Competitive Interactions between the Agarophyte *Gracilaria verrucosa* (Gracilariales, Rhodophyta) and the Problem Alga *Ulva Lactuca* (Ulvales, Chlorophyta) from Saldanha Bay in South Africa

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ABSTRACT

Cast up material of *Gracilaria verrucosa* (Hudson) Papenfuss in Saldanha Bay was recently found to be intermixed with *Ulva lactuca* Linnaeus. This caused problems for the local seaweed industry which makes use of the beach casts of *Gracilaria* as the two species could not be separated resulting in large amounts of seaweed being discarded. The source of the *Ulva lactuca* was found to be a large bed that formed in the vicinity of an effluent plume from a fish factory leading to the hypothesis that the bloom established in response to enhanced nutrient supply.

In this study competitive interactions between the two species at different nitrogen concentrations were investigated. The growth of *Ulva lactuca* at different light levels as well as factors that might promote fertility were also examined to see if these had an influence on the distribution of the species within the Bay.

It was expected that at high, non-limiting levels of ammonium *U. lactuca* would display competitive superiority due to the high surface to volume ratios of the thallus promoting faster uptake. However, little competitive interaction was displayed at both high and low ammonium concentrations. The *U. lactuca* was also found to have much lower growth rates than *G. verrucosa* in all treatments which is in sharp contrast to the findings of other studies.

The growth of *U. lactuca* at different light intensities demonstrated a response typical of a shade adapted plant. The intermittent availability of nutrients appears to be more important in preventing the growth of this species at greater depths as it was found to be much more reliant on a continuous supply of ammonium.

An investigation into factors such as different light levels, temperatures and nutrient availability in promoting fertility over a full lunar cycle was unsuccessful in producing any fertile material.

Thus, it appears that *Ulva lactuca* from Saldanha Bay exhibits features that differentiates it from specimens obtained from other areas. They are found to be slower growing, adapted to low-light levels and do not produce fertile material. These characteristics may be related to their occurrence within a bay system.

INTRODUCTION

Species of *Ulva* have often been noted to be problematic under conditions of eutrophication. A recent bloom of *Ulva lactuca* Linnaeus in Saldanha Bay appears to be linked to the increase in ammonium in effluents released from a fish factory and has interfered in the *Gracilaria* industry in the area. This study was thus initiated to quantitatively assess growth and competitive interactions of these two species under different nitrogen concentrations as well as to investigate growth of *U. lactuca* in response to different light levels and the factors promoting fertility.

Taxonomy and distribution of the two species involved in this study

The genus *Gracilaria* contains seaweeds of commercial value that are utilised for the extraction of agar. In South Africa, species of this genus occur in sheltered habitats in St Helena Bay, Velddrif, Saldanha Bay and Langebaan Lagoon, Table Bay, False Bay and the estuary at the Swartkops River mouth (Stegenga *et.al.*, 1994). They form beds in sandy or muddy areas with the thalli floating near the bottom or partially covered by the shifting sands (Isaac, 1956; Simons, 1977).

The largest populations occur in the Saldanha Bay - Langebaan Lagoon system on the West coast, 100 km north of Cape Town. These form the only natural populations capable of supporting an agar industry (Anderson *et.al.*, 1989; Rotmann, 1990). Langebaan lagoon largely falls within the South West Coast National Park thus commercial exploitation only occurs in Saldanha Bay (Anderson *et.al.*, 1992). The species in Saldanha Bay is commonly referred to as *Gracilaria verrucosa* (Hudson) Papenfuss but there is not absolute certainty as to the correct applicability of this specific epithet.

The scarcity of fertile material in Saldanha Bay impedes identification as species differentiation within this genus is largely based on details of the carposporophyte and the spermatangia (Bird and McLachlan, 1984; Fredericq and Hommersand, 1989a). At one time it was thought that *Gracilaria verrucosa* was confined to the North East Atlantic (Fredericq and Hommersand, 1989a) but subsequent investigations have

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uncovered specimens that correspond to this species *sensu* Fredericq and Hommersand (1989a) in areas as diverse as Europe, Argentina and Japan. These populations were also shown to display similar restriction profiles of the nuclear encoded 18S rDNA (Bird and Rice, 1990; Scholfield *et.al.*, 1991). It has also been suggested that two genera should be delimited, namely *Gracilaria* and *Gracilariopsis* (Fredericq and Hommersand, 1989b, 1990), but this appears to have created much confusion. Furthermore certain studies indicate that the characters used to differentiate the genera (and thus probably the species too) may not be reliable (Bird and McLachlan, 1984; Chang and Xia, 1984; Bird and Rice, 1990; Gargiulo *et.al.*, 1992).

Fertile material has previously been reported in Saldanha Bay when Isaac (1956) noted cystocarpic plants throughout the year and tetrasporic plants for most of the year. He mentioned the presence of tubular nutritive cells within the carposporophyte thus indicating the species to belong to the *Gracilaria* genus *sensu* Fredericq and Hommersand (1989a). Material has been reported in False Bay and Swakopmund that corresponded more to the genus *Gracilariopsis* Dawson (Stegenga *et.al.*, 1994).

Species of *Gracilaria* that occur further north along the West coast at Lüderitz in Namibia that only form vegetative populations have been identified as *Gracilaria verrucosa* using restriction profiles of plastid DNA (Rice and Bird, 1990; Scholfield *et.al.*, 1991) and nuclear sequencing (Bird *et.al.*, 1994). It would be interesting to similarly analyze the populations from Saldanha Bay.

Five species of *Ulva* are reported to occur in South Africa in the mid to lower littoral zone (Joska, 1992). The species occurring in the Saldanha Bay region were identified as *Ulva lactuca* Linnaeus. This is a cosmopolitan species which was found to be uncommon in the South Western Cape occurring only in small discrete populations. It occurs mostly on rocky substrates but in Saldanha Bay it is also found on loose sandy substrates. It is not restricted to sheltered areas as is *Gracilaria*.

A brief history of the Gracilaria industry

South African *Gracilaria* was first used in the agar industry after interruption in the supplies of agar from Japan in the Second World War (Anderson *et.al.*, 1989; Critchley and Rotmann, 1992). Up to 1000 tonnes dry weight per annum were cast up at irregular intervals with the largest deposits occurring after heavy south easterlies of the summer months (Isaac, 1956). This lead to the establishment of two agar processing factories in the 1960s. Unfortunately, after the construction of a large jetty and a breakwater in Saldanha Bay in 1974, the algae ceased to be cast up on the beaches and the industry collapsed (Anderson *et.al.*, 1989). A slow recovery of cast up material ensued with 429 dry tonnes being exported in 1988 (Anderson *et.al.*, 1993). However an abrupt cessation again occurred towards the end of 1988 the reasons for which are uncertain (Anderson *et.al.*, 1993). Studies of the beds by Anderson *et.al.* (1993) indicated a decline in size as the year progressed. An unbalanced system appeared to be occurring with the low abundance of *Gracilaria* being incapable of supporting grazers which rapidly consumed the remaining material preventing the reestablishment of the beds.

Recovery began in 1991 and extensive beds could again be found in 1992 as well as the reoccurrence of cast up material with 191 tonnes of dry weight being recovered (Anderson *et.al.*, 1993). The situation continued to improve and 378 tonnes dry weight were collected in 1993 (Anderson and Monteiro, in preparation).

All the material is collected as beach cast which is then dried, sorted and cleaned. No agar processing plants are in operation at present and the higher quality dried seaweed is exported to Japan, Korea and Chile, with material rejected by these buyers being processed into agar in a factory established at Lüderitz in Namibia (Critchley and Rotmann, 1992). Secondary processing within South Africa may be economically viable but the unpredictability of cast up material indicates the investigation into mariculture techniques (Anderson *et.al.*, 1989, Critchley and Rotmann, 1992).

Problems in the seaweed industry arising from *U. lactuca*

In August of 1993 the cast up *Gracilaria* was intermingled with large amounts of *Ulva lactuca* which on drying was virtually impossible to separate resulting in large amounts of the mixed seaweed being discarded (Anderson and Monteiro, in preparation). This caused considerable financial losses to the seaweed industry.

It has previously been reported that green seaweeds, particulary species of *Ulva*, may cause considerable problems in coastal waters that have become eutrophic (Vollenweider, 1992). These green algal species have also been reported to cause reduced production of *Gracilaria* in tank cultures and in mariculture as a result of epiphytism (Friedlander and Ben Amotz, 1991; Buschmann and Gómez, 1993; Haglund and Pedersén, 1993; Pickering *et.al.*, 1993) or non epiphytically by direct competition where *Ulva* species have been shown to have a competitive advantage at all light and temperature combinations provided nutrients are non-limiting (Enright, 1978; Svirski *et.al.*, 1993). Abundance of these problem algae was shown to decrease with a reduced source of ammonium (Friedlander and Ben Amotz, 1991) although frequency of supply rather than concentration was found to have the more significant effect (Pickering *et.al.*, 1993).

CHONDRUS

Distribution of the two species and nutrient availability in Saldanha Bay

Gracilaria forms extensive beds from about 1.5m or 2.5m to 9m below MLWS with a lower limit of occurrence at 10m whereas *U. lactuca* is found in shallower waters usually growing in isolated patches at a depth of 2m to 5m below MLWS (Anderson and Monteiro, in preparation).

The water column at Saldanha Bay displays two layered stratification with a well developed thermocline in summer. This stratification begins in spring when periodic upwelling of the Benguela system causes an inflow of cold upwelled water of 10°C to 12°C into the Bay area. This cold water moves in under the surface water mass that in summer is warmed to 18°C to 20°C.

The position of the thermocline displays oscillatory behaviour with the inward flow of cold water occurring every 6 to 7 days. This causes shoaling of the thermocline to about 5m to 7m with the concomitant outflow of warm surface water (Monteiro and Brundrit, in press). The lower layer of cold water then retreats and the thermocline deepens to about 10 m with an inflow of warm surface water. The upwelling is driven by summer south easterly winds every six to seven days. Depths of less than 5m to 7m are thus permanently warm in summer with temperatures usually above 18 °C.

