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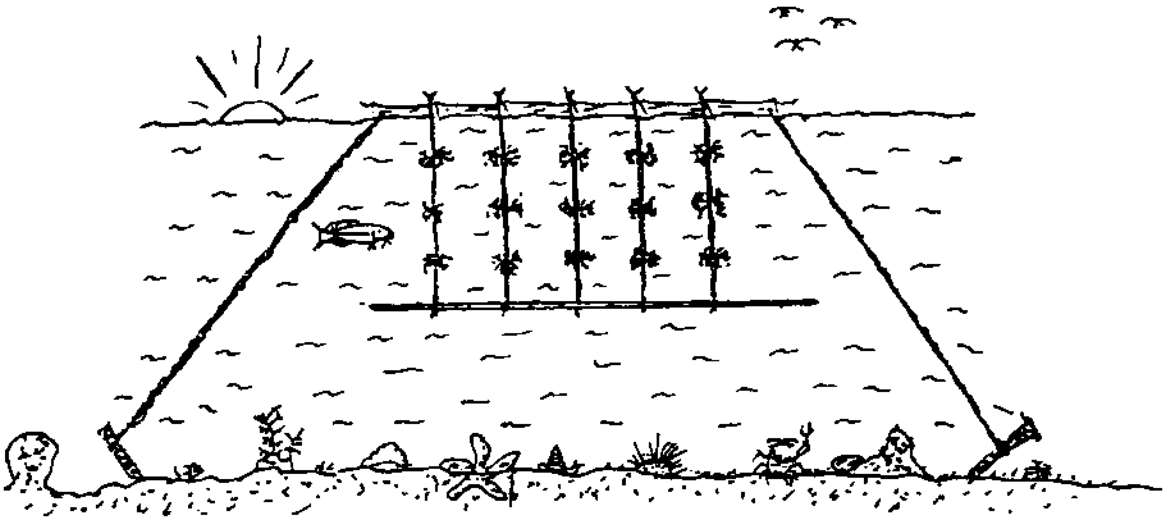

UNIVERSITY OF GUAM MARINE LABORATORY

Technical Report No. 61

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EVALUATION OF SEAWEED MARICULTURE POTENTIAL ON GUAM:
I. AMMONIUM UPTAKE BY, AND GROWTH OF TWO SPECIES OF *GRACILARIA* (RHODOPHYTA)

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ABSTRACT

We examined the specific growth rates of and ammonium uptake by *Gracilaria edulis* and *Gracilaria arcuata* from Guam. Ammonium uptake kinetics were examined in the laboratory at salinities of $13^{o}/oo$, $23^{o}/oo$ and $34^{o}/oo$ for each species. Ammonium uptake was examined over a wide range of substrate concentrations with the highest concentration at 5×10^{3} µg-at $NH_4^{+}\text{-}N \cdot \ell^{-1}$. A strong diffusion component was indicated in the ammonium uptake systems of both species. The nitrogen content of the thalli used in the experiments was variable but averaged 2.07% for *G. edulis* and 3.31% for *G. arcuata*. Specific growth rates for thalli of each species were compared in both an outdoor tank culture system and a raft culture system in Pago Bay, Guam. Growth rates were generally higher in the raft culture system than in the tank culture system. Mean specific growth rates ranged from 2.02% per day for *G. arcuata* in tank culture in the fall to 5.11% per day for *G. edulis* in tank culture in the summer.

INTRODUCTION

Recently there has been an increasing interest in seaweeds and seaweed products on the world market (Michanek, 1978). Concomitant with this growing interest, efforts have been directed toward the development of seaweed mariculture systems throughout the world. The major purposes for seaweed mariculture include phycocolloid production, wastewater purification and human consumption. Some seaweed culture systems have proven so productive that they have also been suggested as a means of biomass production for energy conversion (Doelling, 1978).

One genus of red algae (Rhodophyta) on Guam seems to have potential in each of these categories. This is the genus Gracilaria which is represented by several species on the reef-flat habitats of Guam. This genus is the object of commercial harvest and culture in many areas of the world. In 1976 the worldwide value of the agar from Gracilaria and its direct consumption was valued at approximately US$2,7000,000 (Tsuda and Doty, 1978). One species of this genus, Gracilaria edulis (Gmel.) Silva, is often found in the markets of Guam where it is sold as a fresh vegetable for human consumption.

The two most common species of Gracilaria on Guam are G. edulis and G. arcuata Zanard. Our preliminary studies to evaluate the mariculture potential of seaweeds on Guam have focussed on these two species. The objectives of the present study were 1) to examine the kinetics of ammonium uptake by the two species, and 2) to determine the growth of individual thalli of each species in outdoor tank culture and in reef-flat culture systems. These objectives were chosen to aid us both in the design of future experiments and in the development of a strong research focus for evaluating the potential for the culture of Gracilaria on Guam. The data on ammonium uptake will also be of value in determining the potential value of these species for removal of ammonium from seawater enriched with wastewater.

