AN ABSTRACT OF THE THESIS of William J. FitzGerald, Jr. for the Master
of Science Degree in Biology presented April 9, 1976.

Title: Environmental Parameters Influencing the Growth of Enteromorpha
clathrata (Roth) J. Ag. in the Intertidal Zone on Guam.

Approved: _Roy T. Tsuda_____
ROY T. TSUDA, Chairman, Thesis Committee

Laboratory studies were conducted to determine the light in-
tensity, salinity, temperature, and nutrient levels that would provide
optimum growth for Enteromorpha clathrata. The criteria for deter-
mining growth were the ratio of net productivity to respiration and
mass volume change over a defined incubation period. Environmental
conditions providing optimum growth were found to be a light intensity
of 2600 ft-c or higher; 30°/oo; 25°C; and 150 µg-at/l of N, where the
N:P ratio was maintained at 4:1.

Seasonality, zonation, and the influence of substratum were
examined in the field. E. clathrata at Tumon Bay occurred year-round,
and seasonal variations were correlated to wave height. Additional
factors influencing the presence of this alga were wind-generated
surge and grazing by herbivorous fish. The zone of E. clathrata
growth in Tumon Bay occurred between mean tide level and mean lower
low water. Adequate-sized substratum, which varied with the degree
of water movement, was necessary to maintain a population of
Enteromorpha.

ENVIRONMENTAL PARAMETERS INFLUENCING THE GROWTH

OF ENTEROMORPHA CLATHRATA (ROTH) J. AG.

IN THE INTERTIDAL ZONE ON GUAM

by

WILLIAM J. FITZGERALD, JR.

A thesis submitted in partial fulfillment
of the requirements for the degree of

MASTER OF SCIENCE
in
BIOLOGY

University of Guam
1976

TO THE GRADUATE SCHOOL:

The members of the committee approve the thesis of William J. FitzGerald, Jr., presented April 9, 1976.

Roy T. Tsuda
R. T. Tsuda, Chairman

James A Marsh, Jr
J. A. Marsh, Jr.

Richard H. Randall
R. H. Randall

Patty Jo Hoff
P. J. Hoff
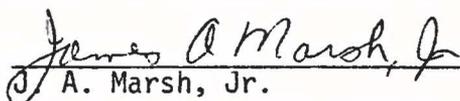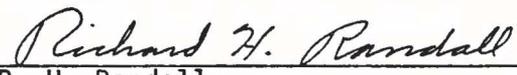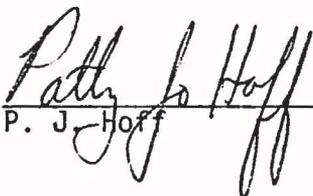
ACCEPTED:
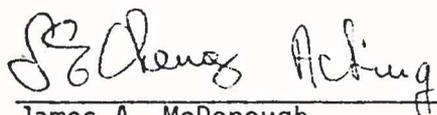
S. Z. Cheung Acting
James A. McDonough
Dean of the Graduate School

ACKNOWLEDGMENTS

I am grateful to Dr. Ted Allen and Dr. Bob Jones for their review of the research.

Marine technicians Ted Tansy and Frank Cushing, Jr.'s innovative technical assistance and forthright commentary were much appreciated.

Cohorts William James Tobias and Michael John Gawel's elaborations of contemplated wisdom pertaining to the subject matter and occasional assistance in the field were held in high esteem.

TABLE OF CONTENTS

LIST OF TABLES

iv

LIST OF FIGURES

INTRODUCTION

Enteromorpha clathrata (Roth) J. Ag., a green alga in the family
Ulvaceae, is common in the intertidal zone of certain bays and estu-
aries on Guam. It is an important alga from several economic points
of view. Previous studies on Guam (Tsuda and Bryan, 1973; Bryan,
1975) reveal that the genus Enteromorpha is the preferred food of the
herbivorous rabbitfishes Siganus spinus and S. argenteus. It is also
used as a food source by people in some Asian countries, such as the
Philippines and Japan (Hoppe, 1966; Tamura, 1970; Velasquez, 1972).
On the other hand, it is viewed as a nuisance by the hotel and tourist
businesses on Guam since this alga accumulates on recreational beaches
and must be raked and removed frequently. Enteromorpha has been
reported as a source of pollution along beaches in Australia by Cribb
(1953).

Despite the recognized importance of this alga, information on
the major environmental factors which affect its growth, although
extensive, is fragmentary. Most of the work concerning these param-
eters has been in determining the extreme tolerances of the alga.
This information is important in ascertaining the possible range of
growth in its natural habitat; however, the eury-tolerances have to
be narrowed to a defined optimum point for maximum growth. The
euryhaline character of Enteromorpha has been shown in various areas
of the world by Nasr and Allem (1949), Biebl (1956, 1962), Carpelan
(1957), Taft (1964), Conover (1964), Umamaheswararao and Sreeramulu
(1964), Salim (1965), Kapraun (1970), Nienhuis (1970), Woodson and

Murley (1970), and Edwards (1972). Kjeldsen and Phinney (1972) found that the salinity tolerance of Enteromorpha exceeded its distribution in its natural habitat. Osterhout (1906) reported this alga's ability to tolerate drastic changes in salinity, i.e., 0 to 35°/oo, in cases of fouling on ships that regularly transit from freshwater rivers to salt water harbors.

The eurythermal properties of E. clathrata are evident by its wide distribution in both tropical and temperate waters (Setchell and Gardner, 1920; Taylor, 1960; Bliding, 1963; Kale, 1966; Kapraun, 1970, 1972; Krishnamurthy, 1972). Its tolerance to nutrient polluted waters has been noted by Grenager (1957), Munda (1967), Edwards (1972), and Tewari (1972).

This genus is extremely productive, as demonstrated by Partington and Jennings (1971), who found that thalli grew 91 cm in six weeks after germination from zygote or zoospores. Kanwisher (1966) found a P/R ratio in excess of 20:1 for Enteromorpha.

The purpose of this study is to ascertain optimal levels for those environmental parameters which are most important in influencing the growth of E. clathrata. The parameters discussed here are light, salinity, temperature, nutrients and substratum. In addition, standing-crop measurements were taken monthly to elucidate seasonal patterns over a 15-month period. The knowledge of how each of these parameters influences the optimum growth of Enteromorpha may make it possible to manipulate conditions for optimum algal growth in fish ponds, thus providing a constant natural food supply for fish mari-culture. Environmental conditions might also be altered in other

areas so as to decrease the growth rate or even eliminate the alga from beaches fronting hotels.

MATERIALS AND METHODS

Field Studies

The field studies were carried out on Guam at Tumon Bay, where lush stands of <u>Enteromorpha clathrata</u> inhabit the intertidal zone. This crescent-shaped bay (Fig. 1), stretching for three kilometers, is fringed by a shallow reef-flat platform and at most places bordered by sandy beaches along the shore. There are numerous natural (e.g., groundwater springs and intertidal seepages) and man-made (e.g., hotel storm drains) freshwater runoff areas along the beach.

Standing Crop and Zonation

A standing crop survey was carried out during the period of October 1973 through December 1974 to determine the seasonal availability of <u>E</u>. <u>clathrata</u> in Tumon Bay. A partial random sampling method (Kershaw, 1964) was employed at three sites selected along the intertidal zone. These sites encompassed the two extremities and the middle of the bay and were selected so that variation in one area would not dominate the cumulative results. A 50 m transect line was run parallel to the shore through the <u>Enteromorpha</u> stand. Ten random samples (0.25 m^2 quadrat) were taken monthly along the transect, and extending 0.5 m to either side. All <u>Enteromorpha</u> within each quadrat was collected, pooled with that from other quadrats within the site, dried at 65°C, and weighed. Necessity of drying large quantities of alga required the use of a plant dryer with a maximum temperature of 65°C.
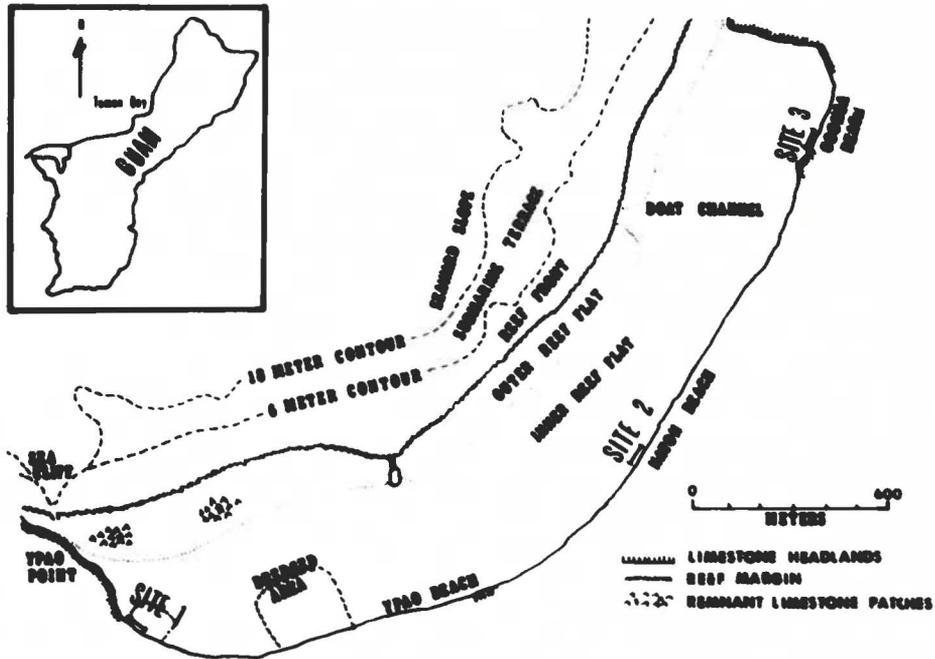
Fig. 1. Map of Tumon Bay, Guam showing standing crop collecting sites.

