

## ***Gracilaria conferta* and its epiphytes: (2) Interrelationship between the red seaweed and *Ulva* cf. *lactuca***

E. Svirski<sup>1</sup>, S. Beer<sup>2</sup> & M. Friedlander<sup>1\*</sup>

<sup>1</sup>Israel Oceanographic & Limnological Research, National Institute of Oceanography, Tel-Shikmona, P.O.B. 8030, Haifa 31080, Israel (\*author for correspondence); <sup>2</sup>Dept. of Botany, Tel-Aviv University, Tel-Aviv 69978, Israel

**Key words:** *Gracilaria*, *Ulva*, epiphytes

### **Abstract**

*Ulva* cf. *lactuca* has been a disturbing competitor of experimental *Gracilaria conferta* outdoor cultures in Israel. The effect of environmental conditions on the competitive ability of *Ulva* versus *Gracilaria*, and the main limiting factors for which these seaweeds compete, were studied here. Single and biculture experiments of both seaweeds showed that *Ulva* outgrew and damaged *Gracilaria* under all irradiance and temperature combinations. The higher competitive ability of *Ulva* cf. *lactuca* in bicultures was not a result of responses to shading or nitrogen shortage, but rather to a shortage of available inorganic carbon, an increase in pH and apparent excretions of *Ulva* which inhibited the growth of *Gracilaria*.

### **Introduction**

One of the main problems of *Gracilaria* sp. cultivation in Israel (Friedlander *et al.*, 1987) and in other places (Lapointe & Ryther, 1978) is the rapid development of accompanying algae (epiphytes), such as *Ulva* sp., in the cultivation tanks. The accompanying algae are mainly thread- or sheet-like, and therefore have a high surface/volume ratio (SA/V, after Littler & Littler, 1980). Due to this structure, these algae show high rates of nitrogen uptake and photosynthesis, resulting in high growth rates (Friedlander, 1992). They are often limited in the ability to accumulate nitrogen and are therefore dependent on a constant high level of nitrogen in the medium. *Gracilaria*, however, has exactly opposite qualities (Ryther *et al.*, 1981; Bird *et al.*, 1982). Performing competition experiments between *Chondrus crispus* and *Ulva lactuca*, Enright (1979) compared their growth in

separate and common dishes, and found that *Ulva* showed dominance under all temperature and irradiance combinations.

In this work we investigated the interactions between *Gracilaria conferta* and its epiphyte, *Ulva* cf. *lactuca*. The two main objectives were to elucidate the effect of environmental conditions on the competitive ability of *Ulva* versus *Gracilaria*, and to find the main limiting factors for which these seaweeds compete in culture conditions.

### **Materials and methods**

The two algal species used in this study were local *Gracilaria conferta* (Schoesboe) J. & G. Feldmann, and *Ulva* cf. *lactuca* Le Jolis. Both species were inoculated in outdoor seawater tanks as reported earlier (Friedlander *et al.*, 1987). For outdoor experiments, water flow was set to 4 ex-

changes per day, daily average photon irradiance was  $130 \mu\text{mol m}^{-2} \text{s}^{-1}$  (12:00 hr, 5 cm under the water), and average daily water temperature was  $26^\circ\text{C}$ . The weekly additional weight was discarded after starting with 200 g per tank, and the initial seaweed ratio was kept stable in the tanks.