MATERIALS AND METHODS

Specimens for the study were collected from two locations on Guam. Thalli of Gracilaria edulis were collected from the reef margin near Talofofo Bay, while those of G. arcuata were collected from the reef flat at Hilaan Point.

Ammonium Uptake

Thalli were acclimated at 32^{o}C from four to six days at salinities of either 13, 23 or 34^{o}/oo prior to determining uptake rates. For the uptake experiments, 0.4 to 1.0 gram dry weight of the seaweed were incubated in jars with 350 mℓ of filtered (0.45µ) seawater in an environmental chamber. The temperature was maintained at 32^{o}C ± 1^{o}C and 200 foot candles of light were supplied from banks of incandescent and fluorescent lights. Ammonium chloride ($NH_4Cℓ$) was added to the jars as an ammonium source. Mean substrate concentrations ranged from 3.0×10^{0} to 5.0×10^{3} µg-at NH_4^+-N·ℓ$^{-1}$. All jars were aerated to ensure adequate mixing. Control jars without plants were also monitored under the same conditions over the range of ammonium concentrations.

The dissolved ammonium levels were determined at the onset and after five hours of incubation, with an Orion ammonia electrode (Model 95-10) attached to a Beckman expanded-scale pH meter (Model 55-2). Gilbert and Clay (1973) found that the ammonia electrode compared favorably with the phenolhypochlorite method (Solorzano, 1969) for determining levels of ammonia in seawater. Since salinity is known to affect the probe, it was necessary to calibrate it using standard solutions consisting of $NH_4Cℓ$ dissolved in each of the experimental salinities. Preparation of the appropriate salinities was accomplished by diluting seawater with distilled water. The response characteristics of the probe are discussed in the Appendix.

After determination of the change in ammonium concentration in the jars, the thalli were rinsed in distilled water and dried at 50^{o}C for 24 hours. Rates of uptake were calculated and expressed as µg-at NH_4^+-N·g^{-1}·h^{-1}. A least-squares analysis (Snedecor and Cochran, 1967) was preformed for each regression of uptake rate on substrate concentration.

Portions of thalli from each experiment were analyzed for organic nitrogen content by a microkjeldahl procedure with replicate samples.

Growth in Tank Culture

Freshly collected thalli of G. edulis and G. arcuata were drained to remove excess water and tagged with a 15 cm piece of electrical wire attached to a mylar tag. The thalli and tags were weighed to the nearest 0.01 g on a triple beam balance. The thalli were then attached to the bottoms of 30 x 45 x 10 cm polypropylene mesh baskets and placed in a shallow wooden tank outdoors. Ten to fifteen thalli were placed in each basket. The tank was supplied with a constant and vigorous flow of seawater from Pago Bay. The baskets floated in the tank and the thalli were maintained at 10 cm below the surface. At approximately weekly intervals, the tagged thalli were reweighed. At the end of the experiments, the tags and wires were removed and weighed and these values were substracted to obtain the actual wet weight of each thallus.

The specific growth rate of each thallus was calculated according to the following expression.

$$\mu = \frac{100\left[\ln (N_t/N_0)\right]}{t}$$

where μ = specific growth rate as per cent increase in wet weight per day.

N_t = final weight on day t.

and

N_0 = initial weight

A least-squares analysis (Snedecor and Cochran, 1967) was performed on the regression of specific growth rate on the initial size of thallus for each species.

Growth in Reef-Flat Culture System

To determine the growth of thalli in a reef-flat culture system, two bamboo rafts were constructed and fastened in position in Pago Bay. Weighed thalli were attached between the strands of 3-ply polypropylene rope. The strands of rope were weighted with pieces of iron rebar and suspended from the bamboo rafts. The raft-culture system is depicted in Figure 1 and the locations of the rafts in Pago Bay are shown in Figure 2.