Destratification occurs in Autumn under the influence of strong northerly winds and a decreased solar flux resulting in mixing of the two relatively shallow layers of the water column leaving similar temperatures throughout (Monteiro and Brundrit, in press).

A relationship between the water temperature and nutrient levels has been established (Monteiro and Brundrit, in press). The deeper cold water layer has a greater nutrient concentration including nitrogen largely in the form of nitrates which is brought to the photic zone during the periods of upwelling. Planktonic blooms form which rapidly utilise the nitrate in about 3 to 4 days. Dead organic matter and detritus then sink to the bottom again where mineralisation takes place and nutrients are again available to be moved higher into the photic zone with the next upwelling. The warm surface water above 5m remains oligotrophic in the absence of anthropogenic influences.

The upwelled water is said to bring 20 to 25μM of nitrate-N to the photic zone whereas the upper oligotrophic layer has very low values close to zero (Monteiro and Brundrit; in press). Ammonium concentrations were found to be 4 to 8μM below the thermocline and less than 1μM above the thermocline.

The biomass of *Gracilaria* is found to be highest in the depth range of 4 to 6m, the same depths at which the upwelled water periodically reaches thus allowing the *Gracilaria* to receive nitrogen rich water in a pulsed manner every 6 days. As this species has been reported to be able to store nitrogen for 7 to 14 days (Ryther *et.al.*, 1981) and maintain non-limiting growth levels for this period in the absence of an external nitrogen source this fluctuating environment would seem to favour the growth of this species and prevent much *Ulva* being established in this region. The species is seldom found at depths less than 2m which would be well out of the range of the upwelled water. The lower limit of occurrence is presumed to be due to light limitations.

The cause of the *Ulva* bloom

A large bed of *Ulva lactuca* was formed in a specific area of the bay at a depth of 2m to 5.5m below MLWS and so occurs in the 'oligotrophic' layer. The bed was first noted in August 1993 and grew in size up until February 1994 but had virtually disappeared by May 1994 (Anderson and Monteiro, in preparation).

Organic waste from a fish factory is released into the bay. The concentration of nitrogen released from the pipe was shown to be considerably higher than usual from July until at least August 1993. No values are available from September to January, but the concentration had decreased to previous lower levels by February 1994 (P. Monteiro, pers. comm.). The bed of *Ulva* is in the path that the effluent takes which, as a result of the surface circulation patterns of spring and summer, is buoyant and within the warm, oligotrophic layer.

Thus the effluent plume appears to be providing an alternative source of nitrogen mainly in the form of ammonia. Past eutrophication events in the area have also been attributed to effluents from fish factories (Monteiro *et.al*, 1990). The approximate nitrogen flux that was calculated using the area of the visible plume was in the order of 1670 μM. m^{-2}. h^{-1} (Monteiro and Brundrit, in press). Thus the anthropogenic supply of nitrogen and high light levels are thought to be sustaining the growth of *Ulva* in the 'oligotrophic' upper layer of the water column. The deep limit of the bed is thought to be due to light limitations (Anderson and Monteiro, in preparation). Analysis of stable isotope ratios indicates that much of the nitrogen source for the *Ulva* bloom appears to be derived from ammonia from the effluent rather than the usual nitrate supply (Monteiro *et.al.*, in preparation).

Objectives of this study

G. verrucosa and *U. lactuca* from Saldanha Bay were analyzed in laboratory cultures to assess the competitive interaction between the two species under different nitrogen concentrations, the hypothesis being that *U. lactuca* exhibits competitive superiority at higher concentrations of ammonium. The species were grown in monocultures and in mixed cultures to determine the optimum concentration for growth and to see if either species showed suppressed or improved growth rates in the presence of the other species compared to when grown alone.

The distributions mentioned above state that the lower limits of the species are determined by light levels and implies that *U. lactuca* may require higher light levels than. *G. verrucosa*. Thus the light curve of *U. lactuca* was also determined in order to compare it with that obtained for *G. verrucosa* (Engledow and Bolton; 1992) with the hypothesis being that *U. lactuca* will exhibit light curves more characteristic of a sun adapted plant.

Conditions that might promote fertility in *U. lactuca* were also investigated to determine if this might have had any influence on the colonisation abilities of this species and so favouring its establishment in an area that may have been cleared of both species as a result of toxic levels of ammonium.

MATERIALS AND METHODS

Maintenance of Stock Cultures

Gracilaria verrucosa and *Ulva lactuca* were collected from Saldanha Bay. A stock culture of seaweed was maintained in separate, square, glass boxes each containing 1.6 litres of seawater and were kept in a constant temperature room at 15°C at low light intensities around 10 $\mu E.m^{-2}.s^{-1}$. Seawater was changed and a nutrient medium based on Provasoli Enrichment Medium was added weekly (R. Anderson, pers. comm.; McLachlan, 1973). The ingredients used to make up the nutrient medium are detailed in Appendix I. An amount of $6ml.l^{-1}$ of nutrient medium was added weekly.

Monocultures grown at different ammonium concentrations

The growth rates of monocultures of *G. verrucosa* and *U. lactuca* were assessed at six different nitrogen concentrations. Nitrogen was added in the form of NH_4Cl as a preference for ammonium over nitrate has been observed widely in seaweeds and above a certain concentration may inhibit nitrate or nitrite uptake (D'elia and DeBoer, 1978; Ryther *et.al.*, 1981; Hanisak, 1990; Haglund and Pedersén, 1993). Ammonium was found to be preferred over nitrate even in plants preconditioned on the latter as a sole source of nitrogen (DeBoer *et.al.*, 1978).

Seawater collected offshore was passed through filters with a pore size of 0.2μm in diameter. The seawater was then heated on two consecutive days for four to five hours at 90°C in a water bath. The intervening period of cooling encourages the germination of spores that may have formed on the first heating episode (Hamilton, 1973; Prescott *et.al.*, 1990). Conical flasks used for growing the experimental cultures were heat sterilised overnight in an oven at 100°C prior to the start of the experiments.

200ml of the sterilised seawater was added to each 250ml conical flask. This lower amount was used to facilitate water movement in the flasks when shaken gently on a shaker. A range of NH_4Cl concentrations were added to each flask. The concentrations used were 1 (the amount naturally present in the seawater with no NH_4Cl added), 10, 100, 200, 500 and 1000 μM NH_4^+-N. Four replicates were used at each concentration.

The mass of seaweed placed in each culture vessel was 100mg of fresh weight. *G. verrucosa* was added in the form of 15mm long tips and *U. lactuca* was cut into circles with a diameter of 11mm using a cork borer. Careful weighing was required as the seaweed pieces needed to be gently blotted dry to remove excess moisture and then quickly weighed and placed in the culture medium before any desiccation started to occur. After the addition of the seaweeds the mouths of the flasks were covered with clear cellophane and placed on a gentle shaker at 50 rpm in the phytotron set so that water temperatures of 15°C and a light intensity of 80 $\mu E.m^{-2}.s^{-1}$ occurred.

The experiment was run for seven days with fresh seawater and nutrients added on the fourth day. The flasks were rotated daily to prevent the possibility of very localised conditions affecting the outcome.

At the end of the seventh day the seaweed was removed from the flasks, carefully blotted dry and weighed as before and the Specific Growth Rate (SGR) determined using the equation below:

$$\text{SGR (\% per day)} = (100(\ln (N_t/N_0)))/t$$

where N_t = final weight in mg

N_0 = initial weight

t = time in days.

The residual ammonium levels remaining within the flasks was measured using a manual method for determining ammonium levels in seawater as set out in Appendix II (Sea Fisheries Research Institute, pers. comm.). The afternoon pH levels at the termination of the experiment were also measured.

Competition Experiment

The experiment was set up as for the previous experiment but using only two ammonium concentrations, namely the unenriched seawater at 1 μM NH_4^+-N, and 200μM NH_4^+-N, the concentration that was indicated by the previous experiment to induce maximal growth in both species. Monocultures with 100mg of seaweed per vessel were again set up to be used as controls as well as mixed cultures containing 50mg of each species. Four replicates were again used for each treatment.

SGRs, residual ammonium concentrations and pH levels were again determined at the end of the experiment. In addition, de Wit replacement series were determined using the final mass data (Begon *et.al.*, 1990). In the competition curves generated by this method if straight lines are produced that species is presumed to be unaffected by the presence of the other species. A concave curve would indicate inhibition of growth and a convex one enhancement of growth in the presence of the other species.

Nitrate and phosphate levels in the seawater prior to the experiment and in the culture media at the termination of the experiment were analyzed by the Sea Fisheries Research Institute using an automated photometric method that is a slightly modified version of that used by Solorzano and Sharp (1980) for nitrate determination and that used by Murphy and Riley (1962) for phosphate determination.

Dry Weight Ratios

To determine the dry weight to wet weight ratios of the two species, pieces of both seaweeds were carefully blotted dry and weighed as before. Four measurements were taken for each species. These were then placed in an oven at 60°C for two days and then reweighed.

SGR and Light Intensity in *U. lactuca*

U. lactuca was exposed to nine different light levels: 3, 10, 24, 40, 76, 146, 250, 310 and 480 $\mu E.m^{-2}.s^{-1}$. Four replicates were used at each light level. Seaweed masses of 100mg were placed in each 250ml conical flasks in 200ml of seawater and covered with clear cellophane. Different light intensities were achieved with the use of shade cloth. The experiment was conducted in the phytotron with water temperatures of 15 °C. Nutrients were added every second day in the form of PEM as used for the stock cultures and the seawater was changed halfway through the experiment. The position of each flask within each treatment was rotated daily. Measurements of pH were taken twice daily in the morning and the afternoon.

Light measurements were also obtained from Saldanha Bay (R. Anderson, pers. comm.) using a Li-Cor underwater spherical quantum sensor. These light levels were measured at noon on a sunny cloudless day in August with good visibility of 2 metres.

