present study, light intensity appears to be the dominant factor influencing lipid concentrations in Caulerpa.

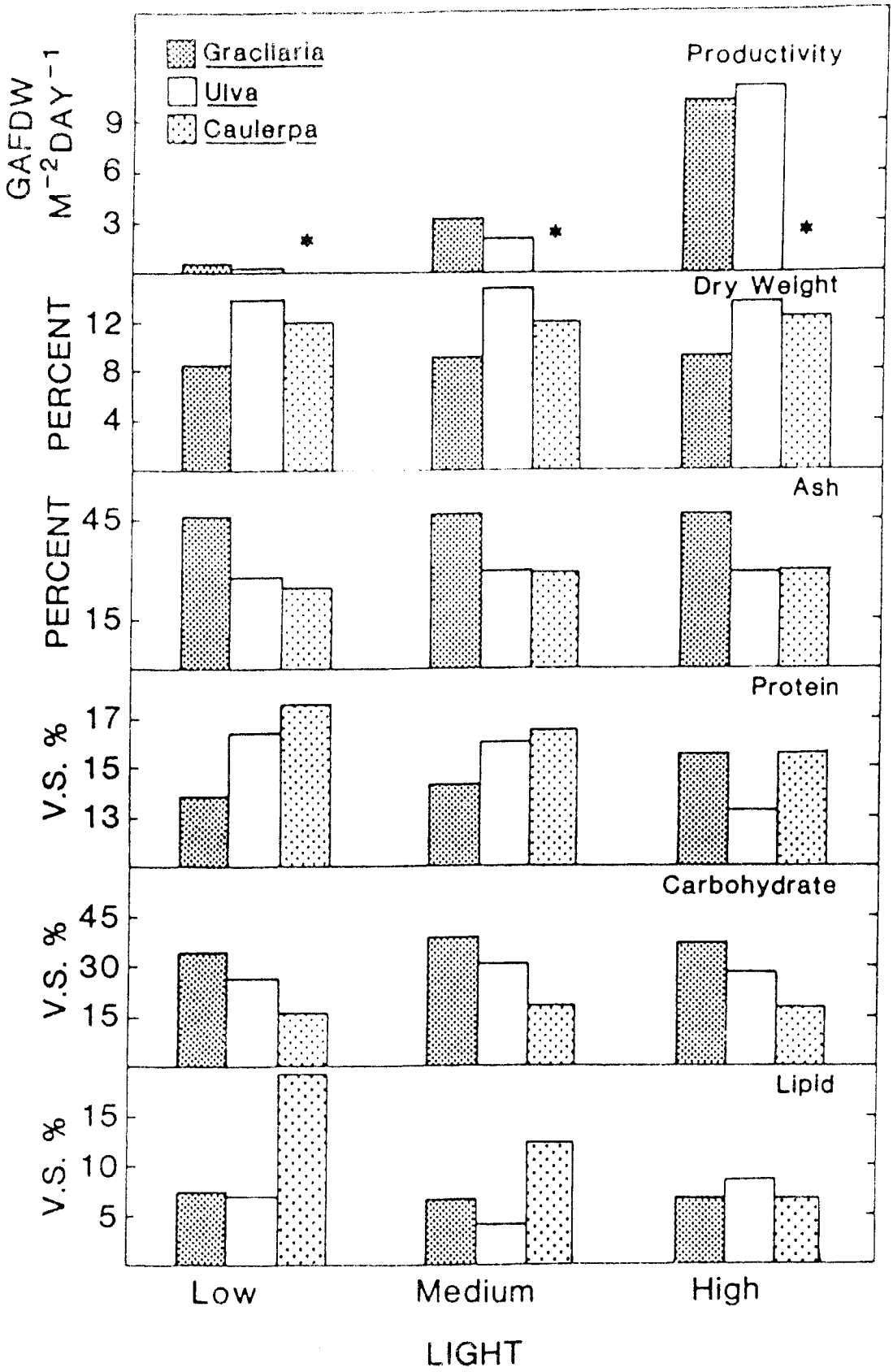

Figure 12. Productivity and proximate composition of three macroalgae cultured at low (3 g cal $cm^{-2}day^{-1}$), medium (30 g cal $cm^{-2}day^{-1}$), and high (150 g cal $cm^{-2}day^{-1}$), levels of irradiance. Parameters not measured for Caulerpa are denoted by an asterisk.

Phosphorus

We have found in prior experiments that nitrogen enriched Ulva and Gracilaria thalli become nitrogen limited (i.e., in terms of growth) within seven to ten days after being placed in a "batch", or stagnant medium deficient in nitrogen. In contrast, several weeks' incubation in a phosphorus "free" medium were required to induce phosphorus limited growth of these two macroalgae in the present study. Trace amounts of phosphorus in the seawater used to formulate the medium, and possibly, stored phosphorus in the plant tissues, supported rapid seaweed growth in the "phosphorus-free" medium during the initial two weeks of this study. Not until week three of this experiment was the productivity of Gracilaria in the phosphorus deficient medium lower than that in the two media amended with high and moderate concentrations of phosphorus. Unlike Gracilaria, Ulva growth was inhibited at high and low phosphorus concentrations (Tables 5 and 6). Few data are available in the literature on affects of phosphorus on the growth of macroalgae, so the reason for this inhibition is unknown. However, phosphorus concentrations of ca. 20 mg l^{-1} (three times higher than the maximum used in the present study) have been found to inhibit the growth of diatoms and green microalgae (Kuhl 1974).

As with the macronutrient nitrogen, luxury consumption of phosphorus is relatively common among microalgae (Kuhl 1974). In the present study, ten- and twentyfold differences in thallus P content were observed between high and low phosphorus treatments for Ulva and Gracilaria, respectively (Tables 5 and 6). However, the unusually high thallus P content of Gracilaria in the high phosphorus treatment may have been caused in part by calcium phosphate precipitation on the thallus, since the fresh algae samples were rinsed in fresh water, rather than dilute acid, prior to drying and analysis.

The ash content of Ulva was not affected by P availability in the growth solution (Table 6). Phosphorus-deficient Gracilaria thalli were low in ash, whereas thalli cultured in the moderate and high phosphorus treatments had an ash content greater than 50% (Table 5). This low ash content was probably caused by the reduction in ion uptake which often accompanies phosphorus depletion in algae (Kuhl 1974). For the Gracilaria cultured in the moderate and high phosphorus media, the extremely high ash content (and hence low volatile solids content) caused the concentrations of protein, carbohydrate, and lipid to be quite high when expressed on an organic matter basis.

Gracilaria grown under phosphorus deficient conditions contained a low concentration of protein, but a high concentration of carbohydrate (Table 5). Thus, with respect to these two proximate constituents, P-deficient Gracilaria thalli are similar in composition to N-deficient thalli. In contrast, the protein content of Ulva was not affected by phosphorus availability, and carbohydrate levels of Ulva actually increased with increasing P in the medium (Table 6). The effects of phosphorus availability on thallus lipid content also differed between the two species. Maximun lipid concentrations for Gracilaria were found in the moderate and high phosphorus treatments, whereas maximum lipid levels for Ulva occurred in the high and low P treatments (Tables 5 and 6).

Table 5. Productivity and proximate composition of *Gracilaria tikvahiae* cultured at low (0 mg L^{-1}), medium (0.7 mg L^{-1}), and high (7.0 mg L^{-1}) phosphorus levels. Values represent $\bar{x}$, (s.d.), n=2 or 3.

	Phosphorus Availability		
	Low	Medium	High
Productivity (g AFDW $m^{-2}day^{-1}$)	3.5 (0.2)	4.3 (0.9)	4.8 (0.5)
Dry weight (%)	9.9 (0.8)	8.2 (0.5)	9.8 (0.1)
Ash (%)	44.8 (3.9)	55.6 (1.4)	52.3 (1.1)
Protein (VS %)	13.0 (1.1)	18.9 (0.5)	18.8 (1.8)
Carbohydrate (VS %)	53.9 (3.6)	42.0 (11.3)	40.1 (7.1)
Lipid (VS %)	7.5 (1.3)	12.3 (5.6)	11.5 (5.9)
Phosphorus (%)	0.11 (0.02)	0.38 (0.11)	1.97 (0.09)[1]

[1]Calcium phosphate may have precipitated on thalli.