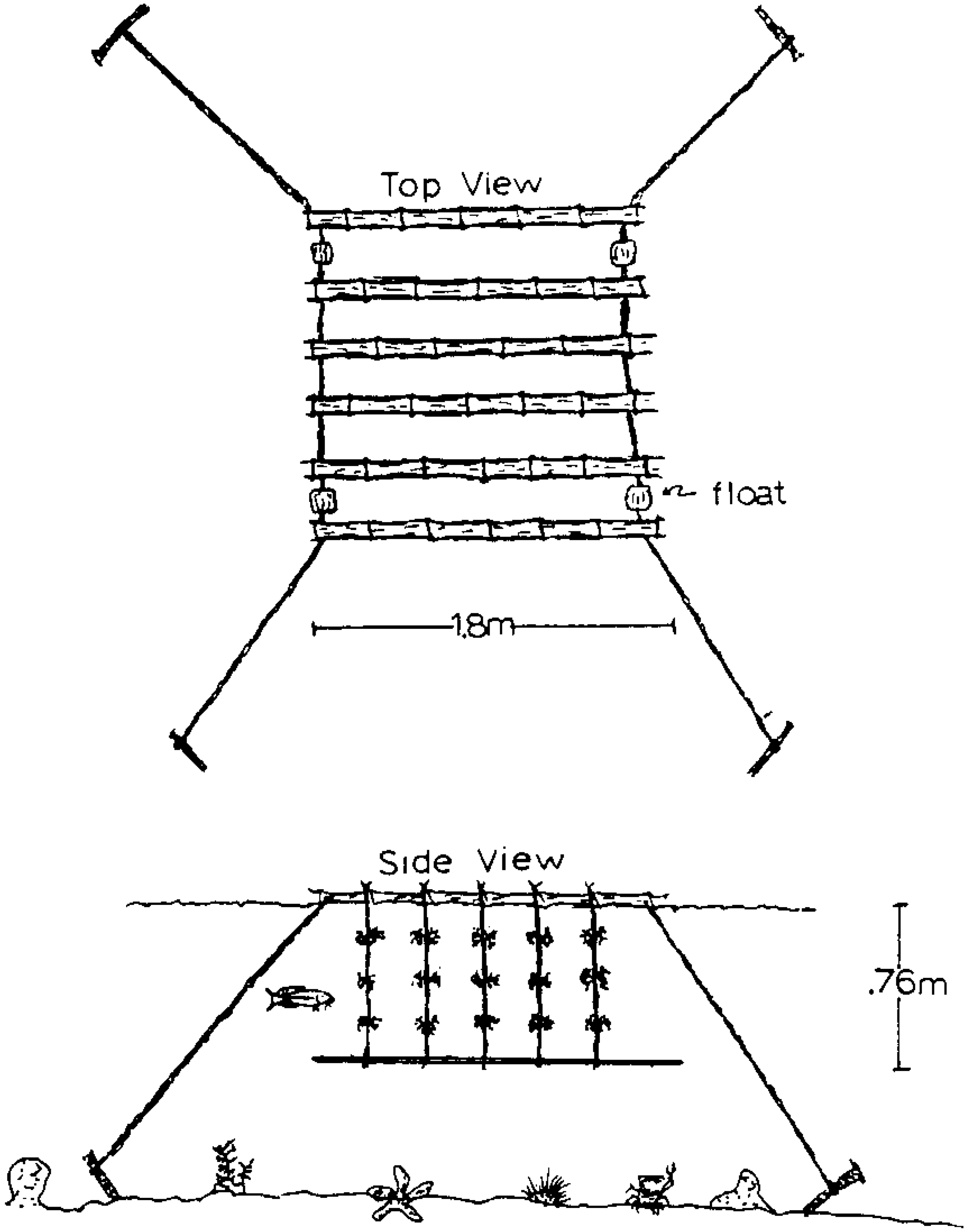

Fig. 1. Design of the bamboo rafts which were used to culture <u>Gracilaria edulis</u> and <u>Gracilaria arcuata</u> in Pago Bay, Guam.