For the gradient table and growth room experiments, fresh healthy thalli were taken from the storage tanks and sectioned to  $2 \text{ cm}^2$  marginal pieces (*Ulva*) or young tips of 2–6 branchings (*Gracilaria*). Four small petri dishes ( $4.5 \times 3.5 \text{ cm}$ ) were placed in every position on the growth gradient table, which was operated as reported earlier (Friedlander, 1992). The dishes in each position were moved clockwise every day. In the growth room, the dishes (200 ml) were placed on a shaker (40 rpm) under  $25^\circ\text{C}$  and  $130 \mu\text{mol m}^{-2} \text{s}^{-1}$ , and the *Gracilaria* was weighed weekly, after which the medium was renewed. In a competition experiment, the limitation of nutrients was excluded by 3 pulse feedings during the one week experimental period. In an attempt to exclude irradiance limitations, a 'biculture' of *Gracilaria* and *Ulva* simulation in the form of green plastic flakes was performed. In a two-week experiment, *Gracilaria conferta* and *Ulva cf. lactuca* were bicultured (3U:1G) or separately cultured with 3 nutrient pulses per week. pH titration to pH 7.5 with diluted HCl or  $\text{CO}_2$  was performed daily. In the  $\text{CO}_2$  titration experiment, the biculture was performed using plastic nets to separate the two species in the dishes. The medium of a dense, week-old *Ulva* batch culture was filtered through Whatman filter paper and called 'Ulva water'. Twenty-eight batches of 'Ulva water' were extracted with ethyl acetate, and the organic phase was dried by evaporation and finally dissolved in seawater to obtain 'Ulva water extract'. The biological effect of both media on the growth rate of *Gracilaria* was investigated in glass dishes.

An initial weight of 1.6–1.8 g seaweeds was introduced into 4 double wall pH controlled glass cylinders with 500 ml enriched growth medium as reported earlier (Friedlander, 1992). Four pieces of *Ulva* and 4 pieces of *Gracilaria*, having an initial weight of 3:1, were individually marked and introduced into the  $\text{CO}_2$  controlled medium. Re-

sults of experiments are presented with standard deviation by numbers ( $\pm \text{SD}$ ) or bars.

## Results

Outdoor cultures of *Gracilaria* (Fig. 1) showed fluctuating growth rates as a function of time. However, when they were bicultured with *Ulva* (1:3), there was a very slow but significant decrease in *Gracilaria* growth rate. Figure 2 shows that the growth rate of *Ulva*, in a biculture (1:1) with *Gracilaria*, was maximal under high photon irradiance ( $354 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) at a relatively wide range of temperatures ( $20\text{--}30^\circ\text{C}$ ). The growth rate of *Gracilaria* under *Ulva*'s optimal conditions was minimal, and its thallus was badly damaged by bleaching and disintegration, showing a significant negative correlation with *Ulva cf. lactuca* ( $r = -0.37$ ;  $p = 0.009$ ). Figure 3 shows that *Gracilaria* in single culture did not show any sign of damage (Fig. 3A), but the larger the ratio of *Ulva* in the medium, the faster and more profoundly was the *Gracilaria* damaged (Fig. 3B–D).

A competition experiment showed that the growth rate of *Ulva cf. lactuca* significantly de-

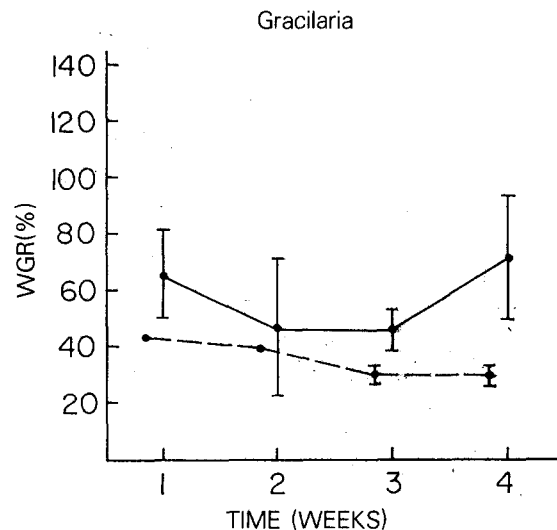


Fig. 1. Average weekly growth rates of *Gracilaria conferta* grown separately (100 g; solid line) and together with *Ulva cf. lactuca* (dashed line; initial biomass ratio of 3U:1G) in tanks under outdoor conditions ( $n = 2$ ).