Table 6. Productivity and proximate composition of *Ulva lactura* cultured at low (0 mg L^{-1}), medium (0.7 mg L^{-1}), and high (7.0 mg L^{-1}) phosphorus levels. Values represent $\bar{x}$, (s.d.), n=2 or 3.

	Phosphorus Availability		
	Low	Medium	High
Productivity (g AFDW $m^{-2}day^{-1}$)	3.1 (1.2)	9.0 (1.2)	5.6 (0.7)
Dry weight (%)	13.9 (0.3)	14.6 (0.6)	14.2 (0.8)
Ash (%)	27.1 (2.5)	27.4 (1.0)	28.2 (1.3)
Protein (VS %)	9.7 (1.2)	9.7 (1.3)	9.5 (0.4)
Carbohydrate (VS %)	27.0 (1.4)	29.4 (1.3)	32.7 (6.2)
Lipid (VS %)	5.7 (2.5)	3.4 (1.1)	6.2 (2.4)
Phosphorus (%)	0.07 (0.01)	0.35 (0.10)	0.9 (0.15)

Seasonal Growth and Composition of Ulva and Gracilaria

Maximum dry matter production by both Gracilaria and Ulva in the outdoor tanks occurred during June, 1984 (Table 7). Ulva growth slowly declined during the remainder of the year, reaching a minumum in December. Gracilaria productivity followed a similar pattern, although an additional growth peak was observed in September and October. Following a period of only moderate growth in early winter, yields of both species increased in March, 1985.

The ash content of Gracilaria (avg. of 44.5%) was consistently higher than that of Ulva (28.0%) during the year (Table 8). Maximum ash levels in Gracilaria were found during the summer, whereas the ash content of Ulva was highest during the spring. The maximum protein content for Ulva occurred during the late fall and early winter, a period when dry matter production was declining (Table 9). The maximum protein content of Gracilaria occurred earlier in the year, during July (Table 9). The mean annual protein content for both species was ca. 11%.

Although carbohydrate concentrations of Gracilaria and Ulva fluctuated during the year, these variations were no greater than the variability between plants found on each monthly sampling date (Table 10). Annual mean soluble carbohydrate concentrations for Gracilaria and Ulva were 17.9 and 14.2%, respectively. Crude fiber (structural carbohydrate) measurements showed the reverse trend between species, with the fiber content of Ulva averaging 10.2% and Gracilaria 5.7% during this study.

When expressed on an ash-free basis, the annual mean productivity of Ulva (16.3 g AFDW $m^{-2}day^{-1}$) was greater than that of Gracilaria (13.6 g AFDW $m^{-2}day^{-1}$). Organic matter production by both species fluctuated seasonally, with production maxima and minima occurring in the summer and winter months, respectively (Table 11). Carbohydrate production by Gracilaria and Ulva generally followed the seasonal fluctuations in dry matter productivity, with annual mean yields of this proximate constituent averaging 4.4 and 3.2 g m^{-2} day^{-1}, respectively (Table 12). Mean protein production by these macroalgae was similar during this study, averaging 2.4 and 2.7 g $m^{-2}day^{-1}$ for Ulva and Gracilaria, respectively (Table 13).

Table 7. Productivity of *Gracilaria tikvahiae* and *Ulva lactura* cultured in 700 l outdoor tanks.

		Ulva	*Gracilaria*
		--Productivity[1] (g dry wt $m^{-2}day^{-1}$)--	
May	(1984)	34.1 (7.6)	20.4 (0.9)
June		33.9 (1.4)	38.4 (3.0)
July		25.5 (0.3)	27.6 (2.1)
August		25.0 (0.0)	27.9 (0.0)
September		22.3 (0.8)	30.2 (5.3)
October		20.8 (0.2)	30.1 (4.2)
November		16.8 (0.3)	23.5 (2.4)
December		10.5 (1.9)	17.7 (0.1)
January	(1985)	15.4 (0.9)	13.3 (1.0)
February		13.1 (2.8)	22.0 (0.5)
March		28.1 (1.1)	24.2 (1.8)
April		26.1 (2.5)	22.4 (0.3)

1 $\bar{x}$, (s.d.), n=2

Table 8. Ash content of *Gracilaria tikvahiae* and *Ulva lactuca* cultured in 700 l outdoor tanks.

		Ulva	*Gracilaria*
		------Ash content[1] (% of dry wt)------	
May	(1984)	28.7 (0.7)	37.7 (0.3)
June		26.5 (0.5)	46.5 (2.2)
July		28.5 (4.0)	51.0 (1.8)
August		26.4 (2.1)	46.9 (3.5)
September		27.4 (0.7)	45.1 (2.8)
October		27.8 (0.3)	47.2 (1.7)
November		27.6 (1.7)	47.1 (0.8)
December		26.9 (0.4)	42.4 (2.7)
January	(1985)	27.0 (0.3)	42.5 (0.9)
February		26.9 (1.5)	41.7 (3.1)
March		29.2 (0.6)	44.5 (2.2)
April		32.9 (1.2)	41.2 (1.1)

1 $\bar{x}$, (s.d.), n=3

Table 9. Protein content of *Gracilaria tikvahiae* and *Ulva lactuca* cultured in 700 l outdoor tanks.

		Ulva	*Gracilaria*
		----Protein content[1] (% of dry wt)----	
May	(1984)	9.9 (0.4)	11.1 (0.5)
June		9.9 (0.5)	12.1 (1.0)
July		10.5 (0.3)	15.0 (1.8)
August		10.3 (0.1)	11.8 (0.9)
September		10.1 (0.4)	8.4 (0.5)
October		10.4 (0.7)	9.0 (0.3)
November		15.9 (3.6)	11.1 (0.2)
December		14.9 (0.5)	10.9 (0.6)
January	(1985)	11.5 (0.5)	11.8 (2.2)
February		10.7 (0.2)	10.9 (0.1)
March		10.0 (0.4)	10.3 (0.4)
April		8.1 (0.6)	8.9 (0.5)

1 $\bar{x}$, (s.d.), n=3

Table 10. Carbohydrate content of *Gracilaria tikvahiae* and *Ulva lactuca* cultured in 700 l outdoor tanks.

		Ulva	*Gracilaria*
		--Carbohydrate content[1] (% of dry wt)--	
May	(1984)	14.5 (1.0)	19.2 (2.7)
June		12.8 (1.2)	14.6 (2.2)
July		14.6 (0.4)	14.4 (0.7)
August		13.6 (1.1)	18.1 (1.8)
September		12.9 (1.4)	19.2 (1.9)
October		18.2 (7.0)	19.9 (0.6)
November		12.5 (0.8)	17.3 (1.1)
December		13.1 (1.4)	20.5 (8.6)
January	(1985)	15.0 (6.0)	20.1 (6.1)
February		13.5 (0.7)	16.6 (1.3)
March		14.6 (1.2)	18.1 (3.1)
April		14.9 (0.1)	16.9 (3.6)

1 $\bar{x}$, (s.d.), n=3

Table 11. Organic matter production by *Gracilaria tikvahiae* and *Ulva lactuca* in 700 l outdoor tanks.

		Ulva	*Gracilaria*
		---Productivity (g AFDW $m^{-2}day^{-1}$)---	
May	(1984)	24.3	12.7
June		24.9	20.5
July		18.2	13.5
August		18.4	14.8
September		16.2	16.6
October		15.0	15.9
November		12.2	12.4
December		7.7	10.2
January	(1985)	11.2	7.6
February		9.6	12.8
March		19.9	13.4
April		17.5	13.2
Annual mean		16.3	13.6

Table 12. Carbohydrate production by *Gracilaria tikvahiae* and *Ulva lactuca* in 700 l outdoor tanks.