Fertility of *U. lactuca*

In order to see if fertile material could be induced, 100 mg masses of *U. lactuca* were placed in 200 ml of seawater in 250 ml conical flasks and placed at various light levels at 15°C and 20°C and in both enriched and non-enriched media. Nutrients were added in the form of PEM described in appendix I to half of the cultures. The light levels used were 10, 40, 80, 180, 300 and 500 $\mu E.m^{-2}.s^{-1}$. Nutrients were added where applicable and the seawater changed weekly. The experiment was run over a full lunar cycle.

Analysis of variance was used to compare different treatments in the various experiments as well as paired t-tests in the case of the competition experiment. Each data set was checked for normality using normal probability plotting and the Kolmogorov-Smirnov test.

RESULTS

Monocultures grown at different ammonium concentrations

The Specific Growth Rates (SGRs) of *G. verrucosa* and *U. lactuca* grown at five different ammonium concentrations are illustrated in figure 1. The final ammonium concentrations remaining in the culture vessels at the end of the experiment are also depicted. Mean values ± Standard Errors (SE) of the SGRs, final ammonium concentrations and pH levels are listed in table 1. (The full data set can be found in Appendix III).

Significantly higher SGRs occurred for both species at an initial ammonium concentration of 200 μM NH_4^+-N. An increase in concentration to 500 μM caused slight to partial disintegration of the thalli in three of the four culture vessels containing the *U. lactuca*. A greater amount of disintegration occurred in all *U. lactuca* cultures at the highest ammonium concentration of 1000μM. *G. verrucosa* displayed no disintegration at these higher levels, nor did any bleaching occur which has sometimes been described as possibly resulting from toxicity (Svirski *et.al.*, 1993). However, *G. verrucosa* did display significantly lower growth rates at the highest ammonium concentration than at all other concentrations. *G. verrucosa* exhibited significantly higher growth rates than *U. lactuca* at all ammonium concentrations.

Final ammonium concentrations, which would have been measured three days after the ammonium additions, indicated that it was virtually used up at the two lowest concentrations of 1 μM and 10 μM for both species, as well as very little remaining in the *U. lactuca* cultures at the 200 μM concentration. Excess ammonium was found to occur in all the other treatments. The residual ammonium levels of the *G. verrucosa* cultures at 200 μM was significantly higher than that of the two lower concentrations and the *U. lactuca* cultures at 200 μM, but significantly less than that of the two highest concentrations for both species.

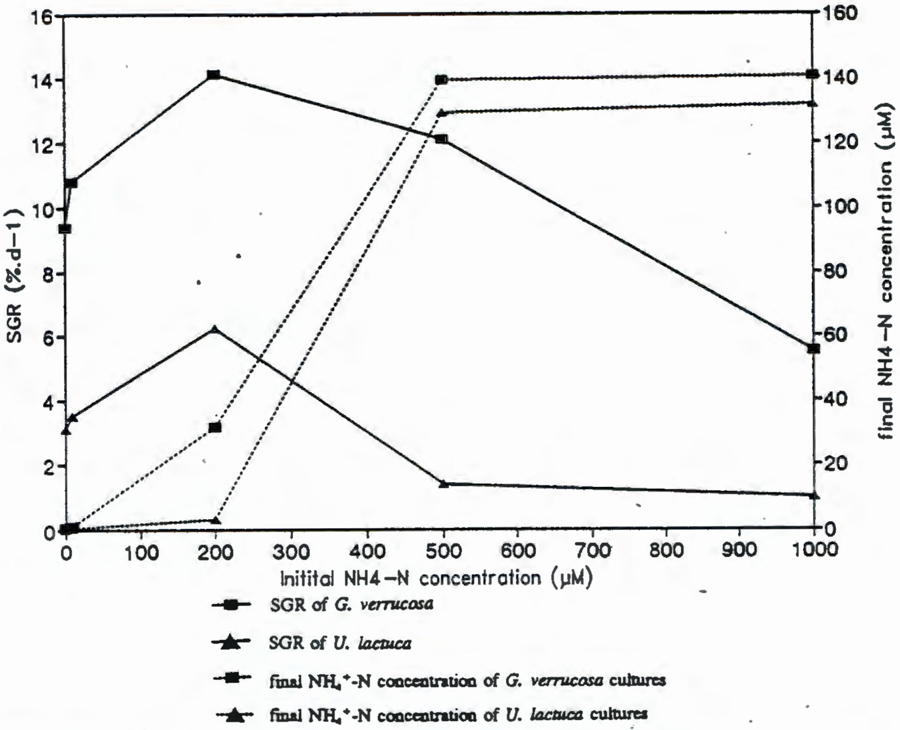


FIG.1: Specific Growth Rates of *G. verrucosa* and *U. lactuca* grown in monocultures at different initial NH_4^+-N concentrations. Residual NH_4^+-N concentrations three days after ammonium addition is depicted on the secondary axis. SGR = specific growth rate.

Table 1: Means and Standard Errors of SGRs, final ammonium concentrations and pH levels for G. verrucoso and U. lactuca grown in monocultures at different initial ammonium concentrations. Different letters express significantly different values by Tukey's test at a significance level of $p<0.05$. (n = 4)

Initial [NH4+] µM	SGR %/d		Final [NH4+] µM		pH	
	Gracilaria	Ulva	Gracilaria	Ulva	Gracilaria	Ulva
1	9.4 ± 0.54 e	3.1 ± 0.97 ab	0.918 ± 0.27 a	0.067 ± 0.04 a	9.01 ± 0.09 bcd	9.31 ± 0.10 cde
10	10.8 ± 0.38 ef	3.5 ± 0.30 bc	1.024 ± 0.28 a	0.381 ± 0.26 a	8.98 ± 0.02 bcd	9.53 ± 0.25 de
200	14.1 ± 0.24 h	6.2 ± 0.24 d	31.754 ± 7.02 b	3.163 ± 2.38 a	8.92 ± 0.08 bc	9.80 ± 0.20 e
500	12.1 ± 0.35 gh	1.4 ± 0.52 ab	139.173 ± 1.54 c	129.054 ± 1.96 c	8.87 ± 0.06 abc	8.69 ± 0.09 ab
1000	5.5 ± 0.35 cd	1.0 ± 0.52 a	140.644 ± 0.18 c	131.711 ± 0.22 c	8.60 ± 0.02 ab	8.32 ± 0.07 a

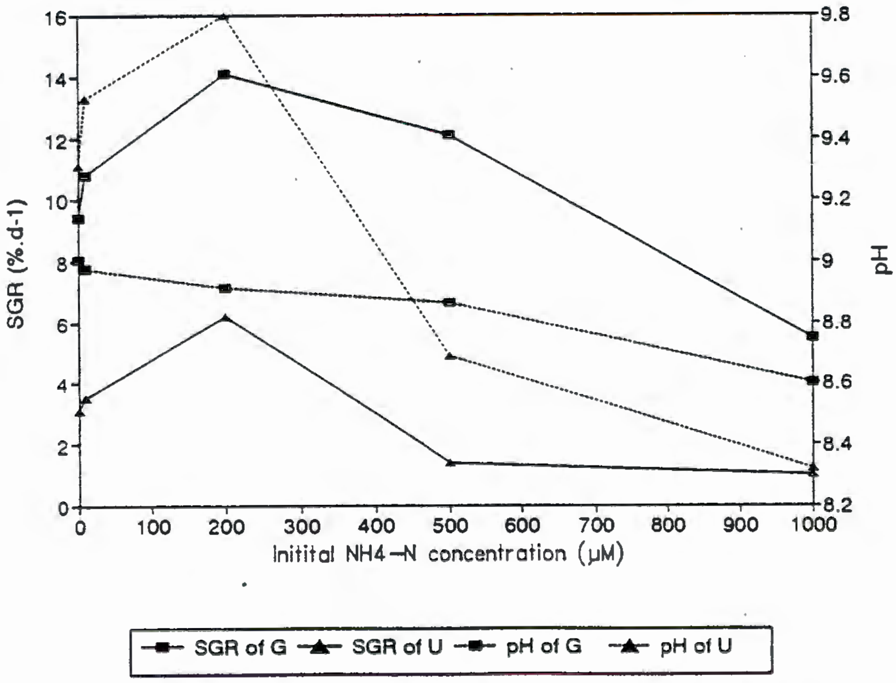


FIG. 2: Specific growth rates and final pH levels of *G. verrucosa* and *U. lactuca* grown at five different NH_4^+-N concentrations. SGR = Specific growth rate; G = *Gracilaria*; U = *Ulva*.

Measurements of pH in the afternoon at the termination of the experiment indicate that the levels tended to follow the SGRs especially in the case of *U. lactuca* with increases in pH occurring as growth rates rise (fig. 2). *U. lactuca* cultures tended to produce much higher pH levels despite lower SGRs than *G. verrucosa* at those concentrations where the thalli were still completely intact. The pH levels are found to be significantly higher in the *U. lactuca* cultures at the 200 μM concentration.

Competition experiment

Specific Growth Rates and final ammonium concentrations of *G. verrucosa* and *U. lactuca* in mono- and mixed- cultures at two different initial ammonium concentrations are indicated in fig. 3. Solid lines indicate the SGRs and final ammonium concentrations in the non-enriched treatments whereas the dotted lines represent that for the enriched treatments. Means and SEs are listed in table 2 (Appendix IV contains the full data set). The appropriate nitrogen limiting and non-limiting concentrations of 1 μM and 200 μM respectively were determined by the previous experiment.

G. verrucosa is again shown to exhibit much higher SGRs than *U. lactuca* with significant differences displayed in all treatments. As with the previous experiment, *U. lactuca* monocultures displayed a similar, significant increase in SGR at the 200 μM concentration with growth increasing from about $3\%.d^{-1}$ to $6\%.d^{-1}$ in both experiments. Although the *G. verrucosa* monocultures also indicated an increase in SGR at the 200 μM concentration, this increase was not statistically significant as in the first experiment. Growth rates increased from an average of $10.8\%.d^{-1}$ at the 1 μM concentration to $11.2\%.d^{-1}$ at the 200 μM concentration whereas in the previous experiment growth increased from $9.4\%.d^{-1}$ to $14.1\%.d^{-1}$.