Zonation was determined by measuring the water depth at the upper and lower range of the Enteromorpha stand. These depths were expressed in relation to datum (0.0 ft.) by recording the time of depth measurement and correcting it to the table of predicted tides.

The measured time and range of tidal fluctuation in Tumon Bay was found to be equivalent to that predicted. It was then assumed that the actual tide level was also the same as predicted.

Substratum and Water Movement

The relationship between the minimum suitable substratum size and the rate of water movement was determined at the sites selected for the standing crop studies. The degree of water movement served as a means of determining the minimum size of the substratum particles capable of providing sufficient anchorage to prevent the thalli from being swept away.

Water movement was quantified twice by the clod card method (Doty, 1971a). Five clod cards, tied to a cement block were submerged for 24 hours at each site within the Enteromorpha stand. During these two 24-hour periods, substratum particles were collected by removing them from Enteromorpha thalli which were 5 cm or greater in length. Thalli of 5 cm or greater in length, which were rarely supported by sandgrains, required a stable substratum for anchorage. Three measurements of diameter with a micrometer were made on each substratum particle to estimate its volume.

Nutrients in Groundwater

Analysis of phosphorus was made by the ascorbic acid method (Strickland and Parsons, 1968) with the use of a spectrophotometer.

Reactive nitrate reduction analysis (Strickland and Parsons, 1968) was used for determination of nitrate. The amounts of iron and manganese from groundwater percolating along the shoreline were analyzed by atomic absorption spectrophotometry.

Laboratory Studies

Laboratory studies were carried out to determine optimum conditions of growth under defined conditions.

Light Saturation Point

Light saturation level was determined and then used as a constant throughout the remainder of the laboratory experiments. The compensation intensity, defined by Jenkin (1937) as the light intensity at which photosynthesis and respiration balance over the period of an experiment, was also determined. Since optimum growth conditions were desired, the light intensity that produced the maximum photosynthetic rate was a desired factor.

The standard oxygen light-dark bottle method was used for measuring productivity. The experimental apparatus consisted of a rack holding 11 bottles (5 light, 5 dark, 1 control), each with a volume of 440 ml, submerged in a 15 gallon (57 liter) aquarium, which served as a constant temperature (ambient 28°C) water bath. The bottles were continuously agitated by a motor-driven system connected to the rack. Net productivity and respiration, over a 30-minute period, were measured with a YSI model 51A oxygen meter through a range of 15 different light intensities (50-5000 ft-c) to determine the light saturation point (Ryther, 1956; Kanwisher, 1966;

Marsh, 1970). Light intensity was measured with a General Electric Type 213 light meter, which was placed in a water-tight housing and submersed to obtain light readings at the point where the alga would be contained in the incubation trough. Two runs per day were made at each light intensity between 1130 and 1330, Guam Standard Time, to limit possible variance in the photosynthetic rate resulting from the diurnal rhythms found in some algae (Sweeney and Haxo, 1961).

A total of 10 light and 10 dark bottles constituted the sample size at each light intensity. Freshly collected Enteromorpha with a wet weight of 1-2 g was placed in the incubation bottles with water of known oxygen content. After the incubation period, oxygen measurements were made and the alga was removed, dried at 105°C for 16 hours, and weighed.

Salinity and Temperature

The alga was acclimatized for 24 hours prior to the experimental run since false results can be obtained with the sudden introduction of an alga into a different environmental setting. Often an initial shock response with a rise in photosynthetic rate is followed by a rapid decrease to a stable rate (Nellen, 1966). Acclimatization was conducted in a recirculating tank system (200-liter capacity, 8.6 liters/minute flow rate) under a light intensity of 1000 ft-c with a normal 12-hour light period. Temperature or salinity was varied to meet the experimental requirements. Holding the alga in the recirculating tank had no obvious detrimental effect after a 4-day period.

The incubation apparatus (Fig. 2) was a trough 150 x 17.5 x 30 cm I.D. Water was pumped from a 200-liter capacity tank into the

9
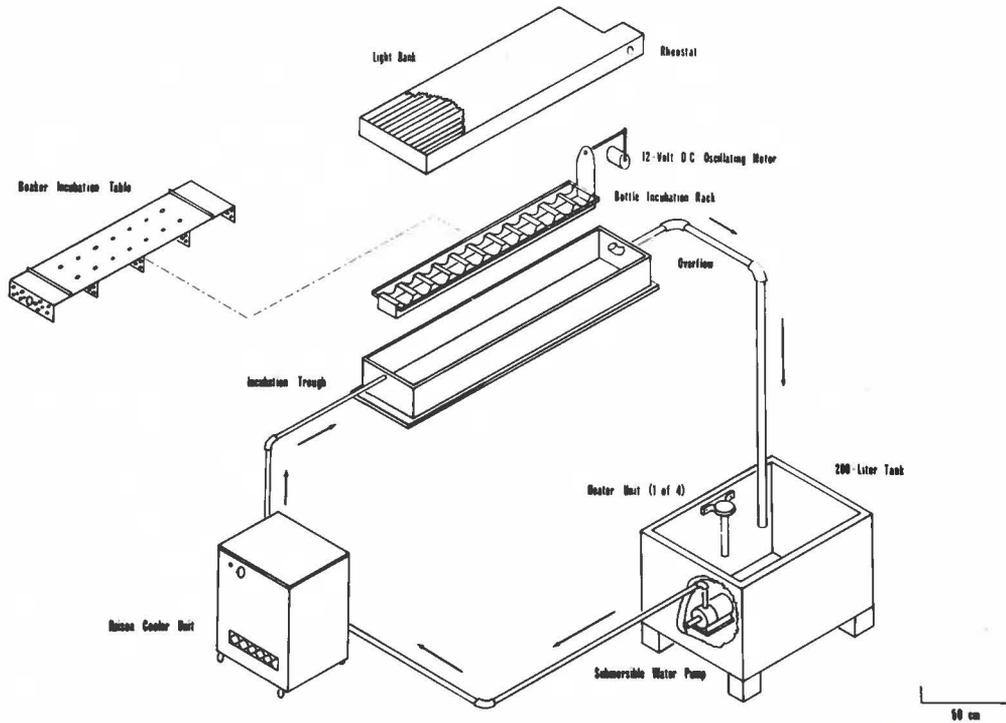
Fig. 2. Apparatus used for the incubation of <u>Enteromorpha clathrata</u> during salinity, temperature, nutrients, and mass growth experiments.

incubation trough and then recirculated back to the tank. A motor-driven rack submerged in the trough provided constant agitation of the incubation bottles (5 light, 5 dark, 1 control; 440-ml volume) to prevent a gradient set up by gaseous diffusion, which might limit oxygen exchange between the algal material and the immediately surrounding water. The same procedures for incubation and measurement of net productivity and respiration (P/R) were used as in the light saturation experiment. The light source was a double layered bank of 12 cool white, 40 watt, fluorescent tubes. This was lowered into position 5 cm above the bottles.

Water samples with six salinity levels (10, 20, 25, 30, 35, and 40°/$_{oo}$) were obtained by dilution of sea water with distilled water, and by concentration of sea water by freezing. Aquarium heaters were used for raising the water temperature and a Reisea Cooler unit was used for lowering the water temperatures (10, 20, 25, 30, and 40°C).

Nutrient Enrichment

Analyses of E. clathrata for nitrogen (N) and phosphorus (P) content were carried out to obtain a N:P ratio. This ratio was used in the enrichment studies.