		Ulva	*Gracilaria*
		-Carbohydrate production ($g\ m^{-2} day^{-1}$)-	
May	(1984)	4.94	3.92
June		4.34	5.61
July		3.72	3.97
August		3.40	5.05
September		2.88	5.80
October		3.79	5.99
November		2.10	4.07
December		1.38	3.63
January	(1985)	2.31	2.67
February		1.77	3.65
March		4.10	4.38
April		3.88	3.79
Annual mean		3.22	4.38

Table 13. Protein production by *Gracilaria tikvahiae* and *Ulva lactuca* in 700 l outdoor tanks.

	Ulva	*Gracilaria*
	---Protein production (g $m^{-2}day^{-1}$)---	
May (1984)	3.38	2.26
June	3.36	4.65
July	2.68	4.14
August	2.58	3.29
September	2.25	2.54
October	2.16	2.71
November	2.67	2.61
December	1.56	1.93
January (1985)	1.77	1.57
February	1.40	2.40
March	2.81	2.49
April	2.11	1.99
Annual mean	2.39	2.72

Relationship between environmental conditions and proximate composition.

In Figs. 13-17, the effects of the four environmental parameters salinity, nitrogen availability, temperature and light intensity are considered for each individual storage product (i.e., dry weight, carbohydrate, etc.). The relative importance of each environmental parameter to the synthesis of a particular storage product can thus be quickly determined. Note, however, that although identical environmental conditions were provided in the control (medium) treatment of each experiment, the composition of the algae under these standard conditions varied among experiments. Relative fluctuations were greater for some parameters (e.g., lipid, dry weight) than for others (e.g., carbohydrate). The algae used in these studies were obtained from outdoor cultures which were exposed to gradual (solar radiation and temperature) and short-term (salinity) fluctuations in environmental conditions. Hence, it is likely that the composition of the initial plant stock varied at least slightly among experiments.

Tables 14-19 were adapted from Figs. 13-17 in order to "standardize" plant composition values in the control (medium) treatment among experiments. Data in these tables represent the difference between proximate composition (or productivity) in the experimental treatment and that in the control treatment. The most prominent effects of environmental conditions on plant composition are described briefly in the following section.

Productivity of Gracilaria and Ulva increased sharply in response to high light conditions (Fig. 13, Table 14). In contrast, the growth of these macroalgae was greatly reduced at the high- and low-temperature extremes, and under conditions of low nitrogen availability.

Nitrogen availability was the dominant factor affecting dry weights of Gracilaria, Ulva, and Caulerpa, with highest % dry weights occurring under low nitrogen conditions (Fig. 14, Table 15). Light intensity had almost no effect on the % dry weight of these algae, while the effects of temperature and salinity varied with species.

Wide fluctuations in ash content, particularly that of Gracilaria, were observed during this study (Fig 15., Table 16). Salinity was the environmental parameter which had the greatest effect on ash content, whereas ash composition remained relatively constant at different light intensities.

The protein composition of Gracilaria, Ulva, and Caulerpa was sharply influenced by nitrogen availability, and to a lesser extent, water temperature (Fig. 16, Table 17). Ulva cultured at high and low temperatures had a high protein content. In contrast, low- and high-temperature cultivation either decreased or had no effect upon the protein levels of Caulerpa and Gracilaria.

The carbohydrate composition of Gracilaria was greatly increased by cultivation under conditions of high salinity, low nitrogen availability and low temperature (Fig. 17, Table 18). Although carbohydrate levels of Ulva and Caulerpa declined at high salinity, carbohydrate composition was increased by low-temperature and low-N cultivation.

Lipid levels in Gracilaria increased slightly in response to high nitrogen and high temperature cultivation (Fig. 18, Table 19). Maximum lipid storage in Ulva occurred at high and low light intensities, whereas lipid levels in Caulerpa were increased primarily by cultivation at low light intensities (Table 19), and high and low temperature extremes.

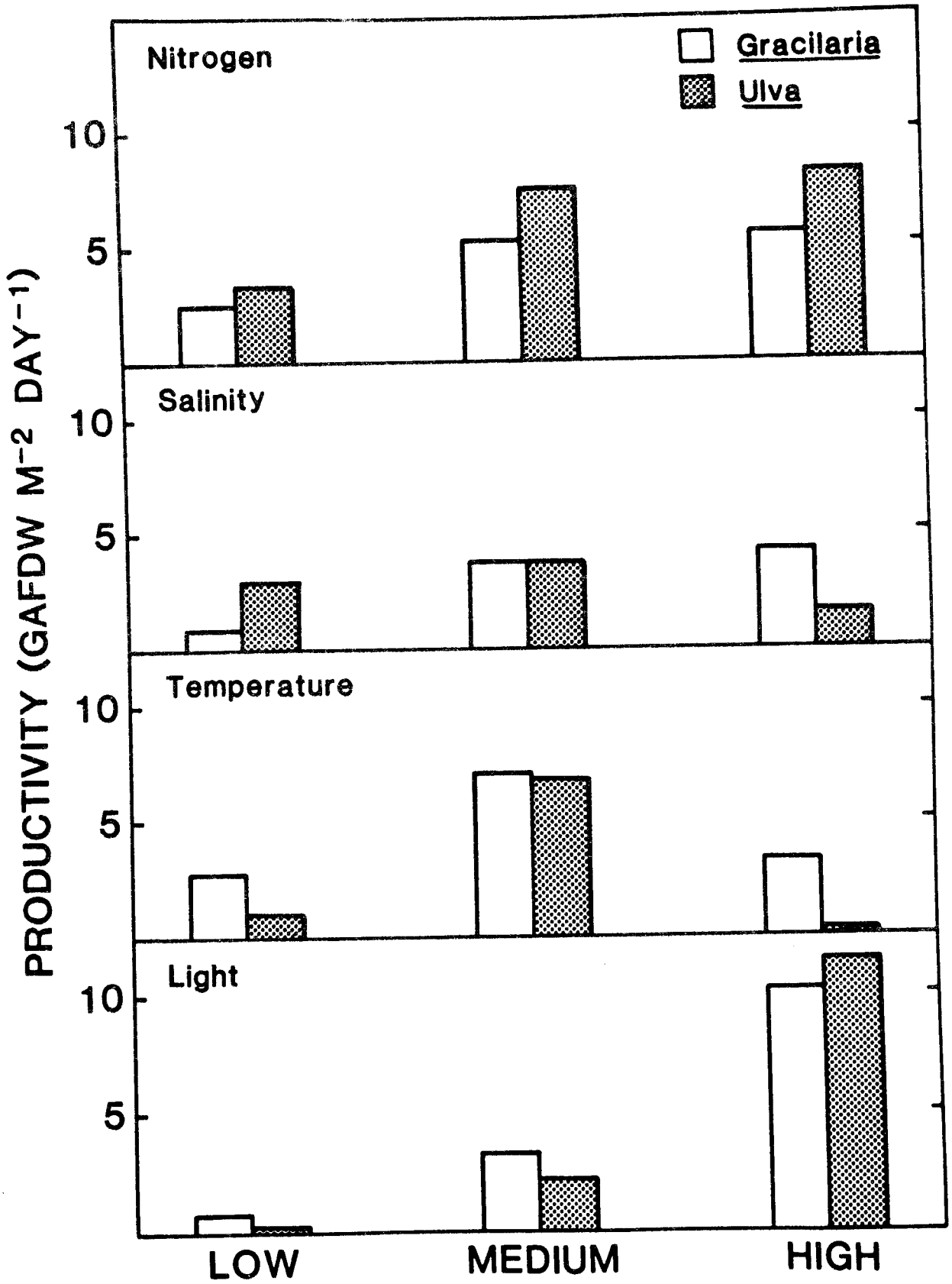

Figure 13. Effect of four environmental parameters on the productivity (on an ash-free dry weight basis) of Ulva and Gracilaria. Low, medium and high treatment regimes are described in text.