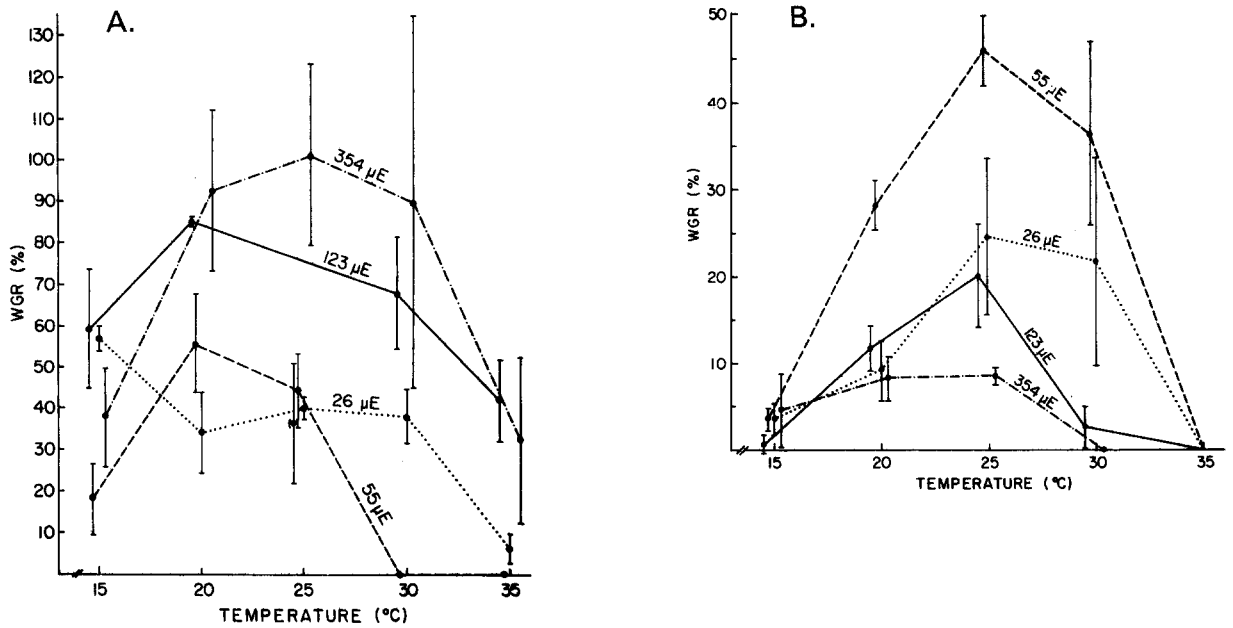


Fig. 2. Effects of irradiance and temperature variation on the weekly growth rate (%) of *Ulva cf. lactuca* (A) and *G. conferta* (B) grown in the same dishes on a growth gradient table. The initial biomass ratio was 1:1 (100 mg total biomass), and the sterile seawater was enriched (PEM, Provasoli, 1968) only in the beginning ( $n = 3-4$ ).

creases as its ratio in the biculture increases (Fig. 4). On the other hand, *Gracilaria conferta*, which showed an overall much lower growth rate than *Ulva cf. lactuca*, exhibited a significant positive relationship between its growth rate and its initial relative ratio in the biculture. A simulation showed (Fig. 5) that the growth rate of *Gracilaria* decreased slightly with its increasing ratio in the

'biculture' medium with green plastic, similar to a control experiment of *Gracilaria* in a single culture.

The growth rate of *Gracilaria conferta* in enriched *Ulva* medium was significantly higher than

Table 1. Average weekly growth rate (%) of *Gracilaria conferta* grown for 7 days in a control or a treated medium. Each medium was enriched with PEM ( $n = 3-4$ ). Different letters express for each row significantly different growth rates between the treatment and control by Duncan's test at a significance level of  $p < 0.05$

Treatment	Week	Average weekly growth rate (%)	
		Control	Treatment
<i>Ulva</i> medium	1	31.4 ± 4.8a	44.6 ± 5.9b
Extract of <i>Ulva</i> medium	1	27.8 ± 4.7a	13.7 ± 1.9b
	2	26.6 ± 14.5a	14.2 ± 2.6b

Table 2. Weekly growth rates of *Gracilaria conferta* and *Ulva cf. lactuca* grown together in glass cylinders with PEM enriched seawater which was pH controlled with CO<sub>2</sub>. Different letters express significantly different growth rates for each species by Duncan's test at a significance level of  $p < 0.05$