Paired t-tests to reveal significant differences between certain of the treatments are indicated in table 3. Comparison of growth of *G. verrucosa* in the monocultures to that in the bicultures indicates that a statistically significant decrease in growth rate occurred when grown with *U. lactuca* at the 1 μM NH_4^+-N concentration whereas at the 200 μM concentration, although a decrease in growth rate occurs, this is not statistically

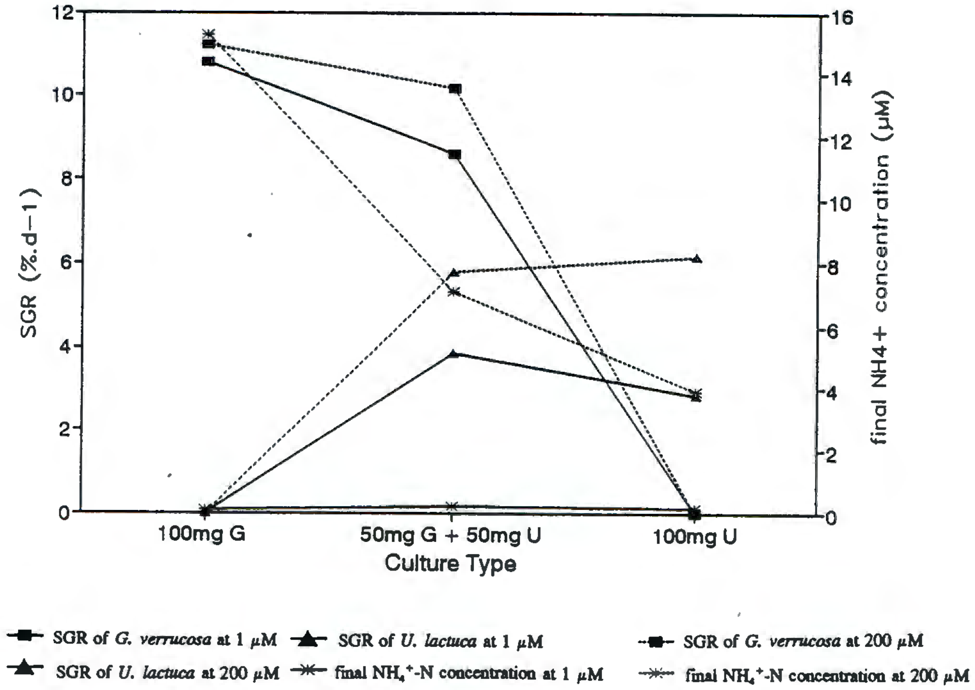


FIG. 3: Specific growth rates and residual ammonium concentrations of *G. verrucosa* and *U. lactuca* cultures grown in mono- and mixed-cultures at two different NH_4^+-N concentrations of 1μM and 200μM.

Table 2: Specific Growth Rates (SGR), final ammonium concentrations and pH values of mono- and mixed-cultures of G. verrucosa and U. lactuca grown at two different initial ammonium concentrations of 1 μM and 200 μM. Different letters express significant differences by Tukey's test at a significance level of $p<0.05$. ($n = 4$).

Initial seaweed mass	Initial concentration of NH4+ = 1 μM			
	SGR(%/d)		Final NH4+ concentration (μM)	pH
	Gracilaria	Ulva		
100 mg G.	10.8 ± 0.37 d	0	0.095 ± 0.095 a	9.0 ± 0.03 a
50 mg G + 50 mg U	8.6 ± 0.24 c	3.8 ± 0.33 a	0.221 ± 0.181 a	9.3 ± 0.09 ab
100 mg U.	0	2.8 ± 0.24 a	0.158 ± 0.066 a	9.2 ± 0.14 ab

Initial seaweed mass	Initial concentration of NH4+ = 200 μM			
	SGR(%/d)		Final NH4+ concentration	pH
	Gracilaria	Ulva		
100 mg G.	11.2 ± 0.19 d	0	15.280 ± 3.29 c	9.1 ± 0.02 a
50 mg G + 50 mg U	10.1 ± 0.63 cd	5.8 ± 0.48 b	7.112 ± 1.22 b	9.4 ± 0.03 ab
100 mg U.	0	6.2 ± 0.29 b	3.926 ± 0.83 ab	9.5 ± 0.12 b

Table 3: p values determined from paired t-tests to determine significant differences in SGR between treatments of the competition experiment.

	G 1	G (G+U) 1	U (G+U) 1	U 1	G 200	G (G+U) 200	U (G+U) 200	U 200
G 1	–	0.0026*			0.351			
G (G+U) 1	0.0026*	–	0.00002**			0.0644		
U (G+U) 1		0.00002**	–	0.0481*			0.0159*	
U 1			0.0481*	–				0.0001**
G 200	0.351				–	0.149		
G (G+U) 200		0.0644			0.149	–	0.0015**	
U (G+U) 200			0.0159*			0.0015**	–	0.512
U 200				0.0001**			0.512	–

G = SGR of Gracilaria in monoculture; G (G+U) = SGR of Gracilaria in bicultures; U (U+G) = SGR of Ulva in bicultures; U = SGR of Ulva in monculture

1 = 1 µM NH4+; 200 = 200 µM NH4+

* $p<0.05$; ** $p<0.005$

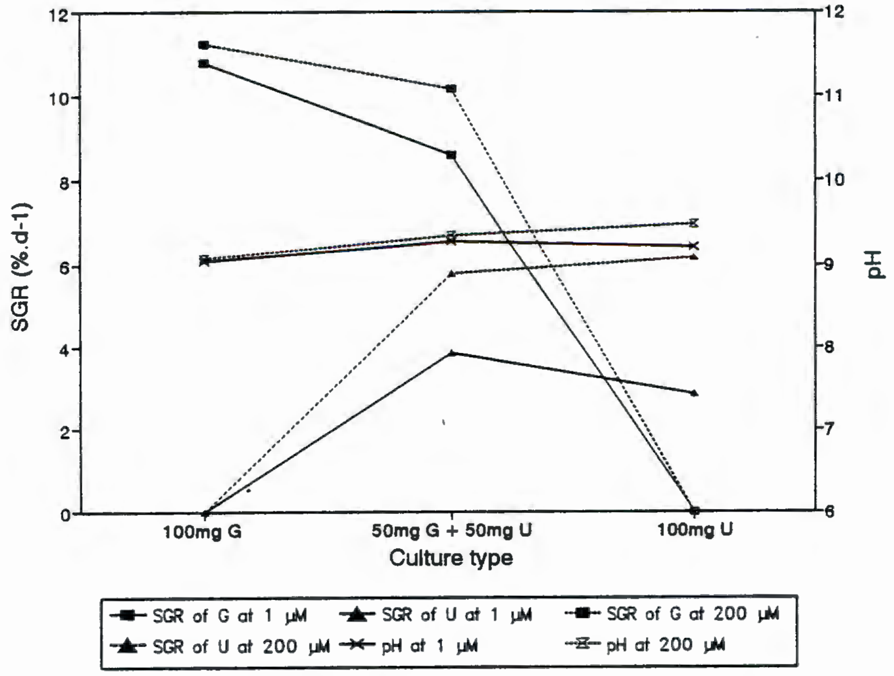


FIG. 4: Specific growth rates and final afternoon pH levels for *G. verrucosa* and *U. lactuca* grown in mono- and mixed-cultures at two different NH_4^+-N concentrations (1µM and 200µM).
SGR = specific growth rate; G = *Gracilaria*; U = *Ulva*.

significant. *U. lactuca* exhibits a slight increase in growth rate when grown with *G. verrucosa*, compared to when grown alone, at the 1 μM NH_4^+ concentration, but also displays a slight decrease in growth when grown in biculture at the higher concentration. However, none of these differences in growth rate was significant.

As in the previous experiment, residual ammonium concentrations at the end of the experiment after growth in the unenriched media, indicated the ammonium to be largely depleted. The treatments enriched with 200 μM of NH_4^+-N again indicated very low levels in the *U. lactuca* monocultures (3.926 μM) despite their exhibiting the lowest growth rates of 6.2%.d^{-1} resulting in an average biomass of 154mg. In the bicultures, intermediate levels of residual ammonium were found (7.112 μM) with average growth rates and thus total biomass also being intermediate at 8%.d^{-1} and 177mg respectively. *G. verrucosa* monocultures, although displaying highest growth rates at 11.2%.d^{-1} and an average biomass of 219mg, contained the highest levels of residual ammonium (15.280 μM).

The pH levels measured in the afternoon at the end of the experiment, which would have been three days after the last water change, indicated all cultures to have high levels, generally above pH 9 (fig. 4; table 2). Again, as in the previous experiment, the cultures containing *U. lactuca* tend to have higher levels despite lower growth rates but this increase was not significant as in the previous experiment.

DEWIT REPLACEMENT SERIES

DeWit competition curves (Fig. 5) were compiled using final mass data (Table 4). Analysis of variance was computed using doubled mass values for the biculture treatments. Significant differences in final mass follows the same pattern as for specific growth rates i.e. a decrease in final mass in *G. verrucosa* when grown with *U. lactuca* at 1 μM NH_4^+-N compared to when grown alone being the only significant difference. Thus the virtually straight lines produced in the competition curves indicate that little competitive interaction is occurring.