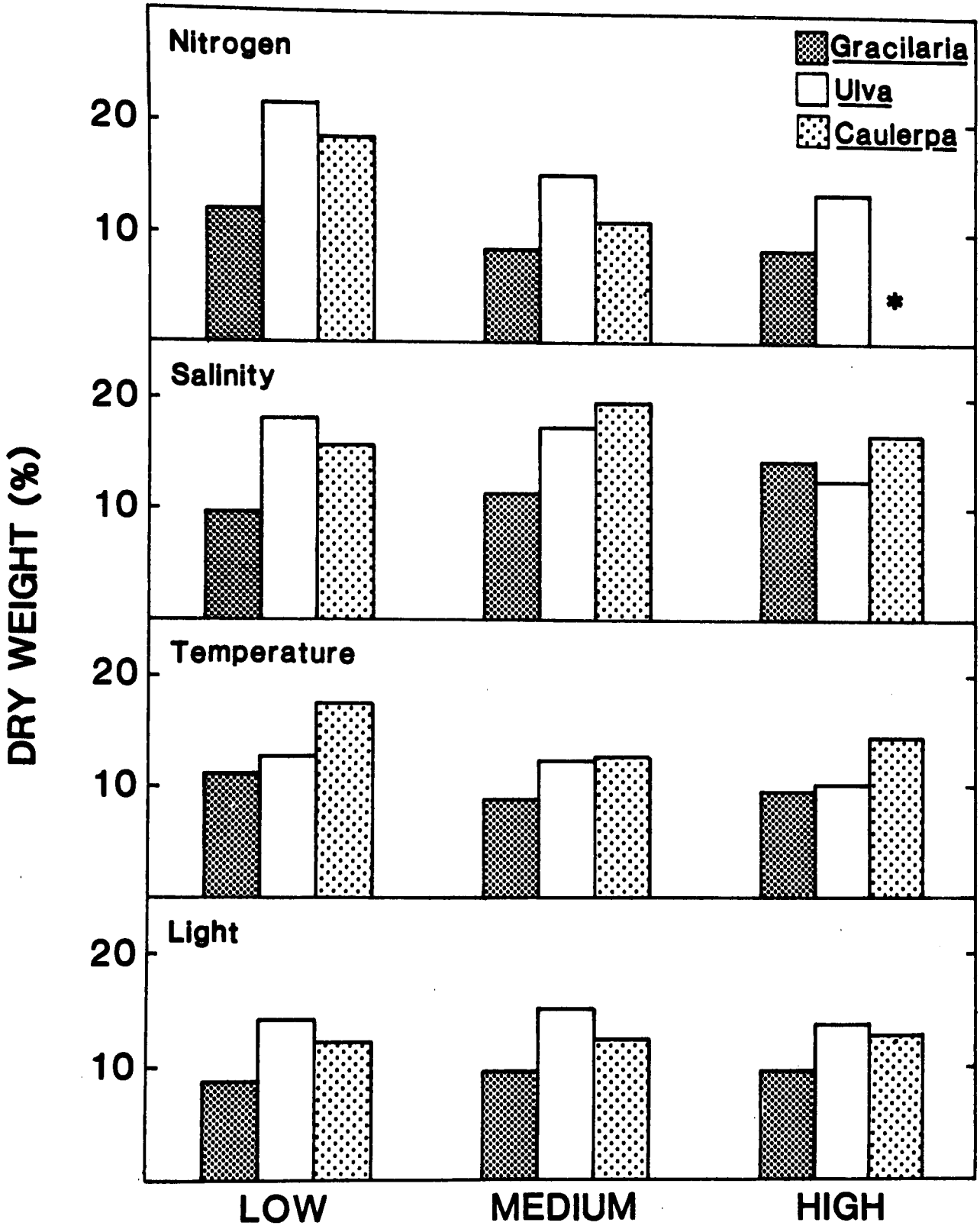

Figure 14. Effect of four environmental parameters on dry weight (as % of wet wt) of three macroalgae. Low, medium and high treatment regimes are described in text.

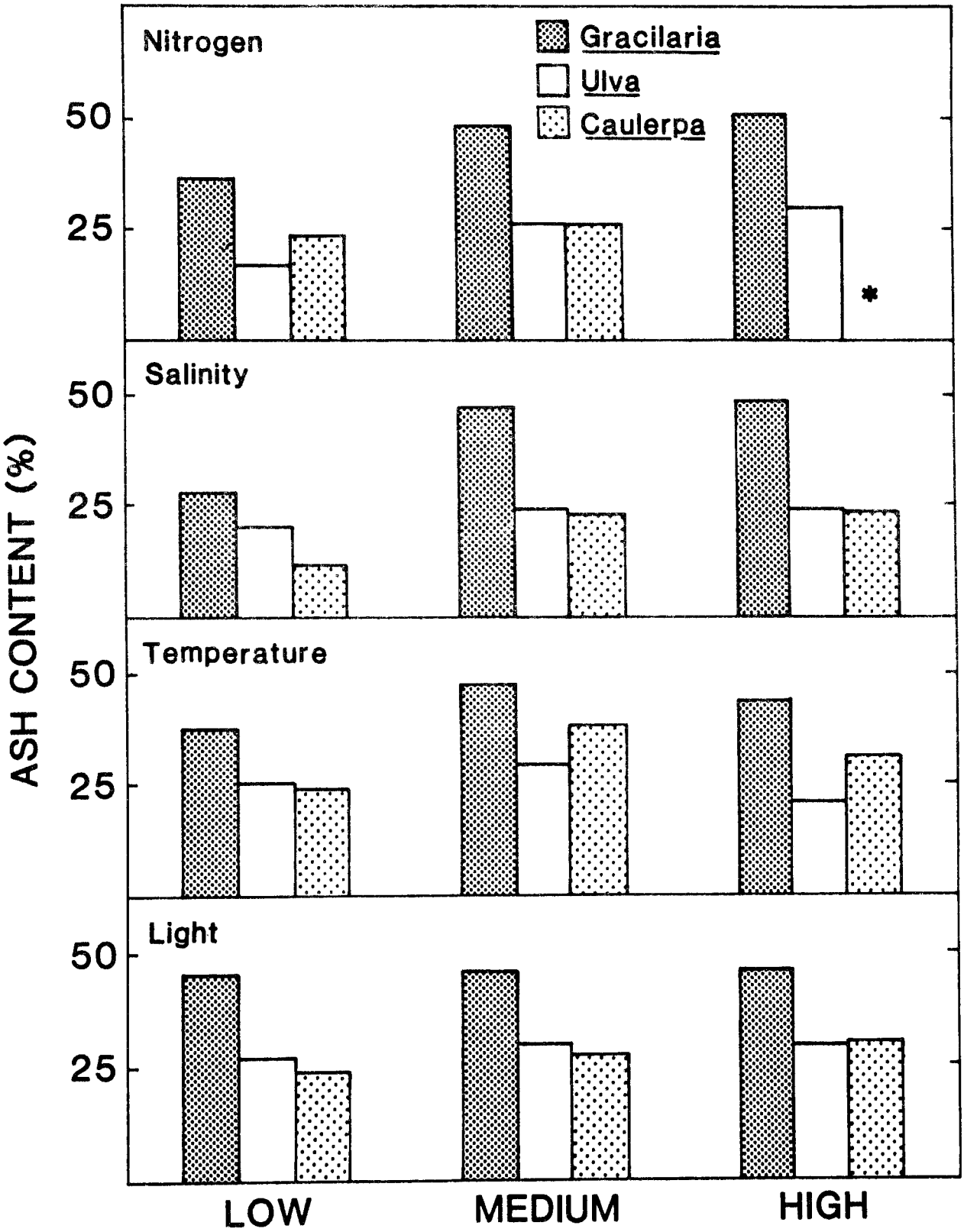

Figure 15. Effect of four environmental parameters on ash content (as % of dry weight) of three macroalgae. Low, medium and high treatment regimes are described in text.