Species	pH	Average weekly growth rate (%)	
		First week	Second week
<i>Gracilaria</i>	8.0	31.8 ± 4.3a	26.7 ± 4.0a
	8.5	28.4 ± 7.0a	18.9 ± 2.9ab
	9.0	13.4 ± 11.2b	14.3 ± 8.2bc
	not controlled	16.9 ± 3.0b	6.8 ± 1.3c
<i>Ulva</i>	8.0	76.1 ± 2.0a	53.2 ± 6.4a
	8.5	82.3 ± 10.2a	40.4 ± 8.3ab
	9.0	85.2 ± 23.8a	48.1 ± 11.8ab
	not controlled	92.3 ± 21.0a	45.6 ± 15.0ab

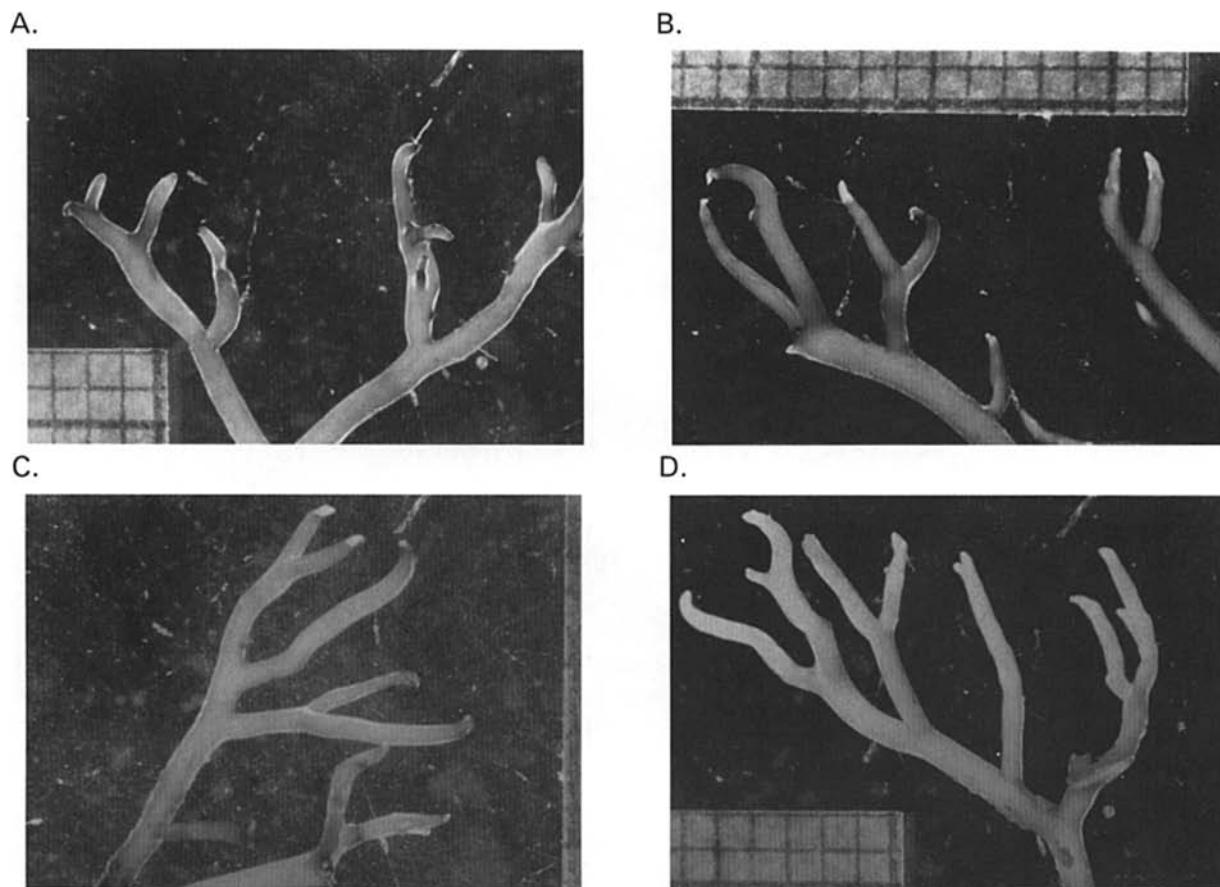


Fig. 3. *Gracilaria conferta* (G) which grew during 9 days in the same dishes with *Ulva cf. lactuca* (U) in different biomass ratios with a total density of 400 mg: A. 4G:0U. B. 3G:1U. C. 1G:1U. D. 1G:3U.