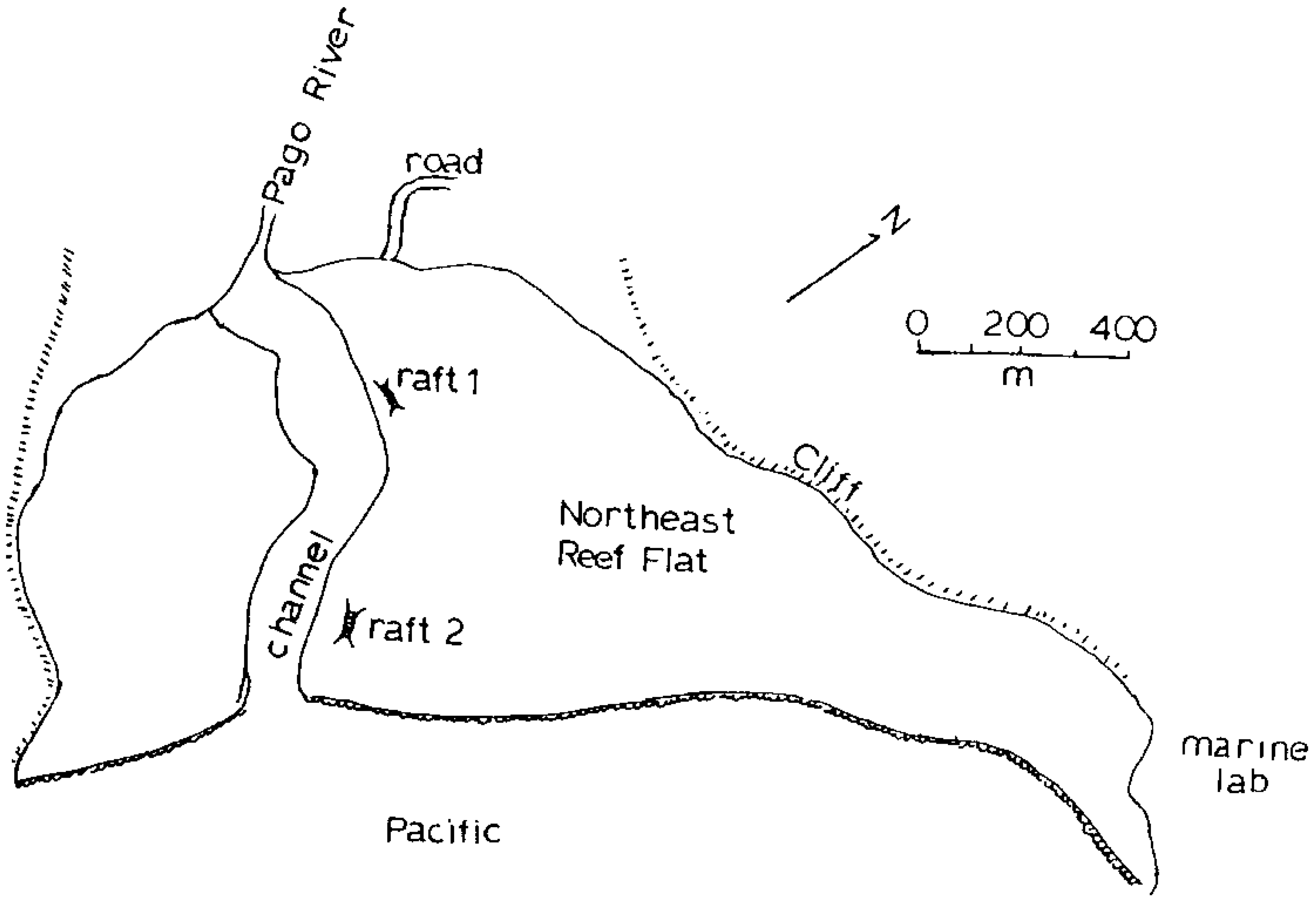

Fig. 2. Map of Pago Bay showing the locations of the rafts.

One raft system and some thalli from the second raft system were lost as a result of wave action when Typhoon Tip passed south of Guam in October 1979. The remaining thalli were used to calculate specific growth rates as described above.

RESULTS AND DISCUSSION

Ammonium Uptake

The uptake of ammonium by both species of *Gracilaria* was linearly related to the mean substrate concentration. Figures 3 and 4 indicate the relation between the rate of ammonium uptake and the mean substrate concentration for each species. Statistics describing the regression of uptake rate (V) on mean substrate concentration (S) are shown in Table 1. The correlation coefficients for these regressions ranged from 0.94 to 0.98. This suggests that a diffusion component in addition to a process of active transport is responsible for ammonium uptake by these species. The studies of D'Elia and DeBoer (1978) also indicated a strong diffusion component in ammonium uptake by the red algae *Gracilaria foliifera* (Forsskal) Børgesen and *Neogardhiella baileyi* (Harvey ex Kützing) Wynne and Taylor.

In studies of other marine macrophytes, NH_4^+ uptake could be adequately described by the Michaelis-Menton equation. This suggests an active transport mechanism is involved. Topinka (1978) showed that the ammonium uptake kinetics of the brown alga *Fucus spiralis* L. could be described by the Michaelis-Menton expression and that the rate of uptake was influenced by temperature. Studies of the green alga *Codium fragile* subsp. *tomentosoides* (Van Goor) Silva (Hanisak and Harlin, 1978) indicated saturation kinetics of ammonium uptake which were influenced by both light and temperature.

The data of D'Elia and DeBoer (1978) showed that ammonium uptake kinetics for *Gracilaria foliifera* and *Neogardhiella baileyi* were probably the result of two uptake systems. The first was characterized as a high affinity system which could be described by the Michaelis-Menton expression. This system predominated at substrate concentrations below 10 µM of NH_4^+ (7.8 µg-at $NH_4^+\text{-}N \cdot \ell^{-1}$). Above this concentration, a strong diffusive component predominated. A similar pattern of NH_4^+ uptake was described for corals which contained symbiotic algae (Muscatine and D'Elia, 1978). The mathematical expression derived to describe the uptake kinetics is as follows:

$$V = V_{max}\, S \cdot (K + S)^{-1} + (K_D \cdot S) \qquad (1)$$

where V and S represent the rate of uptake and the mean substrate concentration, respectively; V_{max} represents the maximum rate of uptake, K represents the substrate concentration when $V = 1/2\ V_{max}$, and K_D is equal to the slope of the linear portion of the plot of V on S.