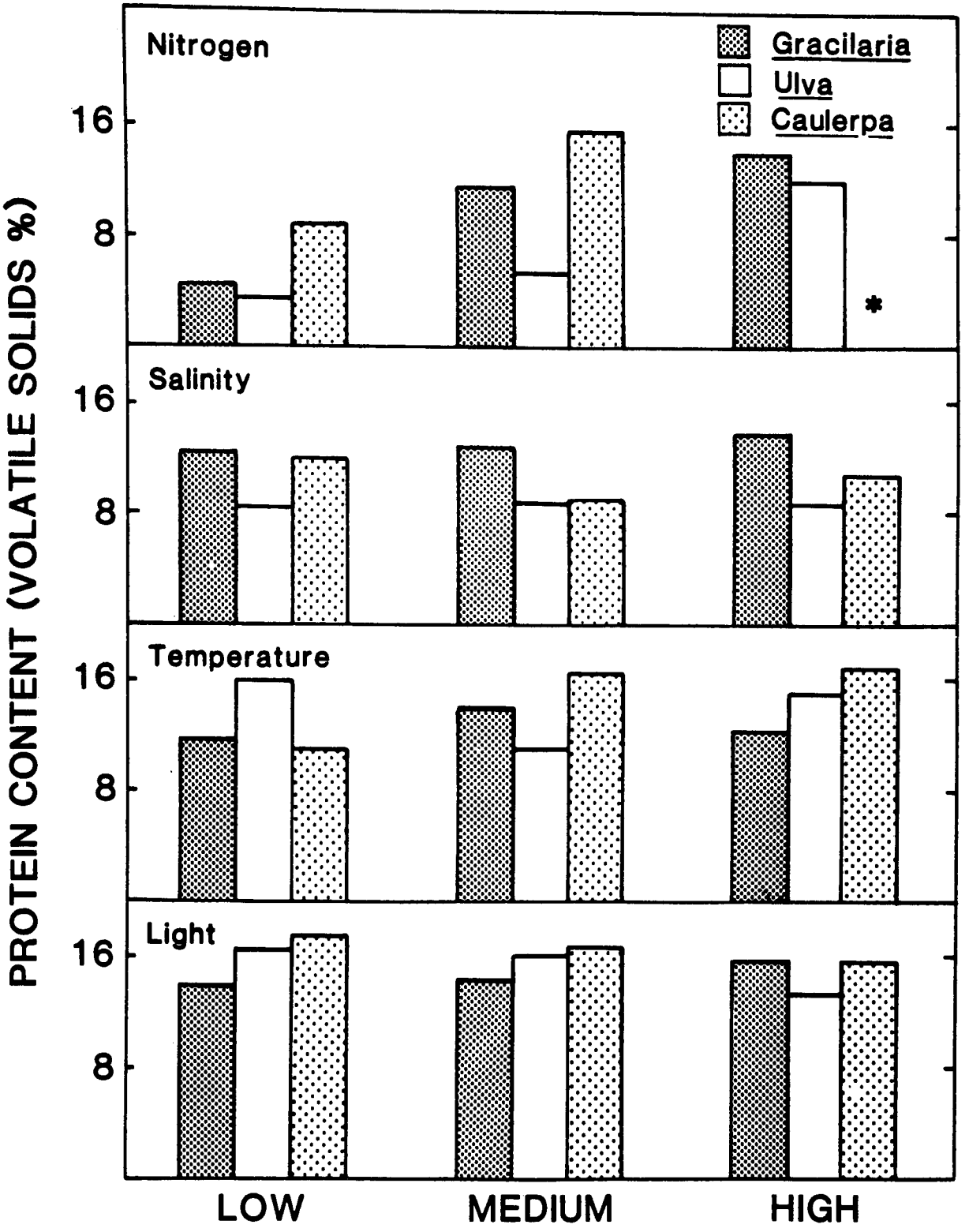

Figure 16. Effect of four environmental parameters on the protein content (as % of volatile solids) of three macroalgae. Low, medium and high treatment regimes are described in text.

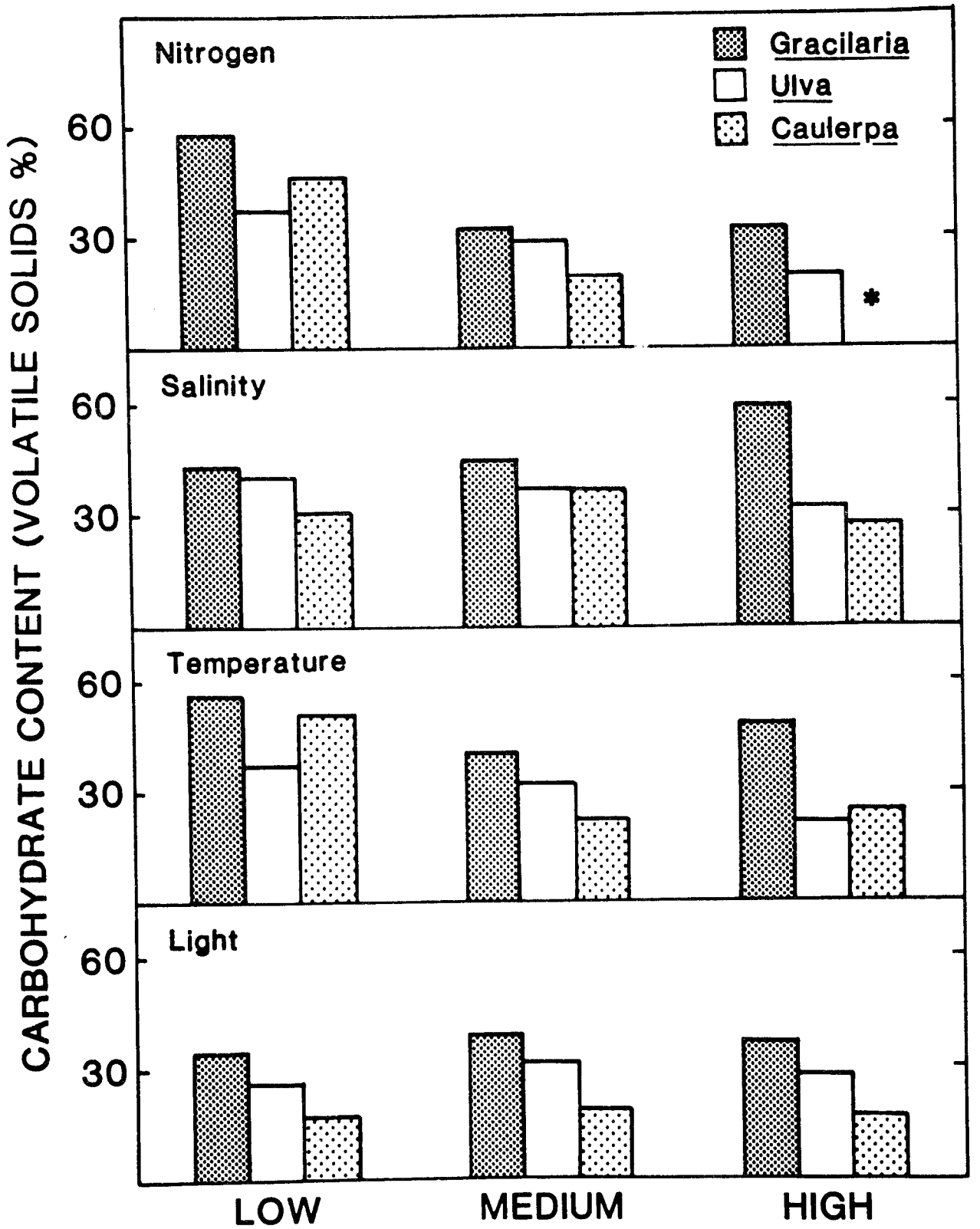

Figure 17. Effect of four environmental parameters on the carbohydrate content (as % of volatile solids) of three macroalgae. Low, medium and high treatment regimes are described in text.