in seawater enriched with PEM (Table 1), while *Gracilaria* growth in extract of *Ulva* medium was significantly inhibited as compared to the control. Biculture growth of *Gracilaria conferta* and *Ulva cf. lactuca* in pH controlled cylinders (Table 2) showed that a change from pH 8 to higher or non-controlled levels decreased *Gracilaria*'s growth rate. *Ulva cf. lactuca* was not significantly affected by the controlled and non controlled pH variable.

Growth rate of both seaweeds in the first week of an HCl titration experiment was highest, and decreased in the following week (Table 3). *Gracilaria* showed higher growth rates in the separate culture (pH 8.8–9.2) than in biculture (pH 9.2–9.9) in both titrated and non titrated treatments. After one week, the first bleached tips appeared in the non titrated biculture, while after

Table 3. Average weekly growth rate (%) of *Gracilaria conferta* (G) and *Ulva cf. lactuca* (U) grown separately and together in the same dishes with an initial biomass ratio of 3U:1G. Dishes were titrated daily (+) or not titrated (-) with HCl to pH 7.5 in the enriched growth medium. Different letters express significantly different growth rates for each seaweed by Duncan's test at a significance level of  $p < 0.05$

Seaweed	Treatment	Weekly growth rate (%)	
		First week	Second week
<i>Gracilaria</i>	G (+)	49.0 ± 10.0a	34.5 ± 2.5a
	G (-) (1)	43.4 ± 6.0a	31.5 ± 9.2a
	G + U (+)	7.6 ± 1.4b	1.8 ± 2.0b
	G + U (-) (2)	10.3 ± 2.6b	-7.8 ± 6.5c
<i>Ulva</i>	U (+)	107.9 ± 8.7a	54.9 ± 11.8a
	U (-) (3)	105.7 ± 11.4a	38.9 ± 8.5a
	G + U (+)	155.7 ± 9.9b	45.7 ± 7.2a
	G + U (-) (4)	164.4 ± 37.2b	46.0 ± 22.0a

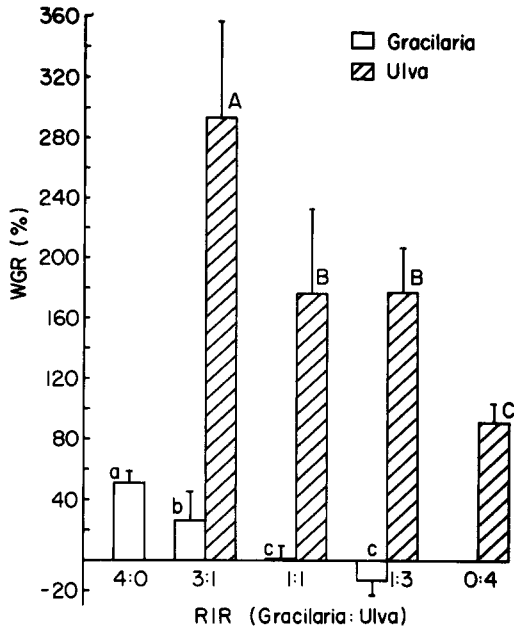


Fig. 4. Average weekly growth rates of *G. conferta* and *Ulva* cf. *lactuca* grown in the same dishes as a function of the initial biomass ratio of the seaweeds (total 400 mg). Growth media were enriched three times during the one-week experiment. Different letters express significantly different growth rates (by Duncan's test, at a significance level of  $p < 0.05$ ;  $n = 3-4$ ).

two weeks they appeared also in the titrated biculture. *Ulva*, on the other hand, showed a higher

Table 4. Average weekly growth rate (%) of *Gracilaria conferta* (G) and *Ulva* cf. *lactuca* (U) grown in separate dishes and together in the same petri dishes with an initial biomass ratio of 3U:1G but separated by a net. Dishes were titrated daily (+) or not titrated (-) with  $\text{CO}_2$  to pH 7.5. Different letters express significantly different growth rates for each seaweed by Duncan's test at a significance level of  $p < 0.05$ .