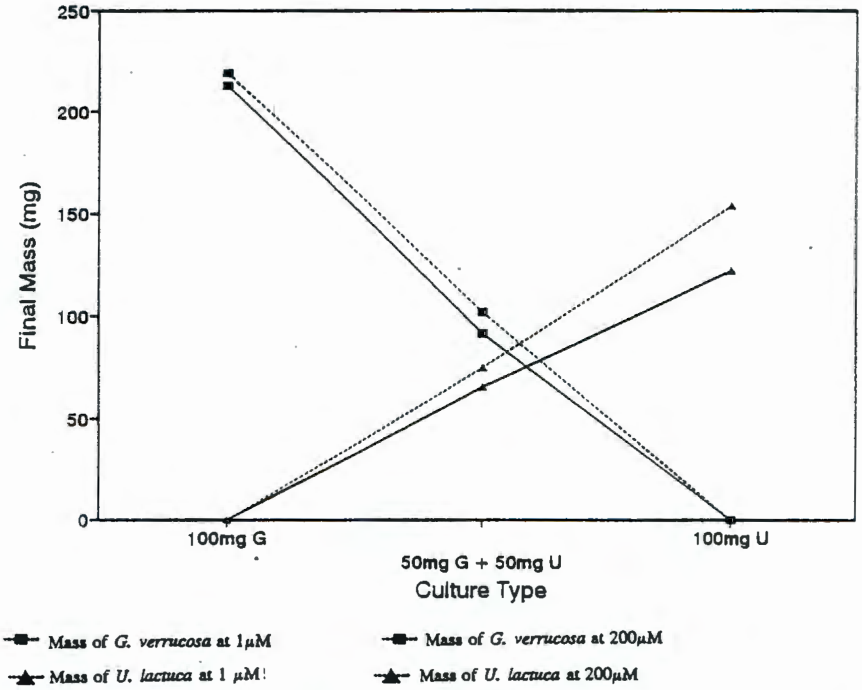


FIG. 5: DeWit competition curves for *G. verrucosa* and *U. lactuca* grown at two different NH_4^+-N concentrations of 1μM and 200μM. G = *Gracilaria*; U = *Ulva*.

Table 4: De Wit replacement series of G. verrucosa and U. lactuca at two ammonium concentrations of 1 μM and 200 μM. Different letters denote significant difference in final mass by Tukey's test at a significant level of $p<0.05$. (n = 4)

Initial seaweed mass	Final mass at 1 μM		Final mass at 200 μM	
	Gracilaria	Ulva	Gracilaria	Ulva
100 mg G	213 ± 5.6 e	0	219 ± 3.0 e	0
50 mg G + 50 mg U	91 ± 1.5 d	66 ± 1.5 ab	102 ± 4.6 de	75 ± 2.5 bc
100 mg U	0	122 ± 2.1 a	0	154 ± 3.2 c

(ANOVA calculated using doubled values for the mixed cultures)

Table 5: Nitrate and Phosphate levels in the competition experiments

(units?)

	PO4	NO3
Initial NH4 concentration = 1 μM:		
100 mg G	0.45	1.11
50 mg G + 50 mg U	0.34	1.39
100 mg U	0.39	1.02
Initial NH4+ concentration = 200 μM:		
100 mg G	0.34	17.73
50 mg G + 50 mg U	0.71	17.88
100 mg U	0.50	18.48
seawater	1.29	22.73

FIG. 5: DeWit competition curves for *G. verrucosa* and *U. lactuca* grown at two different NH_4^+-N concentrations of 1μM and 200μM.

NITRATE AND PHOSPHATE LEVELS

The nitrate levels in the seawater used in the competition experiment indicate much nitrate depletion at the lower ammonium concentration of the unenriched media (table 5). Little depletion occurred at the higher ammonium concentration. Phosphate levels were depleted to the same extent in all treatments.

Dry weight ratios

Significant differences were found in the ratio of dry weight to wet weight in the two species ($p<0.0005$). In *G. verrucosa* the dry weight formed 19 ± 1.9% of the wet weight whereas in *U. lactuca* it formed 34 ± 1.0%.

SGR and Light Intensity in *U. lactuca*

The SGR in *U. lactuca* was seen to increase to a maximum rate of $7\%.d^{-1}$ at a light intensity near 80 $\mu E.m^{-2}.s^{-1}$ (fig. 6; table 6). Regression analysis using the second to fourth data points indicated the compensation point to be at -7.7, i.e. the compensation point probably occurs close to zero at very low light intensities.

The pH levels were monitored twice daily in this experiment (fig. 7; table 6). The levels are seen to rise with increasing light intensity as well as with time from the last water change. Afternoon levels are higher than those measured in the morning. The pH is noted to continue at the higher light intensities even though SGR remains relatively constant.

Fertility in *U. lactuca*

No fertile material could be induced over a full lunar cycle at the range of light, temperature and nutrient levels examined. It was noted, however, that algae grown at the lowest light intensity tended to become very dark green and appeared in much better condition over the entire period than those grown at higher intensities. At higher light

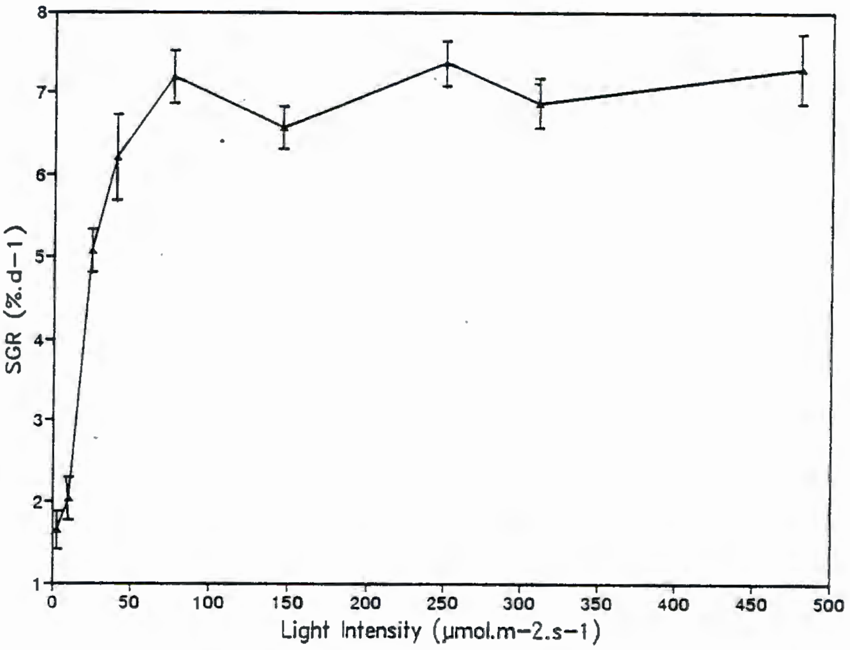
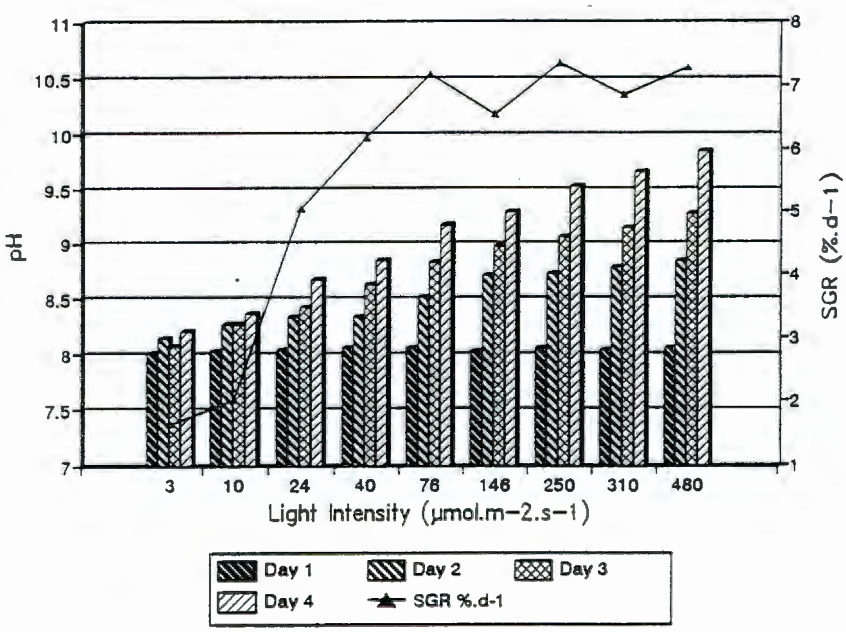


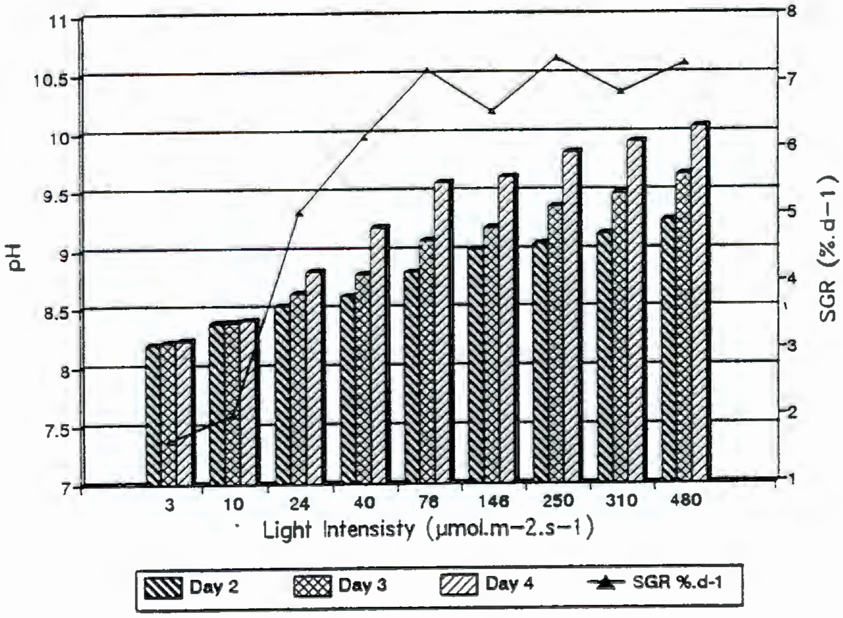
FIG. 6: Specific growth rates of *Ulva lactuca* at nine different light intensities.