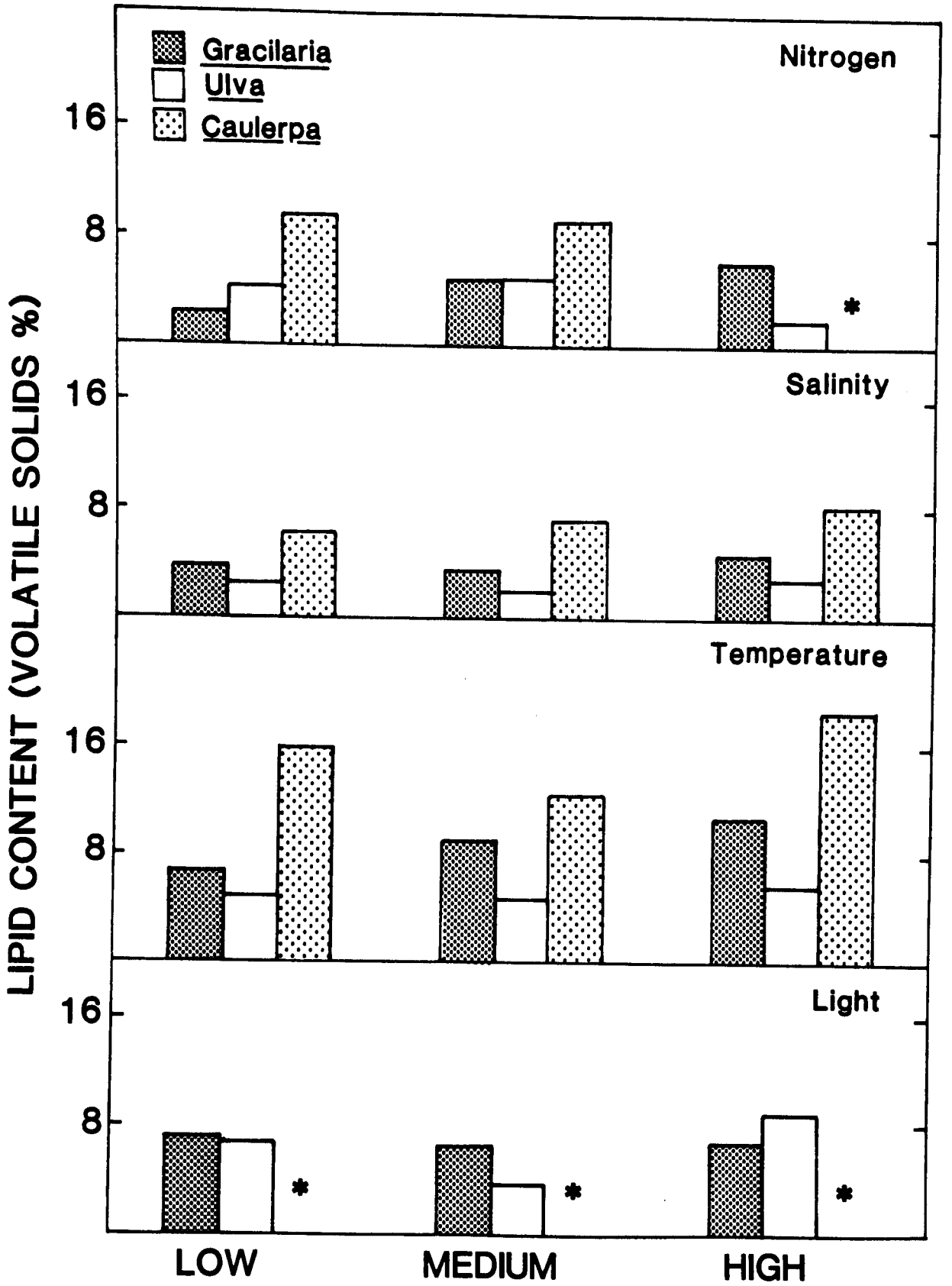

Figure 18. Effect of four environmental parameters on the lipid content (as % of volatile solids) of three macroalgae. Low, medium and high treatment regimes are described in text. The effects of light intensity on lipid content of Caulerpa (not shown here) are depicted in Table 19.

Table 14. Effect of environmental factors in the productivity of *Gracilaria tikvahiae* and *Ulva lactuca*. Values represent difference in productivity (as g AFDW m^{-2} day^{-1}) between experimental and control conditions. See text for description of treatments.

	Gracilaria		*Ulva*	
	Low	High	Low	High
Salinity	-2.8	+0.4	-0.7	-2.2
Nitrogen availability	-2.7	+0.2	-4.1	+0.8
Temperature	-4.2	-3.8	-5.8	-6.8
Light intensity	-2.4	+7.0	-2.1	+9.4

Table 15. Effect of environmental factors on the dry weight of three macroalgae. Values represent difference in dry weight (as % of wet weight) between experimental and control conditions. See text for description of treatments.

	Gracilaria		*Ulva*		*Caulerpa*	
	Low	High	Low	High	Low	High
Salinity	-1.7	+3.0	+0.7	-5.1	-3.8	-2.7
Nitrogen availability	+3.5	-0.1	+6.5	+1.7	+7.6	*
Temperature	+2.3	+0.6	+0.6	-2.2	+4.7	+1.5
Light intensity	-0.6	+0.1	-1.0	-1.4	-0.1	+0.2

*_Caulerpa_ was not cultured under high-nitrogen conditions.

Table 16. Effect of environmental factors on the ash content of three macroalgae. Values represent difference in ash content (as % of dry weight) between experimental and control conditions. See text for description of treatments.

	Gracilaria		*Ulva*		*Caulerpa*	
	Low	High	Low	High	Low	High
Salinity	-19.7	+1.3	-3.8	+0.3	-6.5	-0.2
Nitrogen availability	-11.5	+2.9	-8.3	+4.8	-2.6	*
Temperature	-10.0	-3.9	-3.6	-7.5	-14.0	-5.9
Light intensity	-0.4	+0.2	-2.0	-0.2	-3.9	+2.4

*_Caulerpa_ was not cultured under high-nitrogen conditions.

Table 17. Effect of environmental factors on the protein content of three macroalgae. Values represent difference in protein content (as % of volatile solids) between experimental and control conditions. See text for description of treatments.

	Gracilaria		*Ulva*		*Caulerpa*	
	Low	High	Low	High	Low	High
Salinity	-0.6	+1.0	-0.5	-0.2	+2.9	+1.9
Nitrogen availability	-7.2	+2.3	-1.8	+6.7	-6.8	*
Temperature	-2.1	-1.7	+5.2	+4.1	-5.6	+0.4
Light intensity	-0.4	+1.2	+0.4	-2.8	+1.0	-0.9

*_Caulerpa_ was not cultured under high-nitrogen conditions.

Table 18. Effect of environmental factors on the carbohydrate content of three macroalgae. Values represent difference in carbohydrate content (as % of volatile solids) between experimental and control conditions. See text for description of treatments.

	Gracilaria		*Ulva*		*Caulerpa*	
	Low	High	Low	High	Low	High
Salinity	-2.1	+15.3	+2.4	-5.7	-6.2	-9.4
Nitrogen availability	+25.1	-0.6	+8.1	-9.5	+28.4	*
Temperature	+15.6	+7.9	+4.7	-11.6	+29.3	+2.6
Light intensity	-5.0	-1.8	-4.2	-2.7	-1.7	-1.5

*_Caulerpa_ was not cultured under high-nitrogen conditions.

Table 19. Effect of environmental factors in the lipid content of three macroalgae. Values represent difference in lipid content (as % of volatile solids) between experimental and control conditions. See text for description of treatments.

