



# AMMONIUM

## UPTAKE BY MICRONESIAN SPECIES OF GRACILARIA (RHODOPHYTA)

Stephen G. Nelson

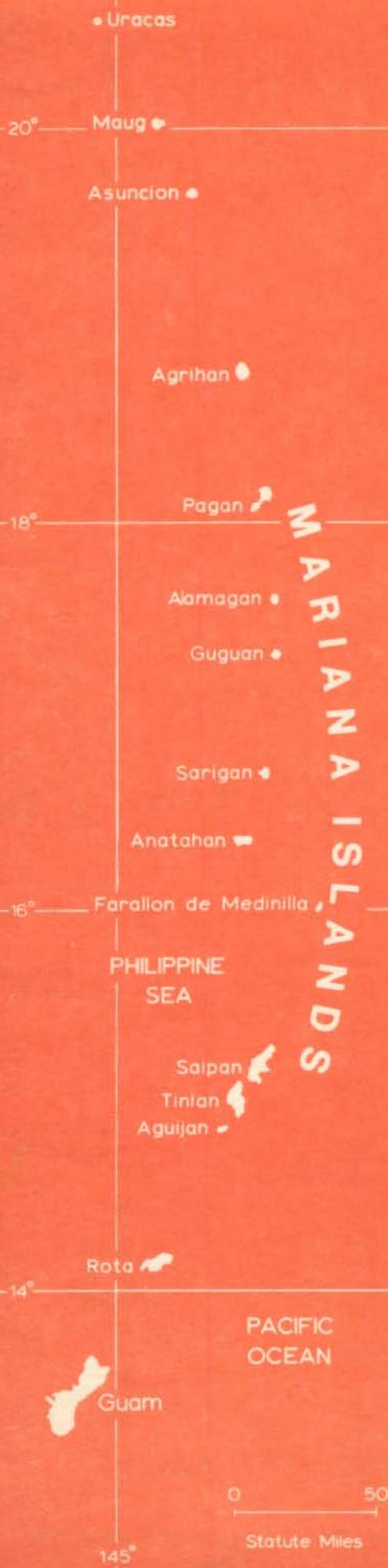
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Water and Energy Research Institute  
of the  
Western Pacific

Technical Report No. 23

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By

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Project Completion Report

for

REMOVAL OF AMMONIUM AND NITRATE FROM DILUTE SEAWATER  
BY SELECTED BENTHIC MACROPHYTES FROM MICRONESIA

OWRT Project No. A-019-Guam, Grant Agreement No. 14-34-0001-0112

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## ABSTRACT

The kinetics of ammonium uptake were examined for several species of Gracilaria from Micronesia. Ammonium uptake was dependent upon substrate concentration. The observed uptake kinetics were consistent with, and suggested a diffusive ammonium uptake process. Rates of uptake were similar during both the day and the night and were not dependent on light. The rate of ammonium uptake was independent of the thalli nitrogen content, which ranged from 0.7 to 3.5% N. Rates of ammonium uptake were generally higher for G. edulis than for other species, a fact which may be related to thallus morphology.

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## INTRODUCTION

In recent years there has been increased interest in the use of seaweeds in mariculture systems for the purpose of nutrient removal (e.g., Ryther et al., 1975 and 1978; Prince, 1974; Ryther, 1977; DeBoer et al., 1978; DeBoer and Ryther, 1978). The removal of dissolved ammonia from mariculture systems which include fish or invertebrates is especially important since high concentrations can result in reduced production and survival.

Several recent studies have focused on the abilities of marine macrophytes to take up ammonium (D'Elia and DeBoer, 1978; Harlin et al., 1978; Nelson et al., 1980). Species of the red algal genus Gracilaria seem especially well-suited for this purpose because of their ammonium uptake kinetics (D'Elia and DeBoer, 1978; Nelson et al., 1980). Ammonium uptake by the species of Gracilaria which have been studied does not follow the familiar pattern of saturation kinetics described by the Michaelis-Menten expression. Instead, these species can continue to take up ammonium even at very high ambient concentrations.