Table 6: Specific growth rate (SGR) of Ulva lactuca and pH levels in culture vessels at nine different light intensities

LIGHT (µE.m−2.s−1)	SGR (%.d−1)	Morning pH levels				Afternoon pH levels		
		day 1	day 2	day 3	day 4	day 2	day 3	day 4
3	1.65 ± 0.23	8.02	8.2 ± 0.01	8.1 ± 0.01	8.2 ± 0.01	8.2 ± 0.01	8.2 ± 0.01	8.2 ± 0.01
10	2.04 ± 0.26	8.04	8.3 ± 0.01	8.3 ± 0.01	8.4 ± 0.04	8.4 ± 0.02	8.4 ± 0.04	8.4 ± 0.05
24	5.07 ± 0.26	8.05	8.3 ± 0.01	8.4 ± 0.04	8.7 ± 0.01	8.5 ± 0.03	8.6 ± 0.01	8.8 ± 0.04
40	6.20 ± 0.52	8.06	8.3 ± 0.04	8.6 ± 0.03	8.9 ± 0.05	8.6 ± 0.02	8.8 ± 0.03	9.2 ± 0.05
76	7.20 ± 0.33	8.06	8.5 ± 0.05	8.8 ± 0.06	9.2 ± 0.03	8.8 ± 0.06	9.1 ± 0.07	9.6 ± 0.05
146	6.35 ± 0.26	8.04	8.7 ± 0.01	9.0 ± 0.03	9.3 ± 0.06	9.0 ± 0.02	9.2 ± 0.04	9.6 ± 0.05
250	7.37 ± 0.28	8.06	8.7 ± 0.02	9.1 ± 0.02	9.5 ± 0.06	9.1 ± 0.01	9.4 ± 0.05	9.8 ± 0.07
310	6.87 ± 0.30	8.05	8.8 ± 0.01	9.2 ± 0.03	9.7 ± 0.04	9.1 ± 0.01	9.5 ± 0.03	9.9 ± 0.03
480	7.30 ± 0.43	8.06	8.8 ± 0.05	9.3 ± 0.07	9.8 ± 0.07	9.2 ± 0.07	9.6 ± 0.08	10.1 ± 0.03



Morning pH levels



Afternoon pH levels

FIG. 7: Morning and afternoon pH levels in still culture at different light levels and on four consecutive days. The line graph represents the SGR at the different light intensities.

WHICH SEAWEED?

levels the thalli became pale green with translucent patches developing at 180 μE.m^{-2}.s^{-1} and above.In light levels of 40 μE.m^{-2}.s^{-1} and above the algae growing in the enriched cultures had a more crinkly appearance. Most growth occurred in the enriched cultures at the light levels of 40 μE.m^{-2}.s^{-1} and 80 μE.m^{-2}.s^{-1}.

Table 7: Light levels measured at different depths at Saldanha Bay at noon on a clear day August

Depth (m)	Light Intensity (μE. m−2.s−1)
surface	2135
just below surface	1767
1.0	1170
2.9	605.6
4.9	229.6
6.9	88.8
8.9	35.9
10.6	16.4

DISCUSSION

1. Monocultures grown at different ammonium concentrations

G. verrucosa and *U. lactuca* exhibited similar optimum ammonium concentrations for growth with highest Specific Growth Rates (SGR) occurring at 200 μM for both species (fig. 1). At this concentration, the external medium is found to possess little ammonium at the end of the experiment, especially in the case of *U. lactuca*, indicating that it is mostly being taken up by the algae. Bacteria present in the cultures could also be converting the ammonium to nitrate however the decrease in nitrate levels as indicated in table 5 would suggest that this is not occurring to any extent. The optimal concentration for *G. verrucosa* would probably be at a slightly lower concentration at which no excess ammonium would remain in the vessel.

At the lower initial ammonium concentrations no to extremely low levels of ammonium remain in the culture vessels indicating that the lower growth rates exhibited result from nitrogen limitation. At higher ammonium levels toxic effects occur that inhibit the growth of both species as well as causing noticeable damage to *U. lactuca* as evidenced by the disintegrating thalli. Very high levels of residual ammonium in the culture vessels three days after addition indicate excess nitrogen availability. The similar levels of ammonium remaining in the vessels at the 1000 μM treatment and the 500 μM treatment may be due to the inability of the higher concentration to go completely into solution in 200 ml of seawater.

Cohen and Neori, (1991) indicated a similar concentration of nitrogen below which *U. lactuca* becomes nitrogen limited. Decrease in growth rates was seen to occur at ammonia fluxes below 10 $\mu M.h^{-1}$ which works out to about 240 μM of ammonium N per day. Maximum uptakes rates in nitrogen sufficient plants have been calculated as 50 to 131 $\mu mol.g^{-1}.h^{-1}$ and in nitrogen starved plants as 252 to 390 $\mu mol.g^{-1}.h^{-1}$ (Fujita, 1985; Cohen and Neori, 1991).

The much lower SGR of *U. lactuca* compared to *G. verrucosa* is surprising as numerous other reports all indicate the ability of *Ulva* to outgrow *Gracilaria* at all light and temperature conditions when nitrogen is non-limiting (Rosenberg and Ramus, 1982; Fujita, 1985; Svirski *et.al.*, 1993). The greater surface area to volume ratio of species of *Ulva* is said to promote faster uptake rates and hence faster growth rates (Fujita, 1985). The use of 15mm growing tips of *G. verrucosa* in this study may be the reason for the much higher SGRs.

However, the species from Saldanha Bay has been reported to be particularly fast growing (J.J. Bolton, pers. comm.). The values obtained for the SGRs do tend to be higher compared to that found in other studies. Haglund and Pedersén (1993) observed average growth rates of 4% per day with a maximum of 9% in *G. tenuistipitata* using an initial biomass of about 1kg of fresh weight, light intensities ranging from 1200 μE.m^{-2}.s^{-1} to 30 μE.m^{-2}.s^{-1} depending on depth in the tank and ammonium concentrations ranging from 40 μM to 820 μM NH_4NO_3 supplied weekly. *G. chilensis* obtained a maximum SGR of 3%.d^{-1} at a light intensity of 25 μE.m^{-2}.s^{-1} and temperature of 20 °C under a variety of ammonium concentrations and pulse rates (Pickering *et.al.*, 1993). In a study that also used 15mm tips of material from Saldanha bay much lower SGRs of only 3%.d^{-1} occurred at 80 μE.m^{-2}.s^{-1}. The availability of nitrogen in the form of nitrates as opposed to ammonium in this latter study may have caused the lower growth rates. ?

A study by DeBoer *et.al.* (1978) using 25g of fresh weight per culture system, obtained comparable growth rates to this study. The SGR of *G. foliifera*, measured at light saturation levels (0.052 ly.min^{-1}) and a water temperature of 22°C, increased with increasing nitrogen concentrations until 210 μM per day was reached, after which SGR remained constant. The growth rates reached after the nitrogen saturation level were 12 %.d^{-1}. Increased nitrogen concentrations up to much higher levels (38.16 μM with flow rate of 140 l.d^{-1}; ie 5342 μM per day) did not cause the SGR to decline but this is probably due to the continuous flow through nature of the system. Studies have indicated though that if C:N tissues ratios reach 6 further addition of ammonium decreases the growth rate (Lignell and Pedersén, 1987). ✓

Rather than the *G. verrucosa* exhibiting unusually high SGRs it might be that the *U. lactuca* used in this study may have lower than usual growth rates. Other studies indicate higher growth rates for this species with a SGR of 8.6%.d^{-1} occurring at light levels of 123 μE.m^{-2}.s^{-1} and 15°C (Svirski *et.al.*, 1993); 7%.d^{-1} when grown at identical light and temperature conditions as in this study (Enright, 1978) and when grown at various ambient conditions throughout the year the SGR was always above 2.9%.d^{-1} and up to 8%.d^{-1} (Rosenberg and Ramus, 1982).

The *U. lactuca* monocultures produced much greater increases in pH in the culture medium at those ammonium concentrations where toxic effects were not evident even though the SGR and thus final biomass of seaweed per culture vessel was much lower than that of *G. verrucosa* (fig 2). It has been hypothesised that the creation of a high pH may be an inhibitory effect of *Ulva* on other seaweeds. However, the production of high pHs in a closed system may have been detrimental to the growth of *Ulva* itself.

? Svirski et al

Svirski *et.al.* (1993) also found the pH of any *Ulva* medium, in monoculture or mixed culture, to be significantly higher than any monoculture of *Gracilaria*. Although *Gracilaria* showed a growth rate that was inversely correlated with pH levels, that of *Ulva* was positively correlated. In batch cultures of *G. tikvahiae* the pH was found to increase from 8.1 to 9.5 and either this or the resultant decrease in available carbon had an inhibitory effect on growth which could be rectified by pH control and carbon addition (DeBusk and Ryther, 1984). The decrease in net photosynthesis above pH 8.0 was thought to indicate that this species may be unable to utilise bicarbonate as a carbon source. ✓

2. Competition experiments

The SGR and DeWit competition curves (figs 3 and 5) indicate a slight depression in the growth of *G. verrucosa* when grown with *U. lactuca* compared to when grown alone, but this was only significant at the lower concentration of 1 μM NH_4^{-1}. *U. lactuca* displayed a slight increase in growth when grown with *G. verrucosa* compared to when grown alone only at the 1 μM concentration but difference in growth rate compared to the *Ulva* monocultures was never significant. Thus the straight lines produced in the de Wit competition curves would indicate that there is little competitive interaction occurring except perhaps at the 1 μM concentration. In this latter instance the thallus shape of *U. lactuca* may promote the fast removal of the scant supply of nitrogen thus inhibiting the growth of *G. verrucosa*.