Seaweed	Treatment	Weekly growth rate (%)	
		First week	Second week
<i>Gracilaria</i>	G (+)	62.7 ± 6.8a	30.0 ± 11.9a
	G (-) (1)	27.8 ± 4.7b	26.6 ± 14.5a
	G + U (+)	26.7 ± 9.2b	15.4 ± 5.1a
	G + U (-)	10.4 ± 6.6c	disintegrated
<i>Ulva</i>	U (+)	51.7 ± 31.9b	92.9 ± 24.8a
	U (-) (3)	161.3 ± 61.2a	111.0 ± 49.9a
	G + U (+)	87.7 ± 15.1b	28.7 ± 31.9a
	G + U (-) (4)	81.3 ± 25.4b	79.0 ± 19.9a

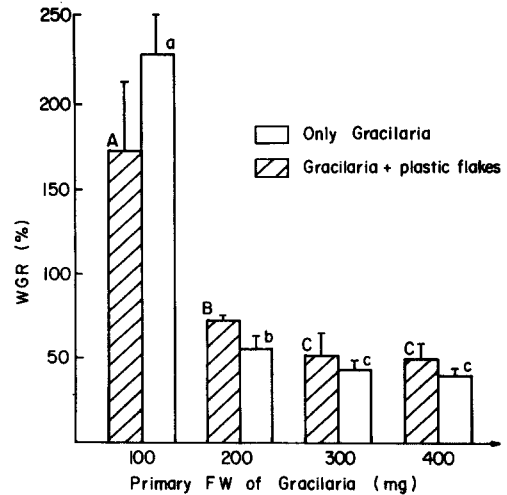


Fig. 5. An average weekly growth rate of *G. conferta* grown separately or together with plastic flakes simulating *Ulva*, as a function of the initial weight ratio (total 400 mg). Growth media were enriched three times during the one-week experiment. Different letters express significantly different growth rates (by Duncan's test, at a significance level of  $p < 0.05$ ;  $n = 3-4$ ).

growth rate in biculture than in separate dishes, in the first week only. No difference was shown in the HCl treated as compared to non treated cultures. The pH of any *Ulva* medium (9.2–9.4) was significantly higher than the separate *Gracilaria* medium (8.6–8.8). Growth rate of *Gracilaria* was inversely correlated with pH levels ( $r = -0.79$ ) and with the calculated  $\text{HCO}_3^-$  concentration ( $r = -0.871$ ), while growth rate of *Ulva* was positively correlated with pH ( $r = 0.71$ ) and with DIC (dissolved inorganic carbon) concentration ( $r = 0.62$ ). Daily titration with  $\text{CO}_2$  increased separate *Gracilaria*'s growth rate as compared to non titrated cultures or to *Gracilaria* in biculture (Table 4). Titration with  $\text{CO}_2$  and biculture decreased *Ulva*'s growth rate significantly as compared to *Ulva* alone, in the first week of the experiment.

## Discussion

Single and biculture growth trials of *Gracilaria conferta* and *Ulva* cf. *lactuca* showed that *Ulva*

had higher growth rates than *Gracilaria* under all irradiance and temperature combinations. These results concur with those of Enright (1979) concerning bicultures of *Ulva* and *Chondrus*. *Ulva* is known as a fast grower, as compared to most algae, and this phenomenon can be explained either by its unique optical characteristics (Ramus, 1983; Rosenberg & Ramus, 1984) or on the wider basis of the 'form-function hypothesis' (Littler & Littler, 1980). The use of plastic *Ulva* simulation or physical separation between the two species showed that *Ulva* did not form a light limiting factor for the *Gracilaria* in any of the experiments. Self shading of *Gracilaria* did, however, appear in single and bicultures. Since bleaching and reduced growth rate of *Gracilaria* continued to appear in biculture, in spite of frequent pulse feeding, it was concluded that nutrient shortage was not its cause.