We became interested in the use of species of this genus for use in Micronesia for several reasons. Firstly, the stability of environmental temperatures of this region would allow a macrophyte nutrient-stripping system to operate year-round. Secondly, Gracilaria can be easily propagated by vegetative means. Additionally, species of this genus are economically valuable sources of agar (e. g., Kim and Henriquez, 1978; Doty, 1979). The object of this report was to examine the kinetics of ammonium uptake by several species of Gracilaria from Micronesia in order to evaluate their relative potential for removing ammonium from mariculture systems.

## MATERIALS AND METHODS

Thalli of several Gracilaria species were collected from various locations on Guam and Saipan, Mariana Islands, and near Malakal Island, Palau. Species collected from Guam were G. edulis (Gmel.) Silva, G. arcuata Zanard, and one unidentified species. Species collected from Saipan were G. lichenoides (L.) J. Ag. and G. salicornia (Mert.) Grev. Species collected from Palau were G. crassa Harvey and G. salicornia.

Thalli collected from Palau were taken to the laboratory at the Micronesian Mariculture Demonstration Center in Palau. Those collected from Guam and Saipan were taken to the Marine Laboratory of the University of Guam. Thalli were maintained in flowing seawater at the laboratories for several days prior to their use in uptake trials.

Prior to these trials the thalli were vigorously washed under running seawater to remove sediment, attached debris, epiphytes, and small invertebrates. For the uptake trials pre-weighted thalli were placed in 2-l beakers with 1.8% of 1- $\mu$ -filtered, 3.3‰ seawater. Each beaker was gently aerated with compressed air delivered through Tygon and glass tubing. On Guam the beakers

were kept in a water bath at  $30 \pm 1^\circ\text{C}$  under  $48 \text{ microeinsteins} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$  of light supplied by fluorescent and incandescent lights. Light intensity was determined with a Licor underwater quantum sensor attached to a Licor model 185-A light meter. At the Micronesian Mariculture Demonstration Center the uptake trails were conducted at ambient light ( $28 \text{ microeinsteins} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$  at one centimeter below surface water) and temperature ( $29 \pm 1^\circ\text{C}$ ) under a fiberglass roof. The light intensity during the daytime trials in Palau was measured with a photographic light meter. This meter was later compared with the Licor light meter and the values were converted to the same units. Ammonium was added to the beakers in the form of  $\text{NH}_4\text{Cl}$ , and the time course of its depletion was monitored with an Orion model 95-10 gas-sensing ammonia electrode attached to either an Orion model 701-A digital pH/mv meter (Palau) or an Orion model 901 ionalyzer (Guam). Measurements were made every 15 minutes from 25-ml aliquots. Several studies have shown that the determination of dissolved ammonia in seawater with the Orion electrode compares favorably with other methods (Gilbert and Clay, 1973; Srna et al., 1973; Merks, 1975). Immediately prior to determination of dissolved ammonia, 0.25 ml of 10N NaOH was added to each 25-ml sample. This shifts the  $\text{NH}_4^+ : \text{NH}_3$  ratio toward the un-ionized form to which the probe is sensitive. Therefore, total dissolved ammonia was measured as the sum of ammonium ( $\text{NH}_4^+$ ) and ammonia ( $\text{NH}_3$ ). However, we refer only to dissolved ammonium ( $\text{NH}_4^+$ ) since most of the total ammonia in seawater at normal pH is in this form. The actual ratio of the two forms is dependent on temperature, pH, and salinity, as described by Bower and Bidwell (1978). After the uptake trails, each thallus was oven-dried overnight at  $50^\circ\text{C}$  and weighed to the nearest 0.001g on a Mettler electronic balance. Thalli nitrogen contents were analyzed with a microkjeldahl procedure.

It has already been demonstrated that ammonium uptake by *Gracilaria* does not exhibit saturation kinetics and that a diffusion model may be more appropriate (D'Elia and DeBoer, 1978; Nelson et al., 1980). A diffusion process would yield a rate of nutrient uptake which is related to the concentration of dissolved ammonium and expressed by the equation:

$$-dC/dt = KC \quad (1)$$

where  $-dC/dt$  is the rate of depletion of ammonium ( $\mu\text{g-at NH}_4^+ \cdot \text{N} \cdot \ell^{-1} \cdot \text{h}^{-1}$ ),  $C$  is the concentration of dissolved ammonium ( $\mu\text{g-at NH}_4^+ \cdot \text{N} \cdot \ell^{-1}$ ), and  $K$  is a diffusion constant. Integration of this equation gives:

$$\log C = - \frac{k}{2.3} t + \text{constant} \quad (2)$$

The diffusion constant ( $k$ ) was estimated by fitting a line by the least-squares method for the regression of the log of substrate concentration with time. These values were used to calculate the rate of ammonium uptake in terms of  $\mu\text{g-at NH}_4^+ \cdot \text{N} \cdot \ell^{-1} \cdot \text{h}^{-1}$ .