This is in contrast to other studies which indicate enhanced growth rates of *Ulva* species in biculture compared to when grown alone (Enright, 1978; Svirski *et.al.*, 1993) and has been attributed to faster uptake rates of nutrients, higher growth rates, increased pH levels and possibly allelopathy (Enright, 1978; Rosenberg and Ramus, 1982; Fujita, 1985; Friedlander, 1992). This competitive advantage was dependent on continual high supplies of nitrogen in the environment as it is less able to accumulate excess nitrogen reserves. The faster growth rates of *Ulva* were noted to remove CO_2 from the external medium with a concomitant increase in pH (Svirski *et.al.*, 1993). Seawater at pH levels above about 9 possess negligible amounts of dissolved CO_2 and bicarbonate forms the major source of dissolved organic carbon (Dring, 1982). It has been speculated that this increase in pH and CO_2 removal may be the factor limiting the growth of *Gracilaria* in cultures containing *Ulva* as it has been reported that *Gracilaria* appears to not contain the membrane bound protein for the transport of bicarbonate which does appear in *Ulva* (Beer quoted by Svirski *et.al.*, 1993, p. 396).

In this study the pH levels at the end of the experiment tended to be high (Table 2) as a result of the experiment being conducted in flasks rather than in open systems. Although values were all fairly similar (fig. 4) ANOVA revealed significant differences between the cultures possessing 100% *Gracilaria* (9.04 at 1μM and 9.08 at 200 μM) and those with 100% *Ulva* at the 200 μM level (9.47). Thus, as in the monoculture experiment, cultures containing *Ulva* did tend to have higher pH values despite lower growth rates. However, it appears that the *G. verrucosa* was still capable of high growth rates at pHs above 9 indicating that it may have been utilising bicarbonate.

As *G. verrucosa* was shown to have higher SGRs than *U. lactuca,* whereas all previous studies found the opposite, this may have accounted for the lack of an inhibitory effect on the growth of *G. verrucosa*. It would be interesting to examine growth rates of larger plants and see if these results still hold true.

A paradoxical increase in final ammonium concentration was found to occur in the treatments supplied with initial concentrations of 200 μM NH_4^+. Contrary to expectation, those cultures with the greatest SGR and hence final biomass had highest residual ammonium concentrations.

The vessels containing only 1 μM NH_4^+ did not exhibit this paradoxical final ammonium concentration as all possessed negligible amounts of ammonium at the termination of the experiment indicating that for *Ulva* this level is limiting as indicated by the decreased SGR whereas in the case of *Gracilaria* it is only just becoming limiting as the SGR was not significantly lower than that of the higher concentration.

The reason for the lower ammonium uptake in the faster growing *Gracilaria* cultures at the higher ammonium concentrations is probably due to the ability to make use of internal reservoirs rather than a lower requirement for nitrogen, whereas *Ulva* is much more reliant on external nitrogen sources. Previous studies have indicated the ability of *Gracilaria* to take up excess nitrogen in periods of high availability (Ryther *et.al.*, 1981; Bird *et.al*, 1982; Pickering *et.al.*, 1993) which can then maintain non-limiting levels of growth when the external supply is removed for a period of 7 to 14 days (Ryther *et.al.*, 1981; Fujita, 1985; Friedlander and Ben Amotz, 1991). *G. foliifera* has been shown to be able to accumulate up to 1020 μg-atN. g dry wt-1 of soluble nitrogen reserves whereas *Ulva* sp. was able to accumulate up to 630 μg-at N. g dry wt^{-1} (Rosenberg and Ramus; 1982).

It has also been shown that uptake rates are dependent on the nitrogen status of the tissues with those possessing high levels showing very little uptake of ammonium whereas uptake rates after nitrogen starvation may be very high (Ryther *et.al.*, 1981; Fujita, 1985). *G. tikvahiae* was found to not take up any ammonium after growth at a high nitrogen flux rate whereas *U. lactuca* grown at the same high nitrogen flux rates did deplete ammonium from the external medium (Fujita, 1985). It has been shown that 1kg wet weight of *U. lactuca* can remove over 90% of ammonia at inflow fluxes up to 10 μM.h^{-1} of ammonium N (216 μM removed per day) and nearly 40% at fluxes of 40 μM.h-1 (384 μM removed per day)(Cohen and Neori, 1991). Maximal uptake rates of 28 μM.h-1 were recorded.

The stock cultures in this experiment were supplied weekly with enrichment medium containing 198 μM of nitrogen in the form of $NaNO_3$. Thus *G. verrucosa* probably stored excess nitrogen from the previous environment on which it was able to draw during the experiments. The more significant increase in SGR that occurred with a change from 1 μM NH_4^+ to 200 μM NH_4^+ in the monoculture experiments compared to the competition experiments may have been due to the culture material in the first experiment having been in the stock culture for a shorter period.

These results thus reiterate the advantage of nutrient pulsing to favour the growth of *Gracilaria* over *Ulva* as mentioned in previous studies (Ryther *et.al.*, 1981; Pickering *et.al.*, 1993). Despite growth in stock cultures with equivalent nutrients supplied, the *Ulva* required much higher ambient levels for growth in the experiments.

With regard to other nutrients, nitrate levels in the seawater prior to the addition of the seaweeds was found to be about 22.73 μM and phosphate 1.29 μM (table 5). The nitrate was greatly reduced in the nitrogen limited cultures whereas little reduction was observed in the cultures with high ammonium concentrations. This supports the observation that preferential uptake of ammonium often occurs if nitrogen is present in non-limiting amounts (D'elia and DeBoer, 1978; Ryther *et.al.*, 1981; Hanisak, 1990; Haglund and Pedersén, 1993). Since ammonium represents a more reduced form of nitrogen and obviates the energetically expensive need for the reduction of nitrate (Prescott, 1990) it should hardly be surprising that ammonium is the preferred source. The phosphates levels were reduced to similar concentrations of 0.34 to 0.71 for all treatments.

Determination of the dry weight ratios indicate that the *G. verrucosa* possesses less dry weight per fresh weight (19%) than *Ulva* (34 %). A study by Ryther *et.al.* (1981) indicated the dry weight of *G. tikvahiae* to be 11% of the wet weight whereas Friedlander and Ben Amotz (1991) demonstrated that a variation in dry weight ratios occurs within individuals with an inverse relationship to growth rate occurring. Higher growth rates of 3.7 to 6.1 $\%.d^{-1}$ possessed dry weight ratios of 9% to 13% whereas low growth rates of 0.9 to $2\%.d^{-1}$ had dry weight ratios of 20% to 30%. Thus the greater dry weight ratios of the *Ulva* used in this experiment may be related to lower growth rates.

SGR and Light Intensity in *U. lactuca*

The light curve obtained for *U. lactuca* indicates that it is adapted for growth at very low light levels as indicated by the low light saturation level at 80 $\mu E.m^{-2}.s^{-1}$ and growth occurring as low as 3 $\mu E.m^{-2}.s^{-1}$. On a sunny day with clear water conditions in Saldanha Bay light levels of 80 $\mu E.m^{-2}.s^{-1}$ occurred at a depth of about 6m and levels of 16 $\mu E.m^{-2}.s^{-1}$ were recorded at a depth of 10m (table 7). Thus light should not be a constraint at these depths. However the *Ulva* is generally only found above 5.5 metres below the MLWS. Light levels at these depths were reported to be about 150 to above 600 $\mu E.m^{-2}.s^{-1}$. The light levels indicated in table 7 do, however, represent optimal conditions and much lower levels are probably available for much of the time.

midday only?

On the other hand, *G. verrucosa*, which exhibits a similar light curve to the *U. lactuca,* occurs at these greater depths. Thus other factors may be involved in preventing the occurrence of *U. lactuca* at these depths. The lack of much wave action in the Bay causes sedimentation to accumulate on the thalli in the deeper waters (R. Anderson, pers. comm.). This would decrease the amount of light reaching the thalli. The flat blades of *U. lactuca* would be more prone to being covered by sediment than the terete thalli *G. verrucosa*. There is also the possiblity that the light quality at lower depth inhibits growth of *U. lactuca* as the green thalli probably absorb highly in the red spectrum which is much less abundant at these greater depths.

The lower depth limit of this species may not be due to light but nutrient limitation and it may be able to grow at greater depths in the region of the thermocline, but the movement of the thermocline with upwelling conditions and resultant nutrient pulsing may favour the growth of *Gracilaria* at these depths, allowing it to outcompete the *U. lactuca*.

A light curve obtained for *G. verrucosa* from Saldanha Bay also indicated maximal growth to occur at 80 $\mu E.m^{-2}.s^{-1}$ (Engledow and Bolton, 1992). Thus this light curve corresponds more with the species position in the natural environment as it occurs with a lower depth limit of 10m and up to 2m below the surface. The curve did not show the usual saturation type of curve as above 80 $\mu E.m^{-2}.s^{-1}$ the growth rate was reduced by

50% and maintained this lower growth rate for all the remaining light levels tested (up to 850 $\mu E.m^{-2}.s^{-1}$). At very low light levels of 4 $\mu E.m^{-2}.s^{-1}$ growth still occurred with a SGR of 1%.d^{-1} (at 21°C) which is lower than that obtained in this study for *U. lactuca* of 1.7 %.d^{-1} at 3 $\mu E.m^{-2}.s^{-1}$ (at 15°C).

Monitoring of the pH levels in the light experiment indicated a definite trend with an increase in pH occurring as light levels increased as well as with time since the water change (fig. 7; table 6). Afternoon pH levels were higher than morning pH levels as photosynthesis removes the dissolved inorganic carbon from the medium. Although the SGR levelled off at a light level of 76 $\mu E.m^{-2}.s^{-1}$, the pH levels continued to increase. Growth rate was not inhibited at these higher pH levels at least for the one week duration of the experiment and demonstrates the ability of *Ulva* to tolerate very high pH levels. The increase in pH with time illustrates the preference for open systems which would more closely approximate the natural situation. Alternatively pH levels could be controlled by the addition of CO_2 (DeBusk and Ryther, 1984; Friedlander and Ben Amotz, 1991).

Fertility in *U. lactuca*

As high ammonium levels were found to inhibit the growth rates of both species it is possible that the release of high levels in the fish factory effluent was toxic for both species and caused a wide area to be completely cleared of either species. As the ammonium was reduced to more tolerable levels this open space would have been available for the more efficient colonisation by *Ulva* which in isolates from other areas usually produces gametes and spores that can be disseminated over a wider area than the vegetative reproduction of the *G. verrucosa*.