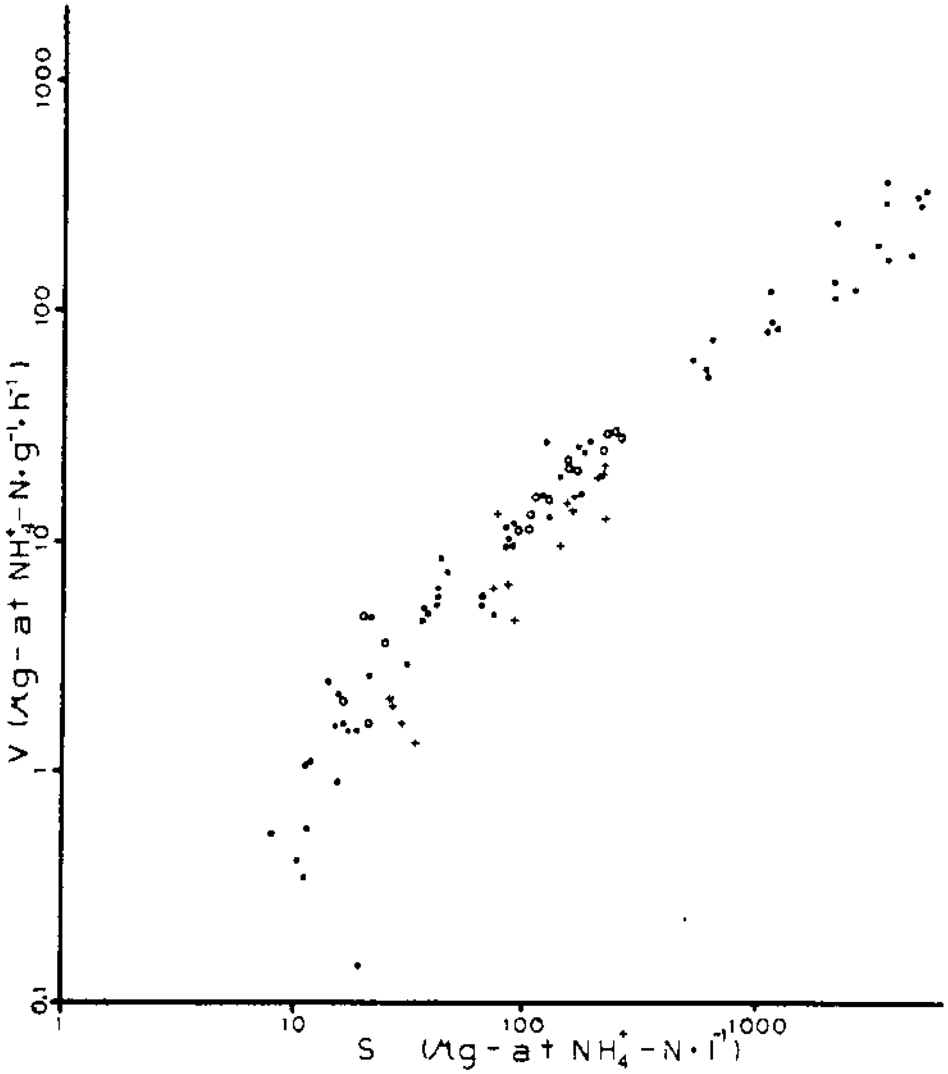

Fig. 3. Plot of ammonium-nitrogen uptake rate (V) on mean substrate concentration (S) for Gracilaria arcuata at 34°/oo (·), 23°/oo (o) and 13°/oo (+).