## RESULTS

The ammonium depletion curves for each species were consistent with the diffusion model described by equations 1 and 2. A typical depletion curve is shown in Figure 1. The regressions of the log of substrate concentration,  $C$  ( $\mu\text{g-at NH}_4^+-\text{N}\cdot\text{l}^{-1}$ ), with time (h) for each uptake trial were linear. The statistics which describe these regressions are shown in Table 1. The correlation coefficients were high and ranged from 0.913 to 0.996.

The value of the diffusion constant ( $K$ ) is equal to the slope of the line multiplied by  $-2.3$ . The values of  $K$  derived from the depletion curves ranged from 0.179 to 6.327. Of course the rate of substrate depletion for a particular species is a function of the ratio of the thallus biomass to the volume of the incubation medium. Since equal volumes were used in the uptake trials, the rate of depletion, and therefore the value of  $K$ , was influenced by the weight of the thalli. The relation between  $K$  and the dry weight of thalli for trials with *G. salicornia* is shown in Figure 2. The correlation coefficient is 0.913. Similar weights of thalli (4.860-7.471g dry weight) were used for each *G. edulis* and *G. arcuata* uptake trial and there was no significant correlation ( $r = 0.663$  and  $0.772$ , respectively) between  $K$  and dry weight for these species.

In order to express the ammonium uptake rates "per gram" for each species, each value of  $K$  was multiplied by the initial incubation volume (1.8l) and divided by thallus dry weight to yield  $K'$ . The uptake rate,  $V$  was then expressed in  $\mu\text{g-at NH}_4^+-\text{N}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$  as a function of substrate concentration ( $C$ ) for each species. This relation can then be expressed as

$$V = K'C \quad (3)$$

The values of  $K'$  for each uptake trial are shown in Table 2. They ranged from 0.810 to 2.184 for *G. edulis* and from 0.086 to 0.722 for other species.

Experiments with *G. salicornia* in Palau showed that the change in uptake rate with substrate depletion was not simply the result of increased internal nitrogen pools within the thalli. After the  $\text{NH}_4^+-\text{N}$  had been depleted from the media, additional  $\text{NH}_4^+-\text{N}$  was added, and the depletion trial was repeated. The resulting depletion curves could be superimposed which indicates that the change in uptake rate, in fact, was responding to the change in substrate concentration and not to an increase in internal  $\text{NH}_4^+-\text{N}$  pools of the thallus.

Experiments with *G. salicornia* on Palau and *G. edulis* and *G. arcuata* on Guam showed that uptake rates are similar during both the day and night. Depletion curves for these experiments are shown in Figure 3.