However fertile material could not be induced under a range of conditions provided in the laboratory. In a taxonomic study of *Ulva* in the South Western Cape no fertile material of *U. lactuca* could be found in Saldanha Bay either in contrast to the more open shorelines (M.A.P. Joska, pers.comm.). Thus it would appear that reproductive characteristics do not afford the *U. lactuca* from Saldanha Bay with a competitive advantage.

CONCLUSION

The economic importance of *Gracilaria* and the financial losses that resulted from the *U. lactuca* bloom indicates the necessity of determining the causes of the bloom. This study confirms the importance of nutrient pulsing to control the growth of *U. lactuca* as indicated by the much higher demand for ambient ammonium in this species even with lower growth rates compared to *G. verrucosa* which appeared to be able to utilise stored nitrogen. Thus the high levels of ammonium released in the effluent could well have sustained the growth of this species in the upper 'oligotrophic' layer of the water column.

The hypothesis that *U. lactuca* would show competitive superiority at high levels of ammonium concentration was not supported with de Wit replacement series indicating little competitive interaction. However, measurements of growth rate did indicate a decline in the presence of *U. lactuca* but this was not always significant. In contrast to other studies *U. lactuca* was found to exhibit much lower SGRs than that of *G. verrucosa*.

This study also indicates that the restriction of *U. lactuca* in Saldanha Bay to depths shallower than about five metres may not be due to light limitations at lower depths. The light curve generated indicates that it is quite possible for it to grow at very low light levels. The lower limitation is possibly due to the fluctuating availability of nutrients with the movement of the thermocline at these greater depths. There is also the possibility that lack of wave action and sedimentation decreases the actual amount of light reaching the flat thalli of *U. lactuca*.

The species of *U. lactuca* from Saldanha Bay appear to form a unique isolate that displays different characteristics to populations growing elsewhere. This may be the result of environmental influences within the Bay. The seaweed is found to have lower than usual growth rates, a light curve indicative of a shade adapted plant and a lack of fertile material.

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APPENDIX I

Substances used to make up the enrichment medium

	Chemical	amount
Solution P_I	$(NH_4)_2(SO_4)_2 \cdot 6H_2O$	0.07g
	Na_2EDTA (titriplex)	0.60g
Solution P_{II}	H_3BO_3	1.14g
	$FeCl_3 \cdot H_2O$	0.049g
	$MnSO_4 \cdot H_2O$	0.164g
	$ZnSO_4 \cdot 7H_2O$	0.022g
	Na_2EDTA	1.0g
	$CoSO_4 \cdot 7H_2O$	0.005g
Stock solution	$NaNO_3$	2.8g
	Sodium glycerophosphate	0.4g
	Thiamine dichloride	0.004g
	Trisaminomethane (Tris buffer)	4.0g
	Vit. B_{12} (1mg/10ml)	0.8ml
	Biotin (1mg/20ml)	0.8ml

Solutions P_I and P_{II}, were made up to 1000 ml using distilled water. For the stock solution 200 ml each of P_I and P_{II} were first added before it too was made up to 1000 ml. The stock solution was then added to the stock cultures of two seaweeds and to the flasks in the light experiment in the amount of 6 ml per litre of seawater.

APPENDIX II

Manual method for the determination of Ammonia in seawater

Reagents

1. Freshly deionised water
2. Low nutrient seawater: seawater collected far offshore
3. Stock standard: Dissolve 0.0535g ammonium chloride (pre-dried at 100°C) and make up to 1000ml with freshly de-ionised water (reagent 1). Preserve with a drop of chloroform.
4. Sodium hydroxide solution (1.0mol.l^{-1}): dissolve 40g sodium hydroxide in deionised water and make up to 1000ml. Store in a tightly stoppered polyethylene container.
5. Citrate buffer: dissolve 240g trisodiumcitrate in about 500ml distilled water. Add 10ml 1.0 molar NaOH. Boil until the volume drops to less than 500ml to remove any ammonia. Make up to 500ml in a volumetric flask. Store in a tightly stoppered bottle in fridge.
6. Phenol reagent: dissolve 10.86g phenol and 0.114g sodium nitroprusside in deionised water and make up to 1000ml. Store in a glass brown bottle in fridge.
7. Oxidant (DTT): dissolve 0.19g dichloro-isocyanuric acid sodium salt in 125ml 1.0 molar NaOH and make up to 250ml with deionised water. Store in refrigerator. This is only stable for one week.

Do not use reagents from Centrolab as they are not of a high enough purity.

Procedure

The analysis for ammonia must be performed in a well ventilated room where no ammonia compounds or solutions are stored. There must be no smoking in this room at all times.

A range of working standards are made up by pipetting the indicated amount of stock standard and making up to volume with the low nutrient seawater in a 100ml volumetric flask.

ml of stock std.	conc. of working std. (μmol.l^{-1})
0.05	0.5
0.10	1.0
0.30	3.0
0.50	5.0
0.75	7.5
1.00	10.0

REAGENT BLANK: To each of 3 tubes add 5.0ml of the deionised water.
BLANK: To each of 3 tubes add 5.0ml of the Low Nutrient Seawater.
WORKING STANDARDS: To each of 3 tubes add 5.0ml of each working standard described above.

SAMPLES: add 5ml of each sample to 2 tubes.

To each of the above tubes add:
0.5ml Citrate Buffer and mix well
0.5ml Phenol reagent and mix well
0.5ml Oxidant (DTT) and mix well

Seal the tubes with NESCO film and set aside in the dark for at least 6 hours or preferably overnight. Measure the absorbence in a 1cm cuvette against a similar cuvette filled with low nutrient seawater at 630nm.

Blanks and standards need to be set up for each new batch of DTT.

Calculations

A = average absorbence of three deionised water blanks divided by 1.08 (salt factor).
B = average absorbence of three low nutrient seawater blanks
C = average absorbence of the three standards
D = absorbence of samples

F FACTOR = conc. of standards used/(C-B)

If several standards are set up take an average of the F factors. Discard any F factors that are too high or too low as this method produces a straight line graph. If the F factors are too erratic new standards must be made up and the process repeated.

Concentration of the samples = F x (D - A) μmolNH_3-N.l^{-1}.

APPENDIX III

ULVA LACTUCA MONOCULTURES

Treatment µM NH4+ –N	Final Mass (mg)	SGR %.d–1	final [NH4+] µM	pH
1	128	3.53	0.135	9.20
1	140	4.81	0.067	9.59
1	129	3.64	0.000	9.23
1	102	0.28		9.20
10	129	3.64	0.875	9.94
10	131	3.86	0.000	8.94
10	131	3.86	0.269	9.94
10	120	2.60		9.30
200	156	6.35	2.827	10.14
200	149	5.70	2.827	9.24
200	161	6.80	3.836	9.77
200	152	5.98		10.04
500	105	0.70	128.543	8.52
500	111	1.49	131.262	8.65
500	104	0.56	124.530	8.64
500	122	2.84		8.94
1000	117	2.24	131.262	8.39
1000	98	–0.29	131.935	8.14
1000	108	1.10	131.935	8.48
1000	107	0.97		8.29

GRACILARIA VERRUCOSA MONOCULTURES

Treatment µM NH4+ –N	Final mass (mg)	SGR %/d	Final [NH4+] µM	pH
1	195	9.54	1.518	9.12
1	187	8.94	0.388	9.05
1	178	8.24	0.530	8.75
1	213	10.80	1.236	9.10
10	206	10.32	1.871	
10	202	10.04	0.741	8.95
10	216	11.00	0.812	8.97
10	228	11.77	0.671	9.01
200	277	14.55	20.087	8.75
200	261	13.71	47.623	8.85
200	277	14.55	19.804	9.12
200	262	13.76	39.503	8.94
500	249	13.03		8.70
500	234	12.15	137.290	8.97
500	222	11.39	142.232	8.95
500	229	11.84	137.996	8.84
1000	141	4.91	140.114	8.61
1000	142	5.01	140.820	8.55
1000	157	6.44	140.820	8.60
1000	146	5.41	140.820	8.64

APPENDIX IV

COMPETITION EXPERIMENTS

Initial concentration of NH4+ −N (µM)	Initial mass (mg)		Final mass (mg)		SGR (%.d−1)		Final NH4+ −N concentration	pH
	Gracilaria	Ulva	Gracilaria	Ulva	Gracilaria	Ulva		
1	100	0	203	0	10.11	0.00	0.000	9.07
1	100	0	229	0	11.84	0.00	0.000	9
1	100	0	209	0	10.53	0.00	0.000	9.1
1	100	0	210	0	10.60	0.00	0.378	8.98
1	50	50	92	68	8.71	4.39	0.000	9.49
1	50	50	92	62	8.71	3.07	0.757	9.04
1	50	50	87	64	7.91	3.53	0.126	9.35
1	50	50	94	68	9.02	4.39	0.000	9.25
1	0	100	0	123	0.00	2.96	0.189	9.39
1	0	100	0	121	0.00	2.72	0.126	9.51
1	0	100	0	127	0.00	3.41	0.000	9.03
1	0	100	0	117	0.00	2.24	0.315	8.93
200	100	0	217	0	11.07	0.00	17.661	9.07
200	100	0	228	0	11.77	0.00	5.803	9.11
200	100	0	216	0	11.00	0.00	16.652	9.12
200	100	0	215	0	10.94	0.00	21.004	9.01
200	50	50	97	80	9.47	6.71	5.109	9.35
200	50	50	103	78	10.32	6.35	5.046	9.42
200	50	50	93	73	8.87	5.41	8.326	9.27
200	50	50	114	69	11.77	4.60	9.966	9.36
200	0	100	0	152	0.00	5.98	6.244	9.16
200	0	100	0	158	0.00	6.53	2.965	9.58
200	0	100	0	146	0.00	5.41	3.974	9.42
200	0	100	0	160	0.00	6.71	2.523	9.73