Since *Ulva*'s superior growth rate in the biculture is likely due to faster metabolism, it might affect the medium by CO<sub>2</sub> removal and by causing a higher pH in the medium, or by excretion of secondary metabolites. The present experiments showed clearly that *Ulva* medium slightly promoted *Gracilaria*'s growth rate, while the extract of *Ulva* medium was found to inhibit *Gracilaria* growth rate, suggesting the probable presence of inhibiting *Ulva* metabolites. It might, however, be also inhibited by a toxic organic acetate complex. In this experiment, growth rates of *Gracilaria* showed a negative correlation with pH, confirming studies with *G. tikvahiae* (DeBusk & Ryther, 1984). At the pH of seawater (ca 8.2), most of the DIC is in the form of bicarbonate. An intensive uptake of bicarbonate or CO<sub>2</sub> increases the pH level and decreases the availability of these forms as well as the rate of photosynthesis (Sze, 1986). Determination of DIC forms in the growth media showed that in the presence of *Ulva*, there is no CO<sub>2</sub> available. Addition of CO<sub>2</sub> increased *Gracilaria* and *Ulva* growth rate, suggesting that the CO<sub>2</sub> was available for *Ulva* and for *Gracilaria*. The fact that CO<sub>2</sub> titration antagonized almost completely the inhibited *Gracilaria* in the presence of *Ulva* suggests that CO<sub>2</sub> is the main limiting factor. This suggestion is supported by the recent

report of Beer (unpublished) claiming that *Gracilaria* does not contain the membrane bound bicarbonate transport protein which appears in *Ulva*.

It may be concluded that *Ulva* dominates *Gracilaria* in a biculture, not because of shading or shortage of nutrients, but presumably by *Ulva*'s possible inhibiting excretion and mainly by a shortage of an available form of inorganic carbon.

## References

- Bird, K. T., C. Habig & J. T. DeBusk, 1982. Nitrogen allocation and storage patterns in *Gracilaria tikvahiae* (Rhodophyta). *J. Phycol.* 18: 344–348.
- DeBusk, T. A. & J. H. Ryther, 1984. Effects of seawater exchange, pH and carbon supply on *Gracilaria tikvahiae* (Rhodophyceae) in large-scale cultures. *Mar. Biol.* 27: 367–362.
- Enright, C. T., 1979. Competitive interaction between *Chondrus crispus* (Florideophyceae) and *Ulva lactuca* (Chlorophyceae) in *Chondrus* aquaculture. *Proc. Int. Seaweed Symp.* 9: 209–218.
- Friedlander, M., 1992. *Gracilaria conferta* and its epiphytes. 1. The effect of culture conditions on growth. *Bot. mar.* (in press).
- Friedlander, M., R. Shalev, T. Ganor, S. Strimling, A. Ben-Amotz, H. Klar & Y. Wax, 1987. Seasonal fluctuations of growth rate and chemical composition of *Gracilaria cf. conferta* in outdoor culture in Israel. *Hydrobiologia* 151/152: 501–507.
- Lapointe, B. E. & J. H. Ryther, 1978. Some aspects of the growth and yield of *Gracilaria tikvahiae* in culture. *Aquaculture* 15: 185–193.
- Littler, M. M. & D. S. Littler, 1980. The evolution of thallus form and survival strategies in benthic marine macroalgae: field and laboratory tests of the functional form model. *Am. Nat.* 116: 25–44.
- Provasoli, L., 1968. Media and prospects for cultivation of marine algae. In A. Watanabe & A. Hattori (eds), *Cultures and Collections of Algae*. Proc. U.S.-Japan Conf., Hakonate, Japan. *Soc. Plant Physiol.*, Sept. 1966: 63–75.
- Ramus, J., 1983. A physiological test of the theory of complementary chromatic adaptation. II. Brown, green and red seaweeds. *J. Phycol.* 19: 173–178.
- Rosenberg, G. & J. Ramus, 1984. Uptake of inorganic nitrogen and seaweed surface area: volume ratios. *Aquat. Bot.* 19: 65–72.
- Ryther, J. H., N. Corwin, T. A. DeBusk & L. D. Williams, 1981. Nitrogen uptake and storage by the red algae *Gracilaria tikvahiae* (McLachlan, 1979). *Aquaculture* 26: 107–115.
- Sze, P., 1986. *A Biology of the Algae*. Wm. C. Brown Publishers, Dubuque, Iowa, p. 178.