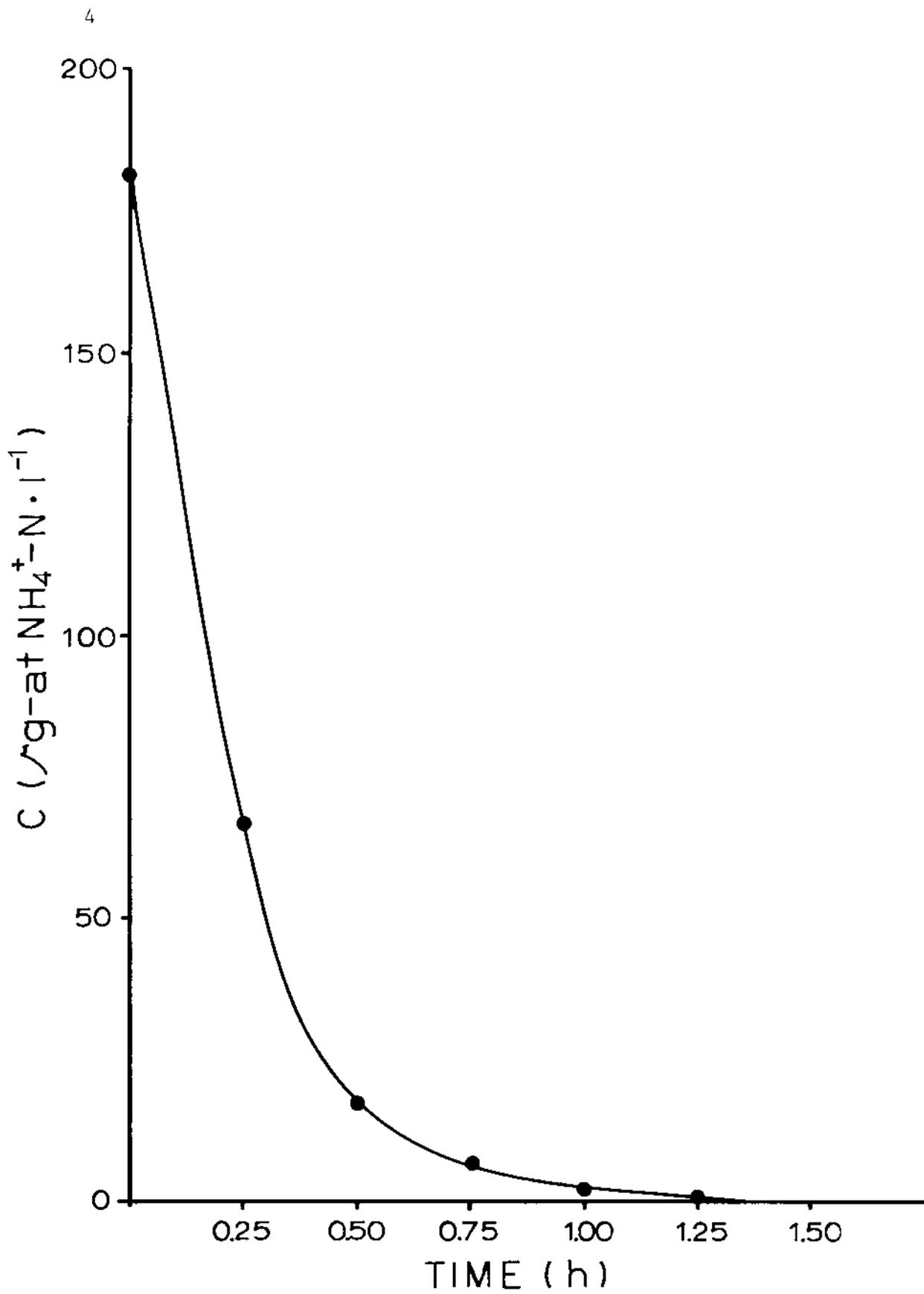


Figure 1. Typical nutrient depletion curve.

Table 1. Statistics describing the regression of the log of substrate concentration, C ( $\mu\text{g-at NH}_4^+\text{-N}\cdot\ell^{-1}$ ), with time (h) for nutrient uptake experiments on Gracilaria species from Guam, Saipan, and Palau.

Species	Location	Date	Slope	y-intercept	Correlation Coefficient	N
<u>G. edulis</u>	Guam	10/26/80	-0.951	1.593	-0.913	9
<u>G. edulis</u>	Guam	11/25/80	-2.751	2.383	-0.995	6
<u>G. edulis</u>	Guam	2/08/81	-2.511	1.928	-0.982	3
<u>G. edulis</u>	Guam	2/08/81	-2.713	1.875	-0.991	3
<u>G. arcuata</u>	Guam	10/26/80	-0.848	2.218	-0.990	10
<u>G. arcuata</u>	Guam	11/25/80	-0.596	2.371	-0.991	11
<u>G. arcuata</u>	Guam	2/08/81	-0.447	1.903	-0.993	13
<u>G. arcuata</u>	Guam	2/08/81	-0.358	1.825	-0.994	15
<u>G. sp.</u>	Guam	11/25/80	-0.399	2.294	-0.973	11
<u>G. lichenoides</u>	Saipan	2/08/81	-0.856	1.905	-0.996	10
<u>G. salicornia</u>	Saipan	2/08/81	-0.552	1.952	-0.989	14
<u>G. crassa</u>	Palau	6/10/80	-0.082	2.965	-0.991	12
<u>G. salicornia</u>	Palau	6/10/80	-0.078	3.134	-0.966	12
<u>G. salicornia</u>	Palau	6/11/80	-1.085	2.201	-0.964	6
<u>G. salicornia</u>	Palau	6/11/80	-1.660	2.175	-0.970	6
<u>G. salicornia</u>	Palau	6/11/80	-1.935	2.295	-0.974	6
<u>G. salicornia</u>	Palau	6/11/80	-2.297	2.230	-0.976	6
<u>G. salicornia</u>	Palau	6/13/80	-1.512	2.341	-0.995	8
<u>G. salicornia</u>	Palau	6/13/80	-1.507	2.220	-0.967	7