Nitrogen analysis was by the Kjeldahl method (Welcher, 1963). The method used for analyses of total phosphorus was a modification of the Fiske and SubbaRow (1925) method. Five milliliters of concentrated sulfuric acid was added to a sample (1-2 g dry wt.). The Enteromorpha was digested by heating the mixture until it turned brown. At this point the mixture was cooled and 2N nitric acid was added drop wise, followed by additional heating and nitric acid additions until

the liquid became colorless and white fumes appeared. After cooling

of the liquid, distilled water was added, bringing the total volume to

100 ml. One-milliliter samples were then placed in test tubes. One

milliliter of molybate solution was added, followed by distilled water

to a final volume of 7 ml. The reducing, agent, stannous chloride,

was added in powder form. After color development for 5 minutes,

absorbency was measured in the colorimeter at 660 nm. Results were

plotted against a standard curve obtained by using serial dilutions of

a solution containing 1.361 g of KH_2PO_4 dissolved in 1000 ml of dis-

tilled water. Controls used were digestion with no sample, with a

known amount of phosphate and alga sample, and with sucrose only.

Upon determination of the N:P ratio, nitrate/phosphate enrichment

was carried out in the same incubation trough as that used for salinity

and temperature. A table baffled to augment uniform water flow for

heat exchange was used to support 25 beakers (350-ml volume). The

trough was used as a water bath to maintain a constant temperature

(25°C ± 1°C). The same light source was used as previously described

(Fig. 2).

The nitrogen concentrations were 1, 10, 30, 70, and 130 times

that normally found in the sea water system (5 μg-at/l). The phos-

phorus was added in amounts which matched the ratio of N to P measured

in the alga (Table 1). To simplify the enrichment procedure the con-

centration of phosphate in normal sea water was assumed to be zero.

To limit experimental bias due to possible variation in physical con-

dition in the trough, a randomization scheme was used to arrange the

samples in the trough. The medium in each of the enrichment beakers

Table 1. Nutrient levels used in enrichment experiments.

Factor Increase	NO_3-N µg-at/l	PO_4-P µg-at/l	Fe µg-at/l	Mn µg-at/l
1x	5	0	0.358	0.592
10x	50	14	0.358	0.592
30x	150	38	0.358	0.592
70x	350	89	0.358	0.592
130x	750	189	0.358	0.592

was changed once a day. The beakers were cleaned at the same time to limit growth of bacteria and diatoms.

Incubation of the samples was at the optimum salinity, temperature, and light saturation. Simulation of the day length on Guam of 12 hours was maintained during the incubation period. Glycylglycine (0.001 M) was used to maintain a pH between 8.2 and 8.6. The incubation period lasted for 5 days. The growth of the alga was measured by change in volume. To determine the volume a graduated cylinder (25-ml capacity) was filled to a given volume, then a blotted dry algal sample was placed in the cylinder and the change between initial and final volume was recorded. The results were reported as a mass growth factor which was obtained by dividing the final volume by the initial volume. This measurement was found by previous trials to be more reproducible than measurement of wet weight.

Maximum P/R Quotient and Maximum Growth

Growth as measured by a P/R quotient would assume that the net photosynthesis in excess of respiration would result in the production of organic matter which would be incorporated into the alga, thus being a measure of growth. However, the use of a maximum P/R quotient as the criterion for optimum growth has been questioned as to whether it indicates maximum growth in terms of mass produced. An experiment was run to determine if the maximum P/R quotient and maximum growth are equivalent.

The experimental apparatus consisted of the same set-up used in the nutrient enrichment experiment. The variable was salinity (10, 20, 30, and 40°/₀₀). Measurement of growth was by volume displacement.

The incubation period was for 7 days. These results were compared to those obtained by using the P/R quotient.

RESULTS AND DISCUSSION

Standing Crop

The standing crop study showed that $\underline{E}$. clathrata is present throughout the year in Tumon Bay; however, there are large quantitative variations from month to month (Fig. 3). The mean standing crops at Sites 1, 2, and 3 were 7.5, 44.1, and 20.7 g/m^2 dry weight, respectively, during the 15-month sampling period.

The major physical factor influencing the presence of Enteromorpha was the surf condition (as recorded by Fleet Weather Central). Surf breaking 6 ft. (1.8 m) in height or greater on the reef margin at Tumon Bay is strong enough to generate a forceful surge and occasionally small breaking waves along the beach. During periods of high surf the standing crop of Enteromorpha is decreased, often to complete elimination. The degree of decrease in the standing crop is dependent on how long the high surf is sustained, and the distance from the reef margin to the beach. Areas with a wide reef flat have the effect of damping the surge generated by the surf, thus decreasing the impact of the surge on the beach. Areas close to the reef margin (Site 1) are very susceptible to surf conditions and are affected even by surf of less height. A highly significant negative correlation (-0.8160) occurred at Site 2 between standing crop and surf heights of 6 ft. or greater. Site 3 showed at all levels tested (4, 5, and 6 ft.) a highly significant negative correlation (-0.7254, -0.7611, and -0.7166) between standing crop and surf heights. This is indicative of Site 3's closer location to the reef margin. The
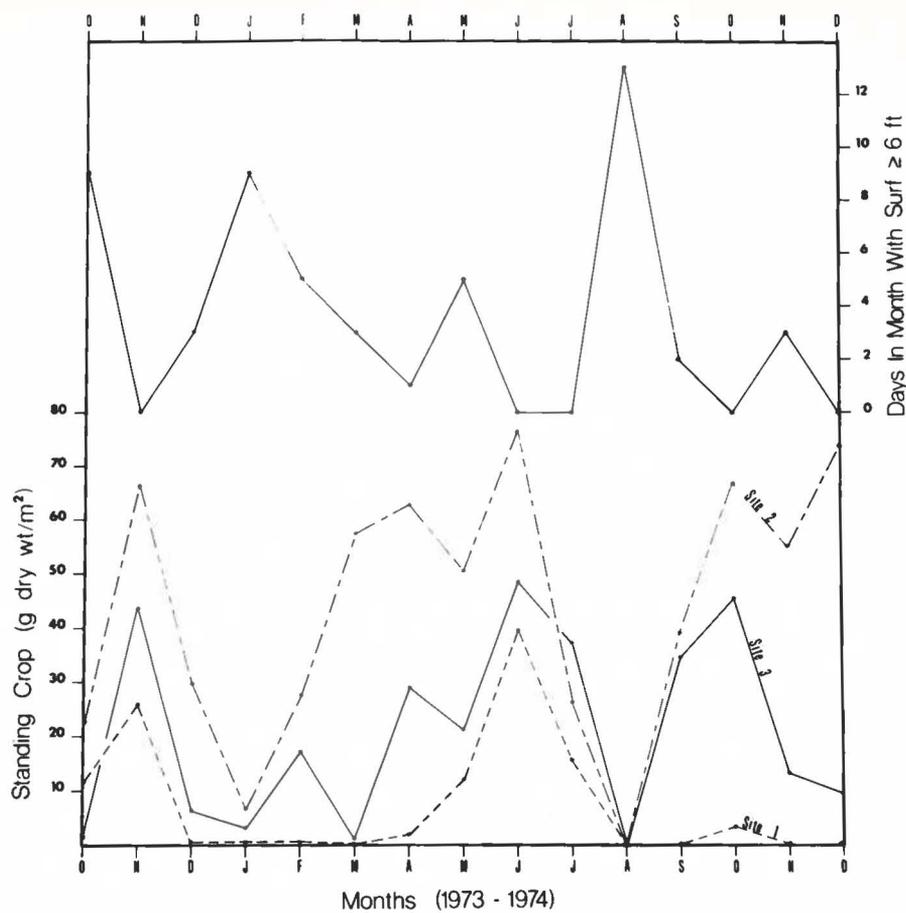
Fig. 3. Standing crop of <u>Enteromorpha clathrata</u> at Tumon Bay during the period of October 1973 through December 1974.

distance from the reef margin to the beach is less here than at Site 2 (Fig. 1). Thus Enteromorpha at Site 3 becomes more susceptible to removal by a moderate surf height.

The importance of wave action has been noted by various authors (Stephenson, 1939; Southward and Orton, 1954; Southward, 1958; Kingsbury, 1962; Jones and Demetropoulos, 1968) to be a major factor influencing the presence, zonation, and structure of attached benthic algae. Doty (1971b) discusses the affects of "antecedent events" on modifying the standing crops of macro-algae. He cites the random occurrence of storm-generated waves as such an "antecedent event" in non-monsoonal tropical areas.