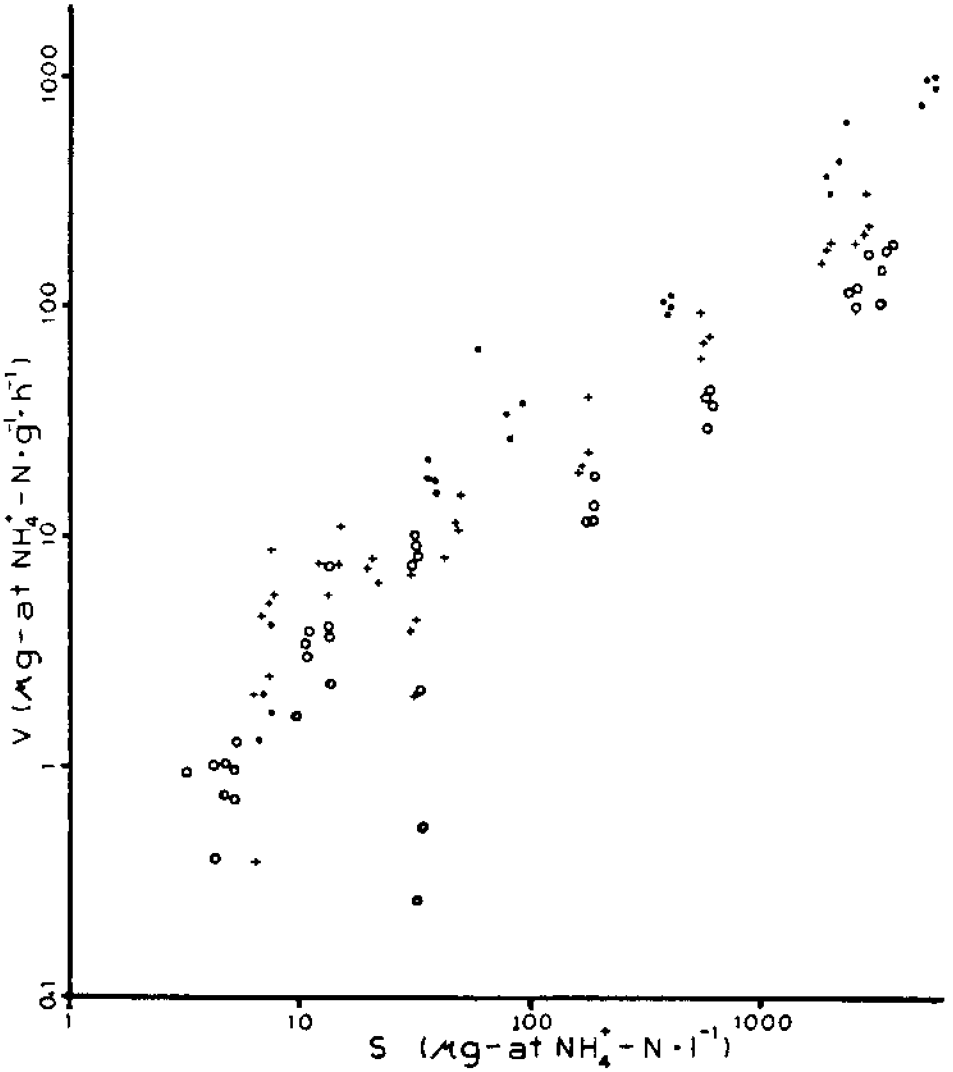

Fig. 4. Plot of ammonium-nitrogen uptake rate (V) on mean substrate concentration (S) for <u>Gracilaria edulis</u> at 34°/oo (·), 23°/oo (o) and 13°/oo (+).

Table 1. Statistics describing the regression of rate of ammonium uptake, V (µg-at NH_4^+-N·g-1·h-1), on the mean substrate concentration, S (µg-at NH_4^+-N·ℓ-1), for *Gracilaria edulis* and *Gracilaria arcuata* at different salinities.

Species	Salinity °/oo	Slope	y-intercept	Correlation Coefficient	N
G. edulis	13	0.089	8.529	0.9816	38
G. edulis	23	0.051	3.754	0.9826	40
G. edulis	34	0.188	24.703	0.9818	24
G. arcuata	13	0.089	-0.303	0.9448	20
G. arcuata	23	0.114	1.050	0.9849	16
G. arcuata	34	0.060	7.910	0.9395	60

At high substrate concentrations, the rate of uptake (V) is essentially determined by the diffusion component ($K_D \cdot S$). This was the prevailing situation in the present study and is evidenced by the high degree of correlation between V and S for both G. edulis and G. arcuata. Since the study of D'Elia and DeBoer (1978) was limited to substrate concentrations below 50 μM of NH_4^+ (38.9 μg-at NH_4^+-$N \cdot \ell^{-1}$), we have demonstrated that non-saturation kinetics are evident even at very high substrate concentrations (up to 5.0 x 10^3 μg-at NH_4^+-$N \cdot \ell^{-1}$).

Since D'Elia and DeBoer (1978) found a strong dependence of ammonium uptake on the C:N ratio of the thalli, we analyzed our specimens for organic nitrogen content. The results are displayed in Table 2. There was a considerable amount of variability in the nitrogen content of the thalli, which we suspect was the result of individual variation. There were no apparent seasonal differences. The percentage of organic nitrogen ranged from 2.20 to 4.23% for G. arcuata and from 1.02 to 3.95% for G. edulis with means of 3.31% and 3.07%, respectively.

Our data cover a wide range of substrate concentrations. We were able to monitor uptake rates at very high substrate concentrations through the use of an Orion ammonia electrode. Many studies of ammonium uptake utilize the method of Solorzano (1969) for determination of dissolved ammonium (Topinka, 1978; Hanisak and Harlin, 1978; D'Elia and DeBoer, 1978). This method is generally limited to ammonium concentrations below 50 μg-at $NH_4^+ \cdot \ell^{-1}$. With the ammonia probe, we were able to monitor uptake rates at substrate concentrations greater than 10^3 μg-at$\cdot \ell^{-1}$ of NH_4^+-N. Of course, these concentrations are much greater than those which would be found in natural Gracilaria habitats, but the data are interesting in light of the potential of Gracilaria for stripping ammonia from wastewater. It appears, from our data, that both species of Gracilaria we studied would be well suited for removal of NH_4^+-N from seawater.