The nitrogen contents of the thalli used in each uptake trial are presented in Table 2. The values of  $K'$  are not correlated with the nitrogen contents of the thalli for G. arcuata, G. edulis or G. salicornia ( $r = -0.067$ ,  $-0.015$ , and  $-0.567$ , respectively).

## DISCUSSION

### Uptake Kinetics

All of the species of Gracilaria which we examined have potential for use in stripping ammonium from mariculture systems. This potential is the result of a strong diffusive component in their uptake kinetics. Other species of marine macrophytes have exhibited ammonium uptake kinetics which could be described by the familiar Michaelis-Menten expression. The species which exhibit this rate saturating form of ammonium uptake include: Codium fragile (Van Goor) Silva (Hanisak and Harlin, 1978), Macrocystis pyrifera (L.) C.A. Agards (Wheeler, 1979), Fucus spiralis L. (Topinka, 1978), and Iridaea cordata (Turner) Bory (Hansen, 1978).

D'Elia and DeBoer (1978) studied ammonium uptake by Gracilaria foliifera (Forsskal) Børgesen and Neoagardhiella baileyi (Harvey ex Kutzing) Wynne and Taylor. They postulated a dual-component uptake system for these species. Rate saturable kinetics predominated at low substrate levels ( $<10 \mu\text{M NH}_4^+\text{-N}$ ), and diffusive kinetics predominated at higher substrate levels. We were primarily concerned with the diffusion component since our interest in Gracilaria was in relation to the reduction of elevated ammonium concentrations. The high coefficient for the regressions of log of substrate concentration with time indicated a reasonably good fit of our data to a diffusive uptake model.

As shown in Figure 3, uptake proceeded similarly during both the day and the night. The study of D'Elia and DeBoer (1978) showed a diel cycle of ammonium uptake under some conditions. The degree of this diel influence was determined by residence time in flow-through systems and by the C:N ratio of thalli in static systems.

The rates of ammonium uptake per gram of thallus are generally faster for G. edulis than for the other species. This may be a function of the thallus morphology. The thalli of G. edulis are thinner than those of the other species and therefore they have a higher surface to mass ratio.

Our study failed to demonstrate that the rate of ammonium uptake was dependent on the nitrogen content of the thalli. D'Elia and DeBoer (1978) however, showed that ammonium uptake by G. foliifera was influenced by the C:N ratio of the thallus. Thalli with lower C:N ratios exhibited lower rates of ammonium uptake. The nitrogen content of the thalli may not be correlated with the C:N ratio as a result of differences in ash-content.