During the period of study the physical conditions of Site 1 were drastically altered. A jetty that ran parallel to the reef margin, which sheltered the beach at Site 1, was removed. This exposed the beach to a swift long-shore current. The area immediately in front of the beach was dredged, thus increasing the slope of the beach. Sand was trucked in and distributed over the beach area, further altering the contour and slope. This construction took place from December 1973 through April 1974.

Wind is a factor that also influences the variability in standing crops at Tumon Bay. The prevailing wind is from the NE, thus having the full length of the bay for its fetch, with the greatest resulting surge at the Hilton-Ipao Beach (Site 1). This heavy surge resulting from the wind keeps Site 1 a less suitable habitat for Enteromorpha. This is shown in the monthly standing crops (Fig. 3) and the clod card values (Table 2). The wind factor in the remaining two sites

usually has less effect; however, with the occasional change in direction of the wind (i.e., from the southwest) Site 3 along with Site 2 to a lesser extent receives the wind blown surge. Nienhuis (1970) cites the tearing and washing away of algae during autumnal gales as the cause of Enteromorpha's disappearance during winter months in the Netherlands.

Grazing by herbivorous fish also affects the standing crop. Large runs of juvenile Siganus spinus and S. argenteus graze on the Enteromorpha resulting in its complete elimination. This was observed during May 1975 and due to continued grazing, Enteromorpha remained absent until September 1975. This phenomenon was also observed in 1972 (P. G. Bryan, personal communication). Such extrinsic influence could lead to the false conclusion that E. clathrata is seasonal. During the period of 1973 through 1974 an extremely small run of siganids occurred (Kami, In Press), thus having a minimal effect on the Enteromorpha standing crop.

Zonation

The range of growth of E. clathrata within the intertidal zone of Tumon Bay was found to be from 1.6 to -0.3 ft. (0.49 to -0.09 m). Guam has a semidiurnal tide with a mean tidal range of 1.6 ft. (0.49 m) and a diurnal range of 2.3 ft. (0.70 m). The extreme predicted tidal range is 3.5 ft. (1.07 m). Mean lower low water is datum (0.0 ft.) for Guam (Randall and Holloman, 1974). Based on this information, the approximate zone of E. clathrata growth is from mean tide level to mean lower low water. During spring mean lower low tides, the upper zone of E. clathrata in Tumon Bay is exposed for approximately 10 hours.

The period of exposure is critical in defining the site a species inhabits in the intertidal zone (Doty, 1946).

Townsend and Lawson (1972), using a tidal simulation apparatus, found that the maximum period of emersion tolerated by E. flexuosa under a semidiurnal tidal cycle was 4 hours (25°C and 65-75% relative humidity). Biebl (1938) found that E. linza tolerated emersion for 14 hours at a relative humidity of 83-86%. In another study, Biebl (1956) found E. clathrata to survive for 14 hours at 83.9% relative humidity, but not at 60.6%.

Relative humidity is thus an important factor in determining the tolerated period of emersion. Humidity levels recorded at the center of the island (by the Aerological Branch, Naval Operations) show a mean maximum of 89% and a mean minimum of 66% throughout the year. Relative humidity at the intertidal zone, by nature of its location, would show an even higher level, often saturation.

Substratum and Water Movement

A direct relationship between water movement and minimum size of substratum required to provide anchorage for thalli greater than 5 cm in length was found. A mean minimum substratum size was 0.70 cm^3 (n = 143, s = 0.28) during surf conditions of 2-4 ft. (0.61-1.22 m) with a clod card value of 6.4 (DF = 11.4) at Site 2. Site 1, which is usually exposed to a greater water movement than the remaining sites (Table 2), had a mean minimum substratum size of 2.70 cm^3 (n = 110, s = 1.2) with a clod card value of 10.2 (DF = 18.2) under the same surf conditions and a NE wind of 8-13 knots.

Table 2. Water movement data obtained by the clod card method on August 7, 1974 and December 25, 1975.

	Seaward Side of Jetty		Site 1		Site 2		Site 3	
	1974	1975	1974	1975	1974	1975	1974	1975
Clod-card Value (C.V.)	7.6 (n = 5, s = 0.46)	4.8 (n = 5, s = 0.2)	4.3 (n = 5, s = 0.31)	10.2 (n = 5, s = 0.15)	4.6 (n = 5, s = 0.93)	6.4 (n = 5, s = 0.29)	8.2 (n = 5, s = 0.39)	7.9 (n = 5, s = 0.32)
Diffusion Index Factor (D.F.)	13.6	26.4	7.7	18.2	8.1	11.4	14.6	14.1
Wind (Knots)	4-7 SW	8-13 NE	4-7 SW	8-13 NE	6-9 SW	2-4 NE	8-12 SW	2-4 NE
Surf Height (ft.)	1-2	2-4	1-2	2-4	1-2	2-4	1-2	2-4

A transplantation experiment was carried out at Site 1 prior to the removal of the rock jetty. The phenomenon of the absence of Enteromorpha on the seaward side of the jetty while it occurred immediately leeward, was examined. Large rocks of 6500 cm^3 or greater with a profuse growth of Enteromorpha were placed at various heights within the intertidal zone on the seaward side of the jetty. After 3-4 days the rocks had been completely denuded of all Enteromorpha, due to abrasion and almost complete sand burial. The factor preventing Enteromorpha's growth on the seaward side of the jetty was the instability of the substratum caused by strong water movement (CCV = 14.8, DF = 26.4).

It becomes evident that the presence of Enteromorpha is dependent upon adequate substratum size to maintain a stable support. The most common substrata supporting Enteromorpha in Tumon Bay were coral rubble, fragments of mollusk shells, and rocks; however, any material that supplied a stable substratum was utilized. Nienhuis (1970) cited the importance of a stable substratum in sandy areas and found a positive correlation between the abundance of Enteromorpha and the presence of suitable substratum. He found an increase of up to 90% in the standing crop of Enteromorpha in areas of adequate substratum. Scoffin (1970) found in his work on the role of m ·ine algae in trapping and stabilizing substratum that the pioneer population of Enteromorpha first became established on large stable objects (e.g., large gastropod shells) followed by the utilization of smaller substratum particles including sand grains as the substratum became stabilized by dense mats of Enteromorpha growth. He found a sand substratum bound by

dense Enteromorpha mats to withstand dislodgement by water currents up to five times the velocity required to dislodge sand particles alone.

Site 2, having a low water movement, has a smaller substratum size requirement than Site 1 which has a greater water movement. This is further exemplified by the lack of Enteromorpha growth in the area adjacent (windward of the jetty) to Site 1, where variables other than water movement - substratum stability are minimal.

Nutrients In Groundwater

The northern half of Guam is formed of limestone which is moderately to highly permeable to water. The water table in this area extends from the shoreline to the interior where it reaches a height of several feet above sea level. Outflow of the groundwater occurs mainly along the shoreline, and is continuous (Randall and Holloman, 1974).

Groundwater introduced along the shoreline by springs and outflows contains high concentrations of nutrients (Table 3). Nitrate showed up to an 8.5 times increase while phosphate did not show a significant increase over reef flat water. Marsh (unpublished data) recorded for groundwater at Tumon up to an 87-times increase of nitrate over reef flat water (due mainly to lower readings on the reef flat). It is evident that the numerous areas of groundwater percolation along Tumon Bay add to the enrichment of the bay, especially the shoreline, and stimulates a rich growth of Enteromorpha (Fig. 4). Boalch (1957) found a positive correlation of the distribution of Enteromorpha with groundwater with high nitrogen levels.

Table 3. Water analysis results. The samples were collected on a -0.4 tide at 0100. Surf conditions were 1-2 ft., with a light NE breeze. These conditions resulted in very little water movement on the reef flat.

Source	Distance From Shore m	NO_3 µg-at/l	NO_2 µg-at/l	PO_4 µg-at/l	Fe µg-at/l	Mn µg-at/l	Salinity °/oo
Runoff Site							
Groundwater	0	116.79	0.056	0.225	0	0.182	0.5
Groundwater	0	106.03	0.078	0.225	0	0.364	0
Drain Water	0	98.70	1.178	0.238	0	0.182	0
Transect (Perpendicular to the Shoreline)							
Groundwater	0	57.52	0.056	0.325	0	0.182	5.5
Reef Flat	5	18.72	0.199	0.825	0	0.729	14.4
Reef Flat	10	14.24	0.099	0.913	0.537	0.546	22.2
Reef Flat	50	13.97	0.033	0.663	0.716	0.911	30.5
Reef Flat	100	13.58	0.155	0.288	0.716	0.546	30.5

Fig. 4. Growth of Enteromorpha clathrata at Site 2, Tumon Bay.