Growth

The specific growth rates of G. edulis thalli in tank culture during September and October of 1979 ranged up to 6% per day with a mean of 2.6% per day. This species had a higher growth rate during June and July 1979 when specific growth rates averaged 5.1% per day. In tank culture, the mean specific growth rate of G. arcuata during September and October of 1979 was 2.02% per day. In field culture during September and October, mean specific growth rates for G. edulis and G. arcuata were 4.8 and 3.5% per day, respectively. The mean specific growth rates for each species and culture system are shown in Table 3.

Table 2. Means and standard deviations of nitrogen content of *Gracilaria edulis* and *Gracilaria arcuata* used in the uptake experiments.

Species	Date	Mean % Nitrogen ± Standard Deviation	Sample Size
G. edulis	9/13/79	1.02 ± 0.00	2
G. edulis	9/28/79	2.87 ± 0.22	2
G. edulis	10/26/79	1.45 ± 0.02	2
G. edulis	10/26/79	1.88 ± 0.116	2
G. edulis	11/ 9/79	1.99 ± 0.12	2
G. edulis	12/ 9/79	3.95 ± 0.00	2
G. edulis	12/16/79	1.33 ± 0.00	2
G. arcuata	7/26/79	4.23 ± 0.17	2
G. arcuata	7/28/79	3.50 ± 1.02	2
G. arcuata	7/29/79	2.20 ± 0.06	2
G. edulis	all data	2.07 ± 0.95	14
G. arcuata	all data	3.31 ± 1.03	6

Table 3. Specific growth rates (% increase in wet weight per day) of *Gracilaria edulis* and *Gracilaria arcuata* in both a tank culture system and a raft culture system on Guam.

Species	Culture System	Date	Mean Specific Growth Rate (u)	Standard Deviation	N
G. edulis	tank	6/79	5.11	± 0.23	30
G. edulis	tank	9/79	2.56	± 2.58	60
G. arcuata	tank	9/79	2.02	± 1.16	19
G. edulis	raft	9/79	4.80	± 3.53	15
G. arcuata	raft	9/79	3.50	± 0.72	8

Table 4 displays the statistics which describe the regression of specific growth rate on initial weight of the thallus for each species, in both the tank culture and the reef-flat culture systems. With the exception of *G. arcuata* in reef-flat culture, there was a negative correlation (r ranged from -0.3075 to -0.4298) between initial size and specific growth rate. In the case of *G. arcuata* from the reef-flat culture system, there was no significant correlation ($r = 0.2353$, $p < 0.05$). However, because of the small sample size ($n = 8$), we feel that the data, in this case, are inconclusive. Most of the reef-flat-grown *G. arcuata* thalli were lost as a result of storm damage from Typhoon Tip.

The specific growth rates found in the present study for *G. edulis* and *G. arcuata* are similar to those described by other investigators for other species of *Gracilaria*. Causey et al. (1946) showed that growth rates of *Gracilaria confervoides* (L.) Greville [now known as *G. verrucosa* (Hudson) Papenf.] in North Carolina were greatest at depths of less than four feet (1.2 m). They found also that growth rates were most rapid at temperatures between 25° and 30° and were unaffected by salinity within the normal range of fluctuation. At depths of less than 1.2 m, plants doubled in weight within 10 days.

In raceway culture systems at Woods Hole, Massachusetts, DeBoer et al. (1978) reported specific growth rates of 3-5% for *Gracilaria foliifera* in unenriched seawater. In the same studies, specific growth rates of over 12% were obtained by enrichment of the media with ammonia. DeBoer and Ryther (1977) reported potential yields of *Gracilaria foliifera* from tank culture in Massachusetts of 28.0 tons (dry weight) $\cdot ha^{-1} \cdot yr^{-1}$ while production of up to 46 tons (dry weight) $\cdot ha^{-1} \cdot yr^{-1}$ were reported from culture systems for *Gracilaria* in Florida (Lapointe et al., 1976). Hoyle (1978) reported mean growth rates of 12.24% and 6.41% per day for *G. bursapastoris* (Gmelin) Silva and *G. coronopifolia* J. Ag., respectively, in tank culture in Hawaii.

It is most likely that the growth rates of *G. edulis* and *G. arcuata* on Guam can be enhanced by nutrient enrichment. This could be accomplished by the addition of nutrients to tank culture systems or by the suspension of bags of nutrients near raft culture systems.

Table 4. Statistics describing the regression of specific growth rate (% increase in wet weight per day) on initial size of thalli of *Gracilaria edulis* and *Gracilaria arcuata*.