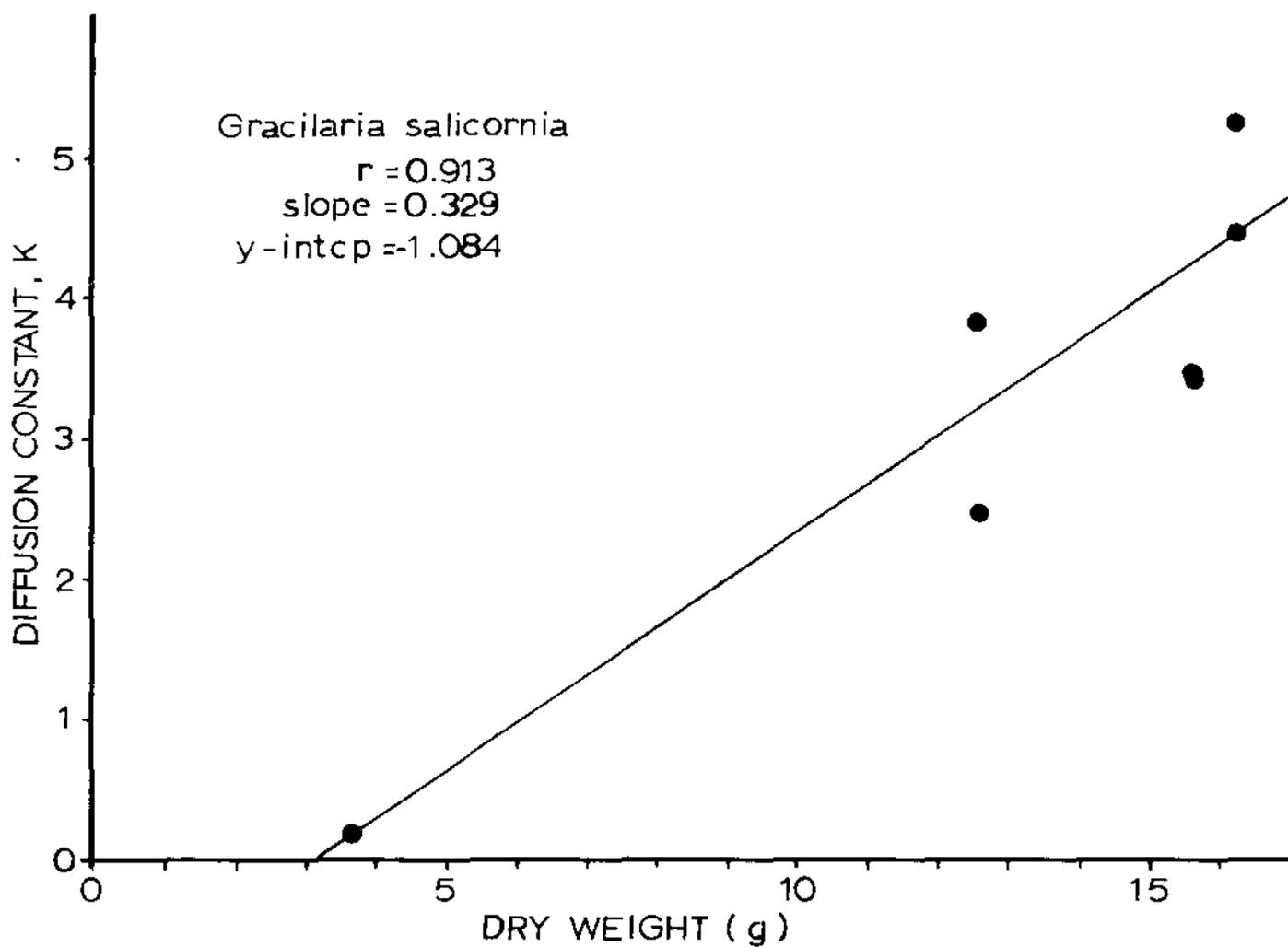


Figure 2. Plot of diffusion constant, K, on thallus dry weight (g) for nutrient uptake experiments on Gracilaria salicornia from Palau.

Table 2. Diffusion constants ( $K'$ ) and mean  $\pm$  standard deviation of nitrogen content of Gracilaria species from Guam, Saipan, and Palau.