Drainage water from the hotels also adds high levels of nitrate and occasionally high levels of phosphate (12.5 μg-at/l) (Marsh, unpublished data). However, drainage water is of a variable nature, and it is not a reliable source of nutrients for Enteromorpha. On the other hand, a lack of Enteromorpha growth was sometimes observed in the immediate area of drainage plumes, with rich growth to either side of the plumes. This could indicate that some drainage waters contain a noxious element that deters Enteromorpha growth.

Light Saturation

Light saturation occurs at 2600 ft-c (Fig. 5). Further increase in the light intensity to 5000 ft-c showed no statistically significant effect. Below the intensity of 2600 ft-c the photosynthetic rate was presumably limited by the photochemical stage and above this intensity the enzymatic stage became the limiting factor. No detrimental effect was observed at 5000 ft-c for the duration of the experiment. Enteromorpha's occurrence in the high intertidal zone means that it is commonly subjected to intensities of this level and higher. Nasr and Aleem (1949) point out Enteromorpha's tolerance to high intensity of light in the natural habitat. The compensation intensity was found to be 150 ft-c. Thus Enteromorpha has a wide range of light intensities under which it is productive.

Salinity

The optimum P/R quotient for salinity was found at 30°/oo. Both the net photosynthesis and dark respiration showed significant differences (Table 4) among the salinity levels ($p < 0.05$). The findings are similar to those of Conover (1964), who found an optimum growth
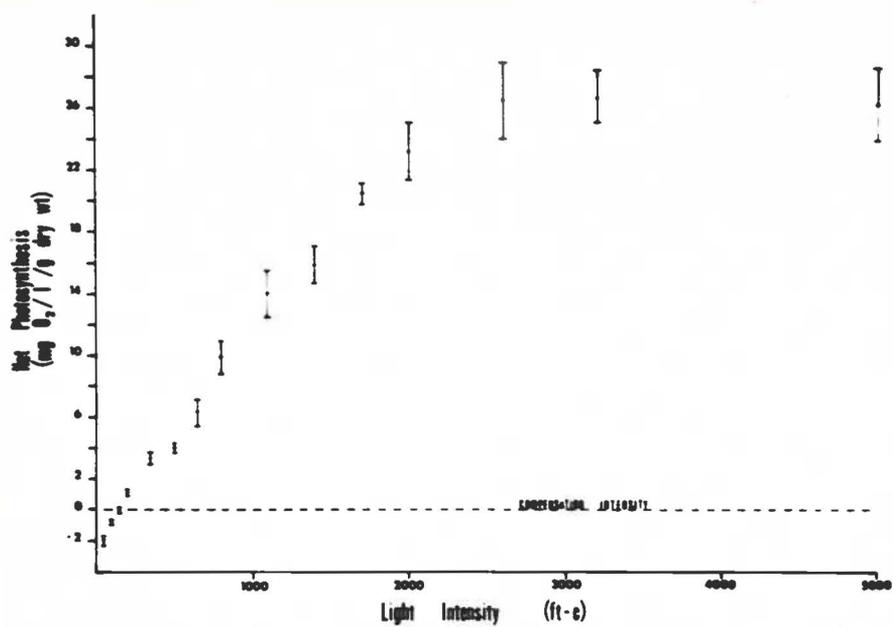
Fig. 5. Light saturation curve for <u>Enteromorpha</u> <u>clathrata</u>
(28°C ± 0.5°C, 34°/oo ± 1°/oo).

between 25 and 35°/oo for E. clathrata. Kapraun (1970) found the development of E. clathrata to be best in a salinity of 27 ± 2.5°/oo, and sporulation at 15-30°/oo. Kjeldsen and Phinney (1972) recorded an optimum growth for E. linza at 30-35°/oo. These findings indicate that E. clathrata grows best in near-to-normal salinity. Significantly lowered salinity by itself has a detrimental affect on the growth of E. clathrata. The euryhaline character of this alga can still be recognized in the P/R quotient (Fig. 6) where within the range of 10-40°/oo it is still above the compensation point. Biebl (1956) found E. clathrata to tolerate 0.5-2.0 times the concentration of normal sea water for up to seven days. It was also able to tolerate distilled water for three days and recover if transferred back into normal sea water. He attributes the ability to resist plasmolysis in E. clathrata to its osmoregulatory mechanism, which is capable of accumulating salts against a diffusion gradient.

The frequent introduction of nutrients along with fresh water may lessen the detrimental effect of lowered salinity. This synergism might be responsible for the frequent occurrence of luxuriant growths of E. clathrata in areas of large fresh water effluents. Kier and Todd (1967) found that blooms of E. prolifera occurred in a lagoon with consistently high phosphate conditions (13 µg-at/1) under conditions of high (45°/oo) or low (12°/oo) salinity.

Temperature

The optimum P/R quotient for temperature (at 30°/oo) occurred at 25°C. Temperature had a significant effect on the respiration (Table 4) with all levels being significantly different ($p < 0.05$).

Lampe (1935) showed that eurythermal marine alga are capable of adapting
to different temperature regimes; this occurs most rapidly under high
light intensities. Associated with a sudden change in temperature
there is generally an increase in respiration within 24 hours.
E. clathrata shows its eurythermal characteristics by its relatively
high P/R quotient through the 10-40°C range (Fig. 6).

Nutrient Enrichment

Nitrogen content of E. clathrata was 4.5% of the dry weight
(n = 10, s = 0.526) and the phosphorous content was 1.1% of the dry
weight (n = 4, s = 0.096). The resulting N:P ratio is 4:1. This
ratio was used as a constant in the enrichment experiment. Imbamba
(1972) found a N:P ratio of 1.92:1 in his analysis of the green alga
Ulva lactuca. This tissue ratio is very close to the optimum growth
enrichment ratio for $NH_3:PO_4$ (2.26:1) as found by Waite and Mitchell
(1972) for U. lactuca. The relative percentages of nitrogen and phos-
phorous incorporated into the alga's tissue should reflect the ratio
required for these nutrients when they are not stored in excess. With
this balance one nutrient will not become the limiting factor in-
fluencing the utilization of the other nutrient. The N:P ratio in
Enteromorpha is probably variable depending on the concentration in
usable form of the elements in the medium, as found for phytoplankton
(Ketchum, 1939; Ketchum and Redfield, 1949; Ryther and Dunstan, 1971).
However, a ratio found in plants growing profusely in a natural
environment should reflect a ratio of these elements that is condusive
and not limiting to growth.

Data from the nutrient enrichment experiment (Fig. 6, Table 4)
showed an optimum at 30x (150 μg-at/l) the nitrate level found in 1x
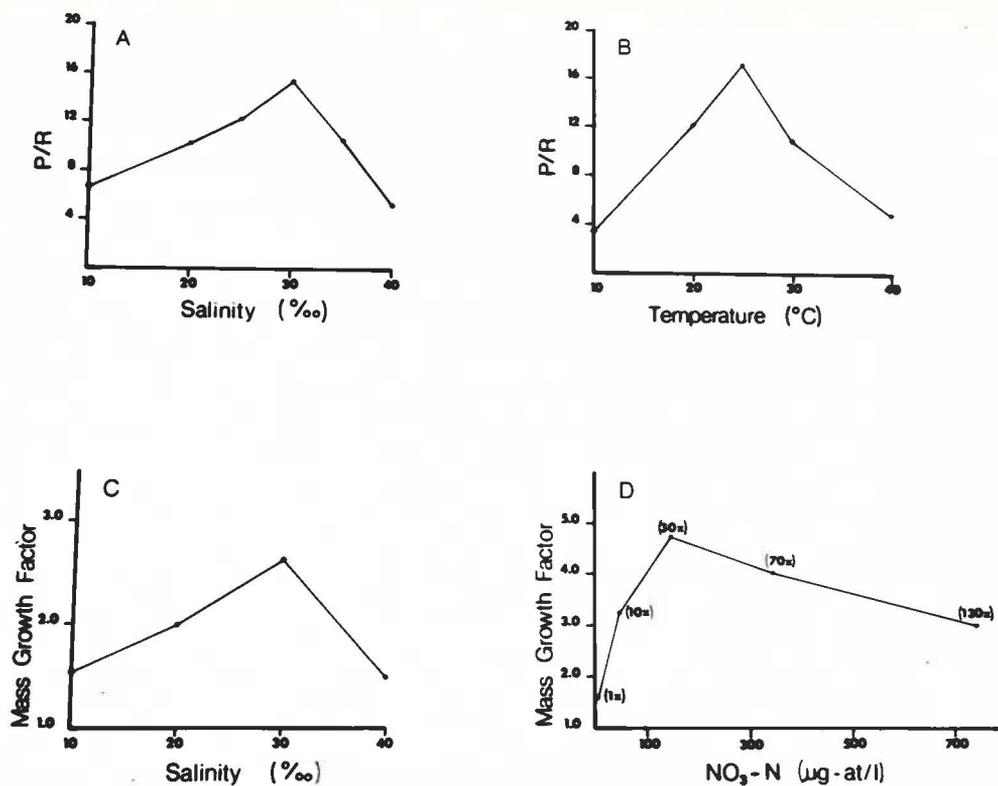
Fig. 6. Optimum growth conditions for <u>Enteromorpha</u> <u>clathrata</u>.
A. Optimum salinity at ambient temperature 28°C ± 1°C,
B. Optimum temperature at 30°/∘∘, C. Optimum salinity
at 25°C using the mass growth factor, D. Optimum
nutrient enrichment at N/. = 4:1, 30°/∘∘, and 25°C.
All experiments were carried out at light saturation
(2600 ft-c).