	Gracilaria		*Ulva*		*Caulerpa*	
	Low	High	Low	High	Low	High
Salinity	+0.4	+0.9	+0.6	+0.7	-0.8	+1.1
Nitrogen availability	-2.4	+1.2	-0.6	-3.1	+0.4	*
Temperature	-2.3	+1.7	+0.3	+1.2	+3.6	+6.6
Light intensity	+0.9	+0.4	+3.0	+4.8	+6.5	-6.5

*_Caulerpa_ was not cultured under high-nitrogen conditions.

Storage product yields.

For each experiment, protein, carbohydrate and lipid concentrations were multiplied by the rates of organic matter production, thus providing a measure of the daily production of these constituents by Gracilaria and Ulva.

Maximum protein production by these two algae occurred under conditions of high light intensity, and high nitrogen availability in the medium (Fig. 19). Although maximum thallus protein levels were not found at the highest light intensity, the rapid productivity of the algae in this treatment resulted in high protein yields (ca 1.4 g $m^{-2}day^{-1}$). In the N-availability experiment, the high nitrogen treatment resulted in highest thallus protein concentrations as well as rapid seaweed growth. Other conditions under which protein production by Gracilaria was maximized were high salinity and medium temperature. The maximum protein production for Ulva also occurred at the medium temperature, but at the medium (29 ppt), rather than the high salinity.

Maximum carbohydrate production by Ulva and Gracilaria occurred under high light conditions (Fig. 20), primarily because of the rapid productivity of the algae in this treatment. In contrast with protein, however, maximum carbohydrate production in the N-availability experiment occurred at medium nitrogen levels. Conditions of medium salinity and temperature resulted in maximum carbohydrate production by Ulva, but for Gracilaria, medium temperature and high salinity were the environmental conditions which favored carbohydrate production.

Conditions of high light and medium temperature stimulated maximum lipid production by these macroalgae (Fig. 21). For Gracilaria, high salinity and high nitrogen conditions also enhanced lipid production, whereas for Ulva, lipid production was maximized at medium nitrogen and medium salinity levels.

Production of useful storage products by macroalgae is dictated by plant growth rate, as well as the concentrations of the particular compounds in the thallus. In the present study, plant productivity was found to be the dominant factor influencing total production of lipids, protein and carbohydrates by Gracilaria and Ulva, with percent composition of these compounds being of secondary importance. Ideally, macroalgae should be cultivated under conditions which maximize both growth rate and tissue concentration of the desired storage product. Unfortunately, our data show that environmental conditions under which relative levels of these useful products are highest are often not conducive to rapid plant growth. Protein production is one exception: high nitrogen levels in the growth medium not only stimulated productivity, they also resulted in algal tissues of high protein content. In contrast, maximum carbohydrate and lipid production may be attained using a different strategy, i.e., that of cultivating the algae under optimum conditions for growth in order to obtain maximum biomass

production, then exposing this plant material to conditions which would stimulate increased tissue levels of the desired storage product (e.g., low light for lipids, low nitrogen for carbohydrate). This technique has not been investigated, but its success will depend largely on the time required to increase tissue concentrations of the various proximate constituents.

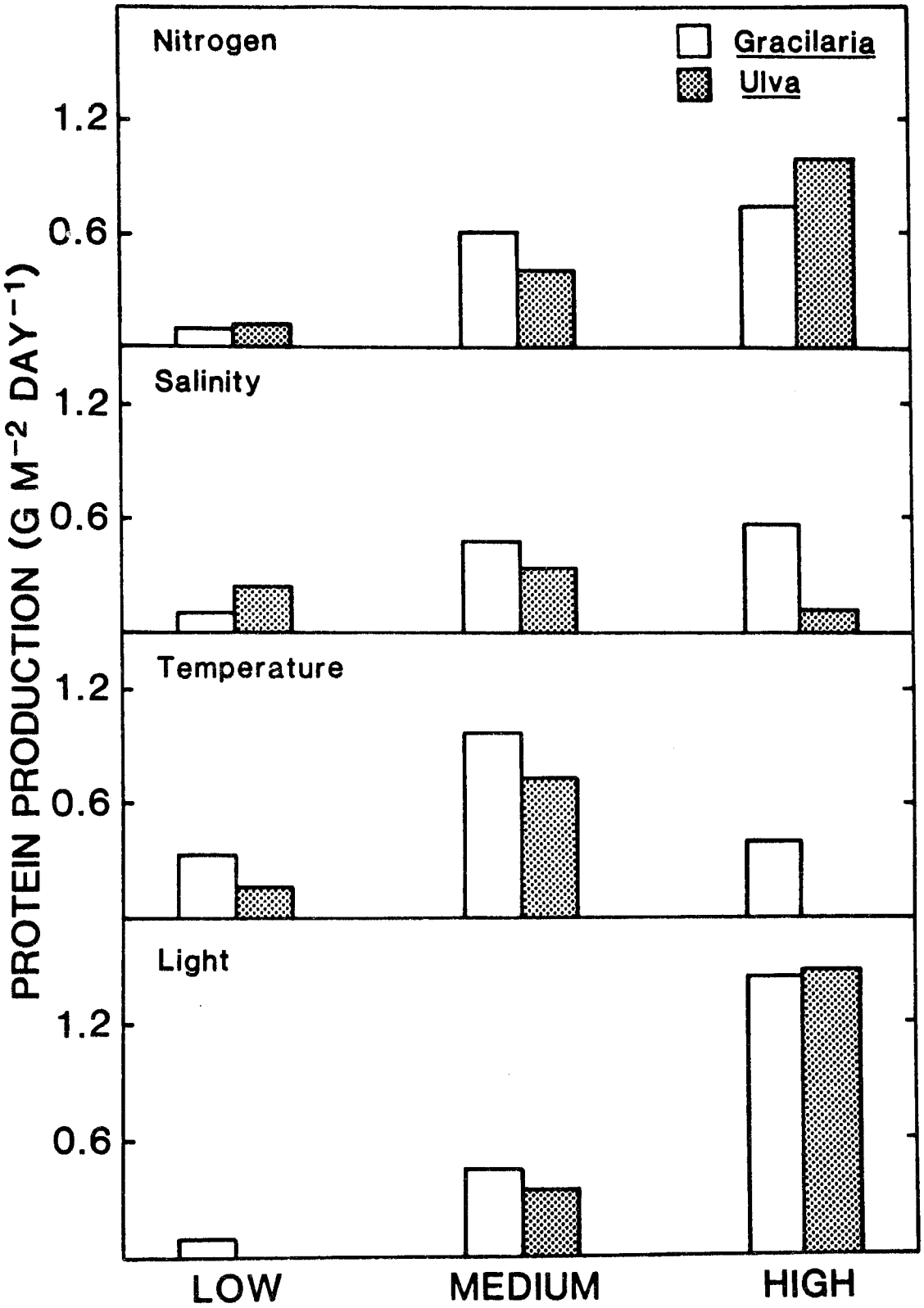

Figure 19. Daily protein production by Ulva and Gracilaria under a range of environmental conditions. Low, medium and high treatment regimes are described in text.