Species	Location	Date	$K'$	Mean % Nitrogen $\pm$ Standard Deviation	Number of Samples
<u>G. edulis</u>	Guam	10/26/80	0.810	1.38 $\pm$ 0.09	3
<u>G. edulis</u>	Guam	11/25/80	2.184	1.55 $\pm$ 0.26	5
<u>G. edulis</u>	Guam	2/08/81	1.677	0.88 $\pm$ 0.06	3
<u>G. edulis</u>	Guam	2/08/81	1.702	0.75 $\pm$ 0.00	3
<u>G. arcuata</u>	Guam	10/26/80	0.504	2.27 $\pm$ 0.00	3
<u>G. arcuata</u>	Guam	11/25/80	0.330	3.50 $\pm$ 0.14	3
<u>G. arcuata</u>	Guam	2/08/81	0.353	1.75 $\pm$ 0.06	3
<u>G. arcuata</u>	Guam	2/08/81	0.289	1.96 $\pm$ 0.08	2
<u>G. sp.</u>	Guam	11/25/80	0.244	1.77 $\pm$ 0.25	3
<u>G. lichenoides</u>	Saipan	2/08/81	0.722	1.87 $\pm$ 0.22	3
<u>G. salicornia</u>	Saipan	2/08/81	0.466	2.26 $\pm$ 0.08	3
<u>G. crassa</u>	Palau	6/10/80	0.094	0.89 $\pm$ 0.07	3
<u>G. salicornia</u>	Palau	6/10/80	0.086	2.27 $\pm$ 1.42	2
<u>G. salicornia</u>	Palau	6/11/80	0.354	0.80 $\pm$ 0.21	3
<u>G. salicornia</u>	Palau	6/11/80	0.542	0.80 $\pm$ 0.21	3
<u>G. salicornia</u>	Palau	6/11/80	0.490	0.68 $\pm$ 0.07	3
<u>G. salicornia</u>	Palau	6/11/80	0.581	0.68 $\pm$ 0.07	3
<u>G. salicornia</u>	Palau	6/13/80	0.397	0.72 $\pm$ 0.00	3
<u>G. salicornia</u>	Palau	6/13/80	0.396	0.72 $\pm$ 0.00	3

The observation that rates of ammonium uptake are similar during both the day and night indicates that uptake is uncoupled from photosynthesis which allows the species to effectively remove nutrients continuously.

### Hydraulic Interactions

We used a continuous flow stirred-tank reactor (C.F.S.T.R.) model (Levenspiel, 1972) to approximate the hydraulic interactions of a circular pond or culture tank. The ammonium level in the tank at steady state is a function of the inflow concentration, the flow rate, and the rate of ammonium uptake by the Gracilaria. The ammonium uptake rate by a particular species is a function of the biomass of the thalli and the concentration in the tank at steady state.

A mass budget for the flow of ammonium can be described by

$$(C_i - C_o) Q = VB \quad (4)$$

where  $C_i$  and  $C_o$  are the concentration ( $\mu\text{g-at NH}_4^+\text{-N}\cdot\ell^{-1}$ ) of the inflow and outflow, respectively,  $Q$  is the flow rate ( $\ell\cdot\text{h}^{-1}$ ),  $V$  is the uptake rate ( $\mu\text{g-at NH}_4^+\text{ N}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$ ), and  $B$  is the dry biomass (g) equivalent of Gracilaria. By substituting  $V$  with its equivalent from equation 4 becomes

$$(C_i - C_o) Q = K' C_o B \quad (5)$$

where  $C$  is the ammonium concentration in the tank. By rearranging the terms, equation 5 becomes

$$C_i \cdot C_o^{-1} = K' \cdot BQ^{-1} + 1 \quad (6)$$

This model assumes that net loss of ammonium to the atmosphere is negligible. At the low concentrations used in this study this assumption is reasonable.

In this study, the values of  $K'$  for Gracilaria ammonium uptake ranged from 0.1 to 2.2. Within this range the sensitivity of  $C_i \cdot C_o^{-1}$  to  $K'$  is diminished as the flow rate ( $Q$ ) is increased. However, the value of  $C_i \cdot C_o^{-1}$  is linearly related to  $K'$  and at low flow rates differences in the values of  $K'$  have a pronounced effect on the efficiency of the system at ammonium removal. Of course at the very high flow rates the value of  $C_i \cdot C_o^{-1}$  approaches 1. These data and their relations may be useful for designing systems for removal of ammonium from mariculture systems.