Table 4. Student-Newman-Kuels Procedure, Multiple Comparisons Test. The means are arrayed in assending order of magnitude. Those sets of means that are underlined are not significantly heterogeneous. Those means that are not connected by a line are considered significantly different ($p < 0.05$).

SALINITY

    Photosynthesis   n = 10

        <u>10°/oo   40°/oo</u>   20°/oo   <u>35°/oo   25°/oo</u>   30°/oo

    Respiration   n = 10

        30°/oo   <u>25°/oo   20°/oo   35°/oo</u>   10°/oo   40°/oo

TEMPERATURE

    Photosynthesis   n = 10

        10°C   40°C   <u>20°C   30°C</u>   25°C

    Respiration   n = 10

        10°C   25°C   20°C   30°C   40°C

NUTRIENTS   n = 15

                130x   10x   70x   30x

       1x   <u>16x   2x</u>   8x   4x

SALINITY (Mass Growth Indicator)   n = 18

        40°/oo   10°/oo   20°/oo   30°/oo

---

sea water with a N:P ratio of 4:1. This value indicates Enteromorpha's tolerance to high nutrient levels, which makes it a suitable species to inhabit and predominate in polluted eutrophic areas (Grenager, 1957; Munda, 1967; Tewari, 1972).

During the preliminary trials of the enrichment experiment, growth was poor at all levels of enrichment. A factor limiting the growth or limiting the utilization of the enriched medium was suspected. Addition of various combinations and amounts of earth extract, Enteromorpha extract (1000 g Enteromorpha boiled in 1 liter of distilled water for 1 hour), $NaHCO_3$ (possible carbon source), and ammonium chloride (alternate nitrogen source) proved to be of no aid in the further stimulation of growth. However, the separate addition of the micronutrients iron (iron citrate) and manganese (manganese sulfate) both gave positive stimulus to the growth (up to 3-fold increase) of Enteromorpha when added to the enriched medium (NO_3/PO_4). No stimulus to the growth occurred in the 1x medium. Thereafter, iron at a concentration of 0.358 µg-at/l (0.020 PPM) and manganese at 0.592 µg-at/l (0.0325 PPM) were added to all incubation beakers (Table 1) including the controls of unenriched sea water (1x).

Kylin (1945) showed that manganese (4.5 µg-at/l) exerted a highly significant influence on the assimilation of nitrogen supplied as sodium nitrate in Ulva lactuca. Assimilation of other nitrogen sources e.g., amides, amino acids, and ammonium, are not influenced by the addition of manganese. Sodium nitrate (72 µg-at/l, N) gave a higher growth rate than ammonium salts when manganese was added. It was suggested (Kylin, 1945) that iron may compensate for the lack of manganese in the medium. Results from this study indicate that iron

does in fact stimulate growth when added to a medium containing sodium nitrate as the nitrogen source. Evidence of iron's involvement in nitrate reduction has been found in Chlorella (Trubachev, 1968) and Anabaena cylindrica (Hattori and Uesugi, 1968). Iron was found to be a constituent of the enzyme that catalyzes the stage in nitrate reduction of nitrite to ammonium in Chlorella (Aparicio et al., 1971). The presence of iron in the growth medium of Ankistrodesmus braunii, and Chlorella fusca was found to increase the capacity for nitrite reduction and the level of nitrite reductase (Kessler and Czygan, 1968; Cardenas et al., 1972). Iron's effect on growth is probably primarily through its incorporation in enzymes and porphyrins and its importance in the energy transport system (Walker, 1954; Hewitt, 1958; Epel and Butler, 1970). Its effect on nitrate reduction is possibly mainly involved with an interaction with manganese (Hopkins, 1930; Noack and Pirson, 1939; Alberts-Dietert, 1941; Treharne and Eyster, 1962). Harvey (1966) and Velichko (1968) review the effects of iron and manganese on algal growth, photosynthesis, and respiration.

Foyn (1934a, 1934b) found poor growth of Ulva lactuca and Cladophora subriana in sea water enriched with nitrate and phosphate until the addition of soil extract, which then produced normal growth. De Valera's (1940) work on culturi· Enteromorpha intestinalis and E. linza with nitrate as the nitrogen source showed that the addition of earth extract further promoted growth in addition to the iron citrate. The addition of earth extract may have supplied a sufficient quantity of manganese to further promote the utilization of the available nitrate. The ill-defined and varying constituents of earth

extract leaves one with no concrete information on what the specific stimulating compound or compounds may have been.

A difference in color of alga samples appeared as incubation progressed. A resulting light green coloration of alga in the 1x medium, a darker green in the 10x, and a very dark green in 30x, 70x, and 130x media were observed. The same result occurred whether the initial alga specimen was of light or dark pigmentation at the beginning of the experiment. De Valera (1940) also noted a color change in cultured E. intestinalis and E. linza, with both species having a pale green color in unenriched sea water and a good green color in enriched medium. This variance in pigmentation intensity occurs naturally in the field. The darkest pigmented specimens were found in areas of drainage or groundwater percolation. Since it was found that groundwater (mainly a nitrate source) and drainage systems are also carriers of enriched nutrients, this pigmentation variance can possibly be used as an indicator of a nutrient-enrichment area. Butcher et al., (1937) found a similar situation for Cladophora glomerata in its natural habitat, with growth in nutrient rich waters a dark green, richly branched specimen occurred, while in unenriched waters a pale green, densely tufted specimen occurred.

Chlorophyll content can vary due to a number of causes (Emerson, 1929; Mandels, 1943; Granick, 1951; Neeb, 1952; Ichioka and Aron, 1955; Yentsch, 1962); however, it appears in this study to be directly related to the nitrate concentration in the media. Yentsch and Scagel (1958) concluded that under culture conditions of high light intensity, a decrease in chlorophyll was due to the exhaustion of nutrients by active photosynthesis and growth. Nitrogen deficient

cells show an increase in chlorophyll content as they recover (Harvey, 1953; Bongers, 1956).

Calculation of the dry weight to volume ratio (0.11:1) of alga specimens grown at the different enrichment levels gave no significant difference. This shows that the growth indicated by the volume change is actually of the increase in tissue matter not interstitial volume by the addition of gaseous spaces of water.

Salinity Mass Growth Factor Indicator

Cultivation of E. clathrata at 10, 20, 30, and 40°/oo showed a significant difference between consecutive salinities in volume change (Table 4). The maximum volume increase occurred at 30°/oo (Fig. 6). This concurs with the results obtained by the P/R quotient method thus supporting the validity of the net excess of photosynthesis above respiration as an indicator of growth (Kanwisher, 1966). However, a direct convergence from a P/R quotient into a corresponding volume of alga is not possible since they are not proportionately equivalent. A ratio for 30°/oo/10°/oo resulting from the P/R method gives 2.27:1, and for the growth factor method gives 1.66:1. This would represent a loss of 27% of the organic matter photosynthesized. The P/R quotient seems to be more sensitive near the optimum range. This discrepancy could possibly be due to an increase in extracellular products (Bergland, 1969), with a higher proportion of energy going towards this production, and less towards growth at 30°/oo as compared to that at 10°/oo. There has been found to be an excretion of 20 to 40% of the total organic matter

photosynthesized in some algae (Lewin, 1956; Allen, 1956; Fogg, 1962; Stewart, 1963; Silburth, 1969; Aaronson, 1971; Guillard and Helleburst, 1971).

CONCLUSIONS

Enteromorpha clathrata shows a wide tolerance to salinity, tem-
perature, and nutrient levels. The optimum conditions for growth are
at a light saturation of 2600 ft-c or higher, 25°C, 30°/oo, 150 µg-at/l
nitrogen, where the N:P ratio was maintained at 4:1. The high P/R
values (e.g., in excess of 17 at 25°C, 30°/oo, and 2600 ft-c) demon-
strate this alga's production capabilities.