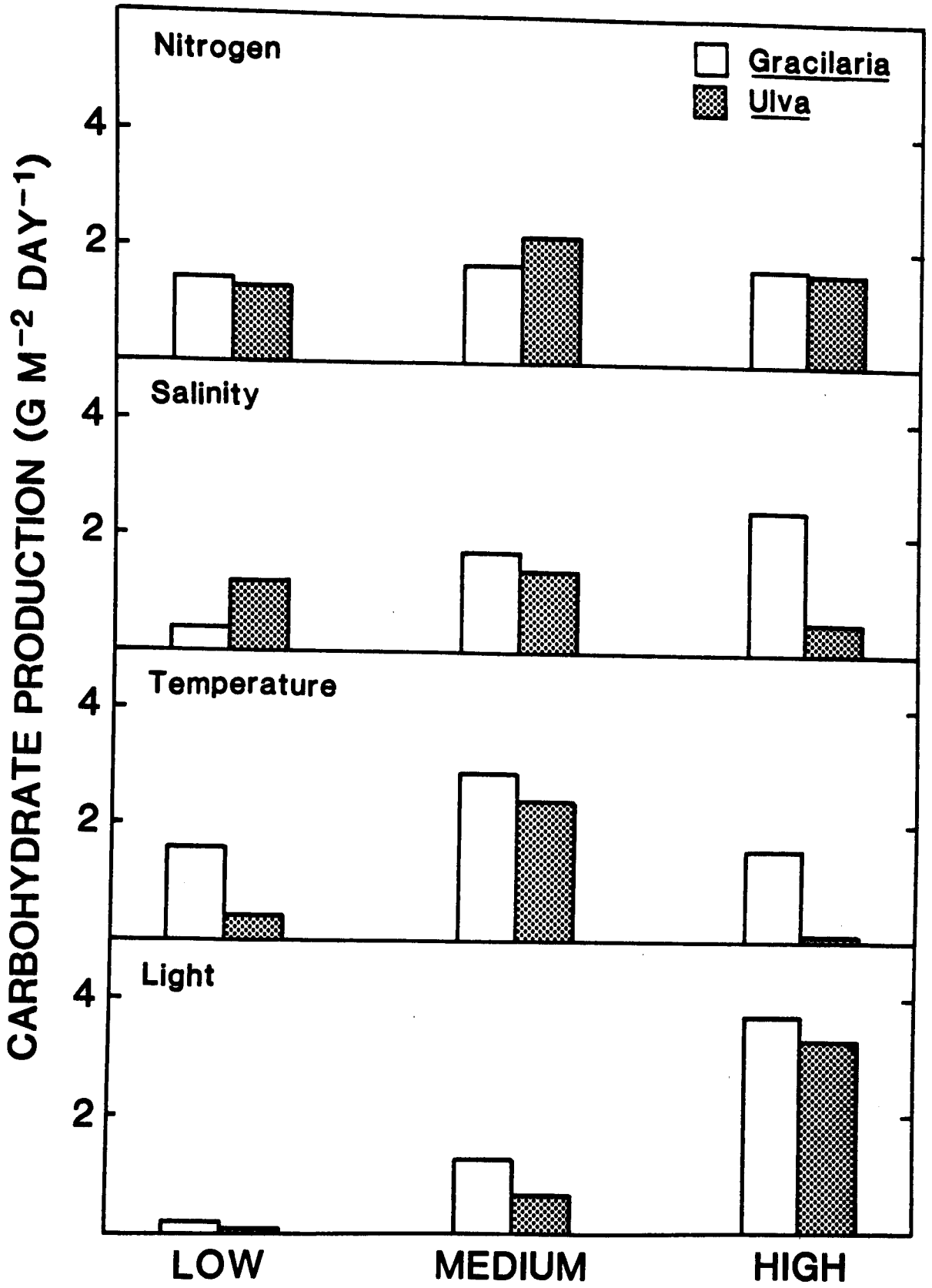

Figure 20. Daily carbohydrate production by Ulva and Gracilaria under a range of environmental conditions. Low, medium and high treatment regimes are described in text.

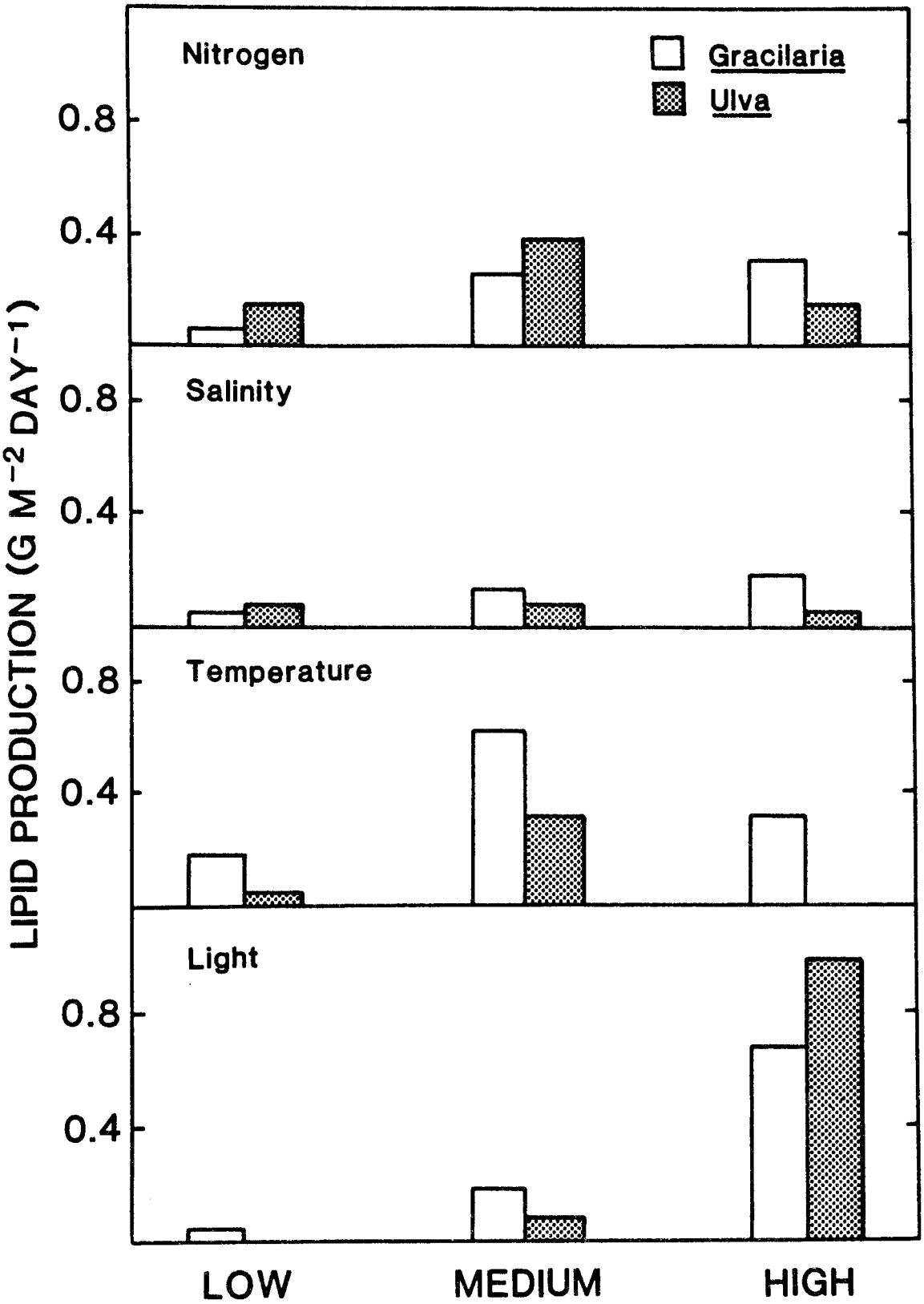

Figure 21. Daily lipid production by Ulva and Gracilaria under a range of environmental conditions. Low, medium and high treatment regimes are described in text.

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16. Abstract (Limit: 200 words)
The objectives of the research for which results are presented in this report were: (1) to determine the growth potential of marine macroalgae cultivated in desert saline waters, and (2) to determine how certain environmental stresses (e.g., nitrogen, salinity, and temperature) affect the proximate composition of several marine macroalgae. Two major desert saline water types were assayed for their ability to support the growth of Gracilaria, Ulva, and Caulerpa. Both water types supported short term growth, but long term growth was not supported. Carbohydrate levels in Gracilaria were increased by cultivation under conditions of high salinity, low temperature, and low nitrogen and phosphorous availability. Data suggests that it may be possible to maximize production of useful proximate constituents by cultivating the algae under optimum conditions for growth, and then holding the resulting biomass under the environmental conditions which favor tissue accumulation of the desired storage products.

17. Document Analysis

a. Descriptors Algae ; Deserts ; Growth ; Nitrogen ; Phosphorous ; Photosynthesis ; Salinity ; Seawater ; Ulva ; Water

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