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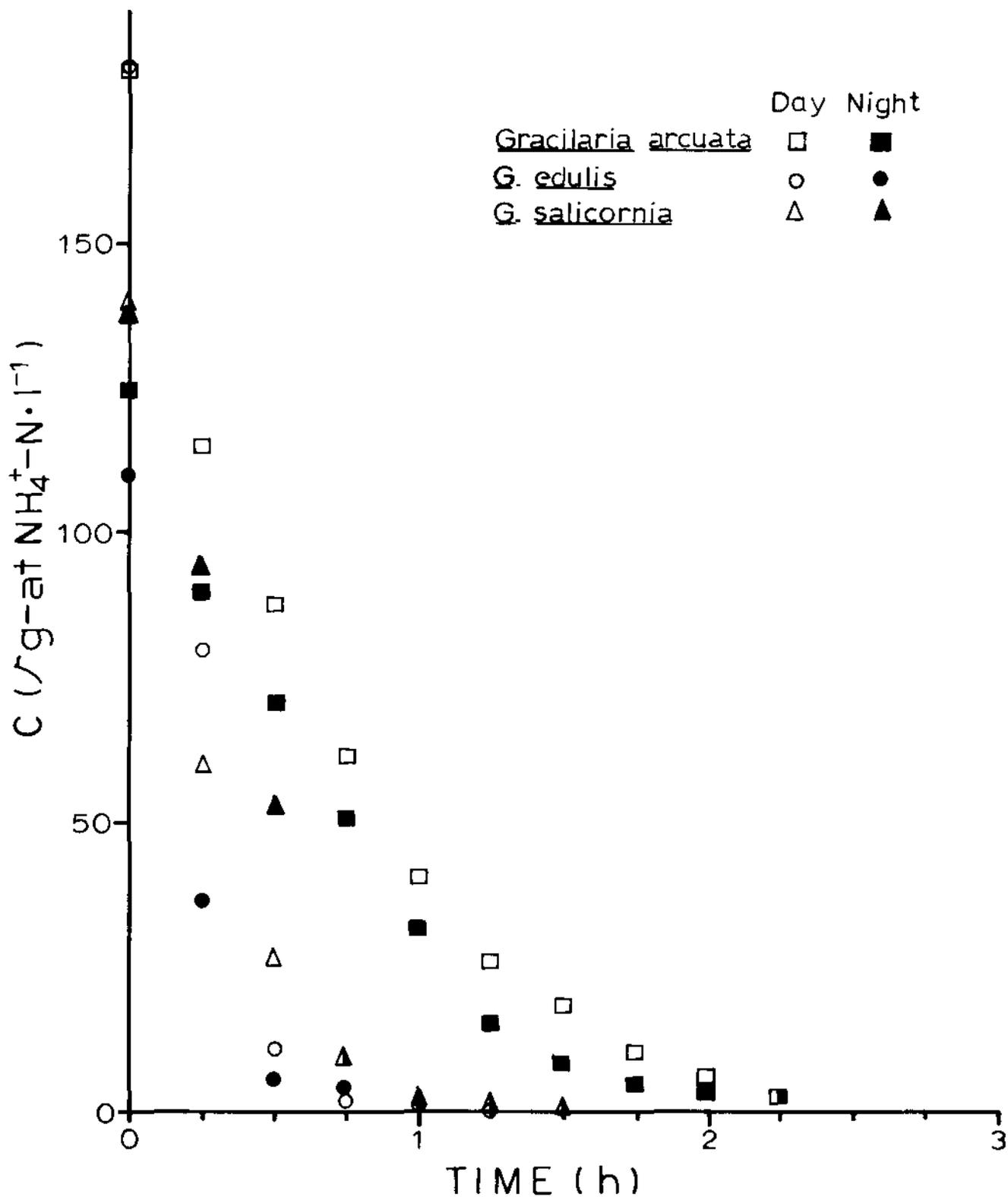


Figure 3. Nutrient depletion curves of *Gracilaria arcuata* (Guam), *G. edulis* (Guam), and *G. salicornia* (Palau) under day and night conditions.

Center. Mr. Pete Dela Cruz, Mr. Joaquín Villagomez and Mr. David Aldan made it possible for us to collect seaweeds from Saipan. Mr. Vaughan Tyndzik and Mr. Frank Lujan assisted with the field work in Palau and Dr. Daniel Matlock assisted us in Saipan. Mr. Vernon Kamiaz helped us in the laboratory as well as in the field and Mr. Barry Smith critically reviewed the manuscript. This study is also referenced as Technical Report No. 71 of the University of Guam Marine Laboratory.

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