Surf conditions, water flow, and grazing by herbivorous fish are
the main causes of fluctuations in standing crop.

Adequate substratum is necessary to maintain anchorage and was
shown to be a function of the intensity of water movement (e.g., surf,
surge, and currents).

Nutrient enrichment of the shoreline by percolating groundwater
stimulates a luxuriant growth of E. clathrata. The slight reduction
in salinity is secondary to its introduction of high nitrate levels.
The optimum salinity (30°/oo), being only slightly less than normal
sea water, indicates that influx of fresh water alone would not be of
a growth stimulating nature, other than to reduce possible competition.

The presence of E. clathrata within the intertidal zone on Guam
is dependent on a number of variables, the major ones being substratum
stability (as influenced by wave height and currents) and nutrient
enrichment. Salinity, temperature, and light intensity would not be
limiting factors in most cases.

LITERATURE CITED

Aaronson, S. 1971. The synthesis of extracellular macromolecules and membranes by a population of the phytoflagellate Ochromonas danica. Limnol. Oceanogr. 16:1-9.

Alberts-Dietert, F. 1941. Die Wirkung von Eisen und Mangan auf die Stickstoffassimilation von Chlorella. Planta 32:88-117.

Allen, M. B. 1956. Excretion of organic compounds by Chlamydomonas. Arch. Mikrobiol. 24:163-168.

Aparichio, P. J., J. Cardenas, W. G. Zumft, J. M. Vega, J. Herrera, A. Paneque, and M. Losada. 1971. Molybdenum and iron as constituents of the enzymes of the nitrate-reducing system from Chlorella. Phytochemistry 10:1487-95.

Berglund, H. 1969. Stimulation of growth of two marine green algae by Enteromorpha linza in unialgal and axenic culture. Physiologia Plantarum 22:1069-1073.

Biebl, R. 1938. Trockenresistenz und osmotische Empfindlichkeit der Meeresalgen verschienden tiefer Standorte. Jahrb. wiss. Botan. 86:350-386.

_____. 1956. Zellphysiologisch-okologische Untersuchungen an Enteromorpha clathrata (Roth) Greville. Bericht der deutschen botanischen Gesellschaft (Berlin) 69:75-86.

_____. 1962. Seaweeds. In R. A. Lewin (ed.), Physiology and biochemistry of algae. Academic Press Inc., New York and London.

Bliding, C. 1963. A critical survey of European taxa of Ulvales. Opera Bot. 8(3):1-160.

Boalch, G. T. 1957. Marine algal zonation and substratum in Beer Bay, South-East Devon. J. Mar. Biol. Ass. U. K. 36:519-528.

Bongers, L. 1956. Aspects of nitrogen assimilation by cultures of green algae. (Chlorella vulgaris, strain A and Scenedesmus.)

Bryan, P. G. 1975. Food habits, functional digestive morphology, and assimilation efficiency of the rabbitfish Siganus spinus (Pisces, Siganidae) on Guam. Pac. Sci. 29(3):269-277.

Butcher, R. W., J. Longwell, and F. T. K. Pentelow. 1937. Survey of the river Tees, III. The non-tidal reaches. Chemical and Biological. Tech. Pap. Wat. Pollut. Res. Lond. 6:1-137.

Cardenas, J., J. Rivas, A. Paneque, and M. Losada. 1972. Effect of iron supply on the activities of the nitrate-reducing system from Chlorella. Arch. Mikrobiol. 8:260-263.

Carpelan, L. H. 1957. Hydro-biology of the Alviso Salt Ponds. Ecology 38(3):375-390.

Conover, J. 1964. The ecology, seasonal periodicity, and distribution of benthic plants in some Texas Lagoons. Botanica Marina 7:4-41.

Cribb, A. B. 1953. Algal pollution of surf in southern queensland. Queensland Nat. 14(6):123-125.

De Valera, M. 1940. Note on the difference in growth of Enteromorpha species in various culture media. Kungl. Fysiografiska Sallskapets I Lund Forhandlinger 10(5):52-58.

Doty, M. S. 1946. Critical tide factors that are correlated with the vertical distribution of marine algae and other organisms along the Pacific coast. Ecology 27(4):315-328.

_____. 1971a. Measurement of water movement in reference to benthic algal growth. Botanica Marina 14:32-35.

_____. 1971b. Antecedent event influence on benthic marine algal standing crops in Hawaii. J. Exp. Mar. Biol. Ecol. 6:161-166.

Edwards, P. 1972. Benthic algae in polluted estuaries. Marine Poll. Bull. 3(4):55-60.

Emerson, R. 1929. The relation between maximum rate of photosynthesis and concentration of chlorophyll. J. Gen. Physiol. 12:609-622.

Epel, B. L., and W. L. Butler. 1970. The cytochromes of Prototheca zopfii. Plant Physiol., Lancaster 45:723-727.

Fiske, C., and Y. SubbaRow. 1925. Method for the estimation of phosphorus. J. Biol. Chem. 66:375.

Fogg, G. E. 1962. Extracellular products. In R. A. Lewin (ed.), Physiology and biochemistry of algae. Academic Press Inc., New York and London.

Foyn, B. 1934a. Lebenszyklus und Sexualitat der Chlorophycee Ulva lactuca. L. Arch. Protistenkd. 83:154-177.

_____. 1934b. Lebenszyklus, Cytologie und Sexualitat der Chlorophycee Cladophora subriana. L. Arch. Protistenkd. 83:1-56.

Granick, S. 1951. Biosynthesis of chlorophyll and related pigments. Ann. Rev. Plant Physiol. 2:115-144.

Grenager, B. 1957. Algological observations from the polluted area
of the Oslofjord. Nytt. Magazine Bot. 41-60.

Guillard, R., and J. A. Hellebust. 1971. Growth and the production
of extracellular substances by two strains of Phaeocystis
poucheti. J. Phycol. 7(4):330-338.

Harvey, H. W. 1953. Synthesis of organic nitrogen and chlorophyll by
Nitzschia closterium. J. Mar. Biol. Assoc. U. K. 31:477-487.

_____. 1966. The chemistry and fertility of sea waters.
Cambridge Univ. Press, New York. 240 p.

Hattori, A., and I. Uesugi. 1968. Ferredoxin-dependent photo-
reduction of nitrate and copper ions are applied jointly to
Chlorella vulgaris. Physiologia Plantarum 22:304-311.

Hewitt, E. J. 1958. The role of mineral elements in the activity of
plant enzyme systems. In W. Ruhland (ed.), Handbuch der
Pflanzenphysilogie vol. IV. Springer, Berlin. 427-481.

Hopkins, E. F. 1930. Iron-ion concentration in relation to growth
and other biological processes. Botan. Gaz. 89:209-240.

Hoppe, H. A. 1966. Nahrungsmittel aus meeresalgen. Botanica Marina
9(Supplement):18-40.

Ichioka, P., and D. Aron. 1955. Molybdenum in relation to nitrogen
metabolism. II. Assimilation of ammonia and urea without
molybdenum by Scenedesmus. Physiologia Plantarum 8:552-560.

Imbamba, S. 1972. Mineral element content of some benthic marine
algae of the Kenya Coast. Botanica Marina 15:113-115.

Jenkin, P. M. 1937. Oxygen production by the diatom Coscinodiscus
excentricus Ehr. in relation to submarine illumination in the
English Channel. J. Mar. Biol. Assoc. U. K. 22:301-343.

Jones, W. E., and A. Demetropoulos. 1968. Exposure to wave action:
measurement of an important ecological parameter on rocky shores
on Anglesey. J. Exp. Mar. Ecr¹. 2:46-63.

Kale, S. R. 1966. On some green algae from the channels and re-
servoirs of Saurashtra Salt Works, Porbandar. Curr. Sci.
35:180-181.

Kami, H. In Press. Notes of the annual juvenile siganid harvest in
Guam. Micronesica.

Kanwisher, J. W. 1966. Photosynthesis and respiration in some sea-
weeds. p. 407-420. In Harold Barnes (ed.), Some contemporary
studies in marine science. George Allen and Unwin Ltd., London.

Kapraun, D. F. 1970. Field and cultural studies of Ulva and Enteromorpha in the vicinity of Port Aransas, Texas. Contrib. Mar. Sci. 15:205-285.

_____. 1972. Notes on the benthic marine algae of San Andres, Colombia. Carib. J. Sci. 12(3-4):199-203.

Kershaw, K. 1964. Quantitative and dynamic ecology. American Elsevier Pub. Co., Inc., New York.