Species	Culture System	Date	Slope	y-intercept	Correlation Coefficient	N
G. edulis	tank	6/79	-0.047	5.77	-0.368	30
G. edulis	tank	9/79	-0.069	2.56	-0.303	60
G. arcuata	tank	9/79	-0.048	2.29	-0.365	19
G. edulis	raft	9/79	-0.377	7.572	-0.430	15
G. arcuata	raft	9/79	0.057	3.035	0.2353	8

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APPENDIX

Ammonia is present in solution in seawater in two forms, ionized (NH_4^+) and un-ionized (NH_3). The equilibrium equation for ammonia in aqueous solution is:

$$NH_4^+ + H_2O \rightleftharpoons NH_3 + H_3O^+$$

The relative proportions of NH_4^+ to NH_3 are strongly dependent on pH. Increased pH shifts the equilibrium curve to the right. The Orion ammonia probe was used to determine the amount of total ammonia dissolved in seawater by treating the samples with NaOH to convert the NH_4^+ to the un-ionized form (NH_3). The hydrophobic membrane on the ammonia probe is permeable to NH_3. When NH_3 diffuses from the sample across the membrane the pH of the internal solution of the probe is changed. This pH change was read as millivolts on a Beckman expanded scale pH meter. The change in millivolts can be converted to $\mu g\text{-at} \cdot \ell^{-1}$ of NH_4^+-N, or other convenient units by recording the probe's response to a series of standard solutions. A typical calibration curve is shown in Figure A-1.

The probe response is very precise. The response curve is linear at substrate concentrations greater than approximately 5 $\mu g\text{-at}\ NH_4^+\text{-N} \cdot \ell^{-1}$. At lower concentrations the response is curvilinear. The response of the probe is affected by temperature and salinity so the calibration curve should be constructed prior to each group of samples.

We found that the ammonia probe was a convenient method for monitoring ammonium uptake by seaweeds.

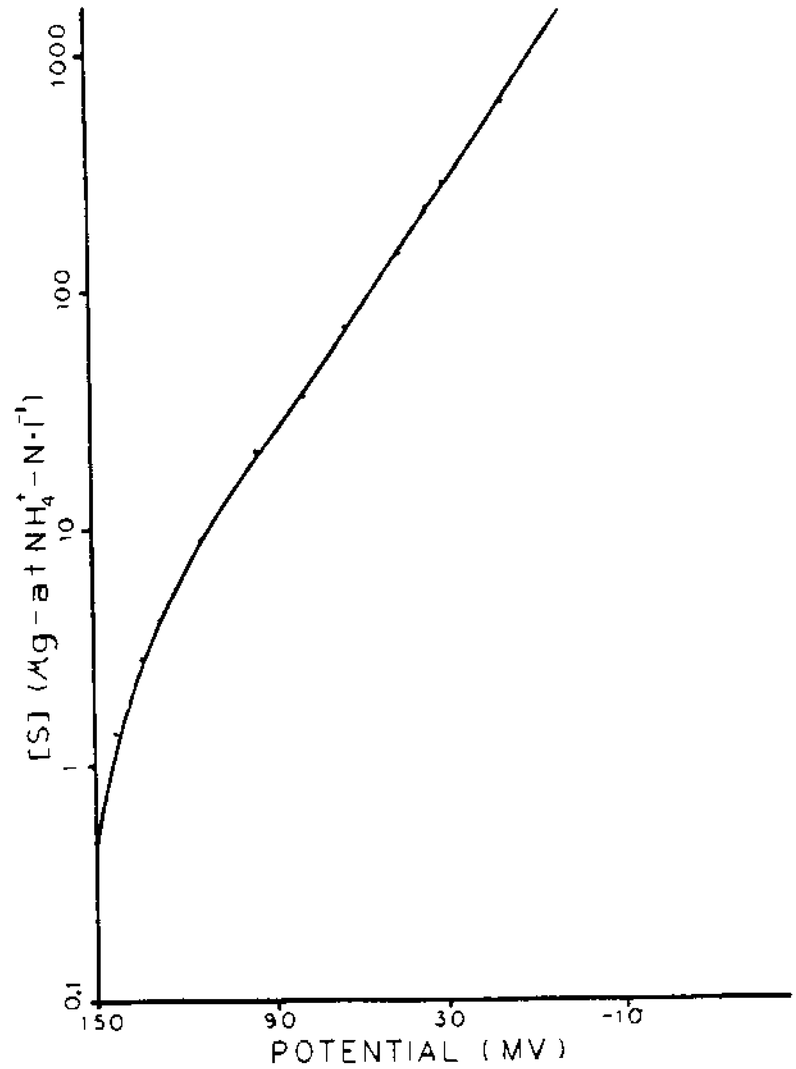

Fig. A-1. Response of the Orion ammonia electrode. The relation between dissolved ammonium-nitrogen and electrical potential.