Kessler, E., and F. C. Czygan. 1968. The effect of iron supply on the activity of nitrate and nitrite reduction in green algae. Arch. Mikrobiol. 60:282-284.

Ketchum, B. H. 1939. The absorption of phosphate and nitrate by illuminated cultures of Nitzschia closterium. Am. J. Bot. 26:399-407.

Ketchum, B. H., and A. C. Redfield. 1949. Some physical and chemical characteristics of algae growth in mass culture. J. Cell. Comp. Physiol. 33:281.

Kier, A., and E. S. Todd. 1967. Self-regulatory growth in the green alga Enteromorpha prolifera Formae. Bull. S. Calif. Acad. Sci. 66(1):29-34.

Kingsbury, J. M. 1962. The effect of waves on the composition of a population of attached marine algae. Bull. Torrey Bot. Club. 89(3):143-160.

Kjeldsen, C. K., and H. K. Phinney. 1972. Effects of variations in salinity and temperature on some estuarine macro-algae. Proc. Seventh International Seaweed Symp. Univ. Tokyo Press, Tokyo.

Krishnamurthy, V. 1972. The species of Enteromorpha from India. Bot. J. Linn. Soc. 65:119-128.

Kylin, A. 1945. The nitrogen sources and the influence of manganese on the nitrogen assimilation of Ulva lactuca. Kungl. Fysiografiska Sallskapets I Lund Forhandlingar 15(4):27-35.

Lampe, H. 1935. Die Temperatureinstellung des Stoffgewinns bei Meeresalgen als plasmatische Anpassung. Protoplasma 23:534-578.

Lewin, R. A. 1956. Extracellular polysaccharides of green algae. Can. J. Microbiol. 2:665-672.

Mandels, G. R. 1943. A quantitative study of chlorosis in Chlorella under conditions of sulfur deficiency. Plant Physiol. 18, 449-462.

Marsh, J. A., Jr. 1970. Primary productivity of reef-building calcareous red algae. Ecology 51(2):255-264.

Montfort, C. 1931. Assimilation und Stoffgewinn der Meeresalgen bei AussuBung und Ruckversalzung. I. Phasen der Giftwirkung und die Frage der Reversibilitat. I. Typen der funktionellen Salzunstellung. Ber. deut. bot. Ges. 49:49-66.

Munda, I. 1967. Changes in the algal vegetation of a part of the Deltaic Area in the Southern Netherlands (Veerse Meer) after its closure. Botanica Marina 10(1/2):141-157.

Nasr, A. H., and A. A. Aleem. 1949. Ecological studies of some marine algae from Alexandria. Hydrobiologia 1(3):251-281.

Neeb, O. 1952. Hydrodictyon als Objekt einer vergleichenden Untersuchung physiologischer Grossen. Flora (Jena) 139:39-95.

Nellen, U. R. 1966. Uber den Einflub des Salzgehaltes auf die photosynthetische Leistung verschiedener Standortfromen von Delesseria sanguinea und Fucus serratus. Helgolander wiss. Meeresunters 13:288-313.

Nienhuis, P. H. 1970. The benthic algal communities of flats and salt marshes in the South-Western Netherlands. Neth. J. Sea Res. 5(1):20-49.

Noack, K., and A. Pirson. 1939. Die Wirkung von Eisen ung Mangan auf die Stickstoffassimilation von Chlorella. Ber. deut. bot. Ges. 57:442-452.

Osterhout, W. J. V. 1906. The resistance of certain marine algae to changes in osmotic pressure and temperature. Univ. Calif. Publ., Botany 2(8):227-228.

Partington, A., and F. J. Jennings. 1971. Growing Enteromorpha in laboratories. J. Appl. Ecol. 8(1):269-271.

Randall, R. H., and J. Holloman. 1974. Coastal survey of Guam. Univ. Guam, Marine Laboratory, Tech. Rept. 14.

Ryther, J. H. 1956. Photosynthesis in the ocean as a function of light intensity. Limnol. Oceanogr. 1(1):61-70.

Ryther, J. H., and W. M. Dunstan. 1971. Nitrogen, phosphorus and eutrophication in the coastal marine environment. Science 171(3975).

Salim, K. M. 1965. The distribution of marine algae along Karachi Coast. Botanica Marina 8(Fasc 2-4):183-198.

Setchell, W. A., and N. L. Gardner. 1920. The marine algae of the Pacific Coast of North America. Univ. Calif. Publ., Botany 139-374.

Scoffin, T. P. 1970. The trapping and binding of subtidal carbonate sediments by marine vegetation in Bimini Lagoon, Bahamas. J. Sedimentary Petrology 40(1):249-273.

Silburth, J. M. 1969. Studies on algal substances in the sea. III. The production of extracellular organic matter by littoral marine alga. J. Exp. Mar. Biol. Ecol. 3:290-309.

Southward, A. J. 1958. The zonation of plants and animals on rocky sea shores. Biol. Rev. 33:137-177.

Southward, A. J., and J. H. Orton. 1954. The effects of wave-action on the distribution and numbers of the commoner plants and animals living on the Plymouth breakwater. J. Mar. Biol. Assoc. U. K. 33:1-19.

Stephenson, T. A. 1939. The constitution of the intertidal fauna and flora of South Africa. Zoology (J. Linn. Soc.) 40:487-536.

Stewart, W. D. R. 1963. Liberation of extracellular nitrogen by two nitrogen-fixing blue-green algae. Nature, Lond. 200:1020-1021.

Strickland, J. D. H., and T. R. Parsons. 1968. A practical handbook of seawater analysis. Fish. Res. Bd. Canada, Ottawa. 71-80.

Sweeney, B., and J. Haxo. 1961. Persistence of a photosynthetic rhythm in enucleated Acetabularia. Science 134(3487):1361-63.

Taft, C. E. 1964. The occurrence of Monostroma and Enteromorpha in Ohio. Ohio J. Sci. 64(4):272-274.

Tamura, T. 1970. Marine aquaculture. Nat. Sci. Foundation PB194 OSIT Part II. Washington, D. C.

Taylor, W. R. 1960. Marine algae of the eastern tropical and sub-tropical coasts of the Americas. Univ. Mich. Press. 870 p.

Tewari, A. 1972. The effect of sewage pollution of Enteromorpha prolifera var. tubulosa growing under natural habitat. Botanica Marina 15:167.

Townsend, C., and G. W. Lawson. 1972. Preliminary results on factors causing zonation in Enteromorpha using a tide simulating apparatus. J. Exp. Mar. Biol. Ecol. 8:265-276.

Treharne, R. W., and H. C. Eyster. 1962. Electron spin resonance study of manganese and iron in Chlorella pyrenoidosa. Biochem.

Trubachev, I. N. 1968. Ob uchasti askorbinovoi kisloty, perekisi vodoroda i zheleza v. vosstanovlenii nitratov klorelloi. Fiziol. Rast. 15:658-664.

Tsuda, R. T., and P. G. Bryan. 1973. Food preference of juvenile Siganus rostratus and S. spinus in Guam. Copeia 3:604-606.

Umamaheswararao, M., and T. Sreeramulu. 1964. An ecological study of some intertidal algae of the Visakhapatnam coast. J. Ecology 52:595-616.

Velasquez, G. T. 1972. Studies and utilization of the Philippine marine algae. Proc. Seventh International Seaweed Symp. 62-65. Univ. Tokyo Press, Tokyo.

Velichko, I. M. 1968. The role of iron and manganese in the vital activities of blue-green algae of the genus Microsystis. Mikrol. Lem. Selskokhoz Med. Respub. Mezhvedom SB. 4:11-17.

Waite, T., and R. Mitchell. 1972. The effect of nutrient fertilization on the benthic alga Ulva lactuca. Botanica Marina 15:151-156.

Walker, J. B. 1954. Inorganic micronutrient requirement of Chlorella. II. Quantitative requirements for iron, manganese, and zinc. Arch. Biochem. Biophys. 53:1-8.

Welcher, F. J. (ed.). 1963. Standard methods of chemical analysis. Vol. 2 part B. D. Van Nostrand Co., Inc., Princeton, New Jersey.

Woodson, B. R., and J. F. Murley. 1970. The effect of halides on Chlorophyta distribution in the James River, Virginia. Adv. Frontier Plant Sci. 25:187-197.

Yentsch, C. 1962. Marine plankton. In R. A. Lewin (ed.), Physiology and biochemistry of algae. Academic Press, New York. 771-795.

Yentsch, C., and R. F. Scagel. 1958. Diurnal study of phytoplankton pigments. An in situ study in East Sound, Washington. J. Mar. Res. 17:567-584.