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Environmental Parameters Influencing the Growth of *Enteromorpha clathrata* (Roth) J. Ag. in the Intertidal Zone on Guam[1]

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Abstract

Laboratory studies were conducted to determine the light intensity, salinity, temperature, and nutrient levels that would provide optimum growth for *Enteromorpha clathrata*. The criteria for determining 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 ‰; 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*.

Introduction

Enteromorpha clathrata (Roth) J. Ag., a green alga in the family Ulvaceae, is common in the intertidal zone of certain bays and estuaries 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 parameters 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), Kapraum (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 ‰, in cases of fouling on ships that regularly transit from freshwater rivers to salt water harbours.

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*.

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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 mariculture. 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 *Enteromorpha clathrata* 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 *E. clathrata* in Tumon Bay. A partial random sampling method (Kershaw 1964) was employed at three sties 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 *Enteromorpha* stand. Ten random samples (0.25 m^2 quadrat) were taken monthly along the transect, and extending 0.5 m to either side. All *Enteromorpha* 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.

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.

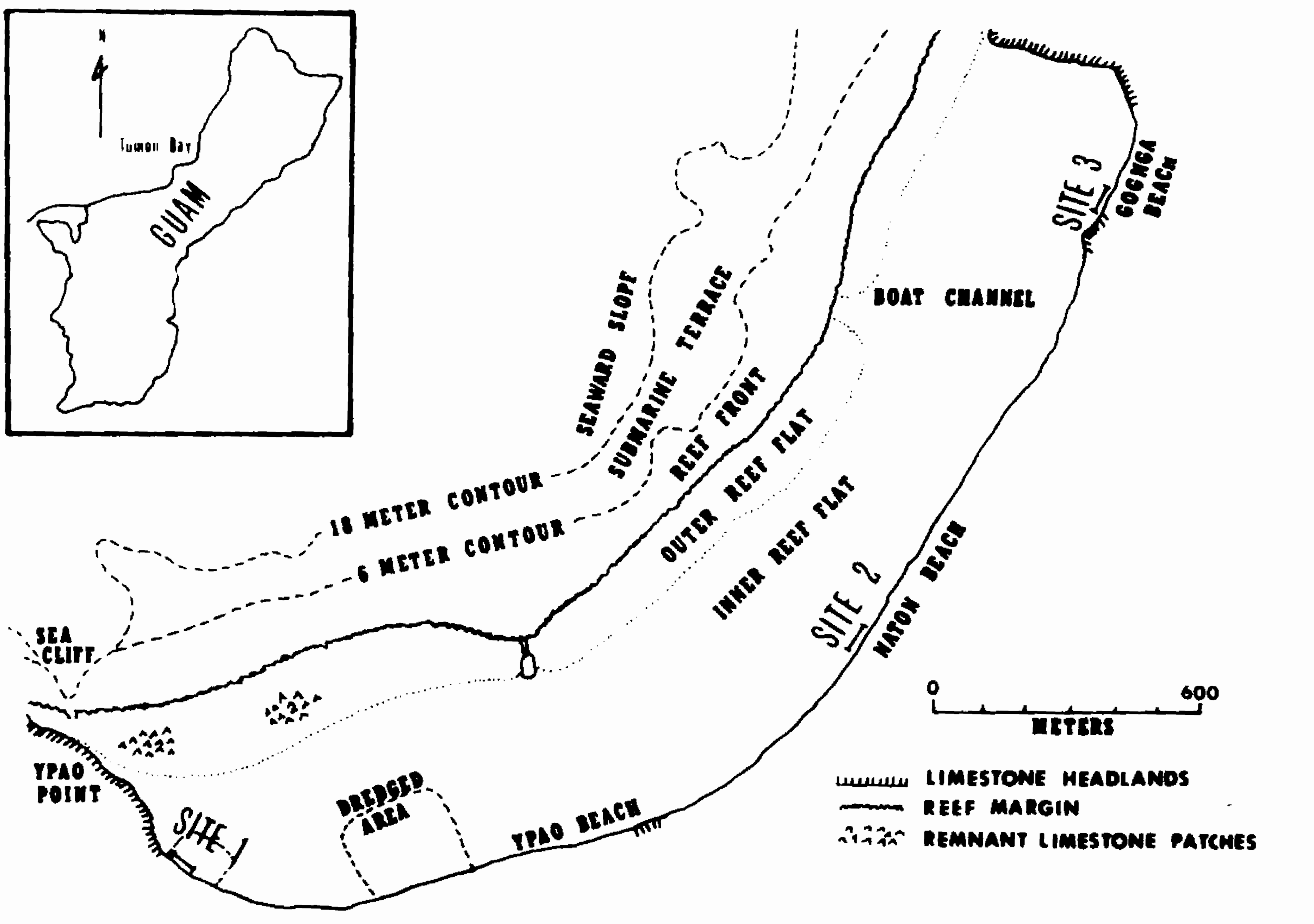

Fig. 1. Map of Tumon Bay, Guam showing standing crop collecting sites.

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 sand grains, 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 was 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, defiend 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 × 17.5 × 30 cm I. D. Water was pumped from a 200-liter capacity tank into the 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 ‰) 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).

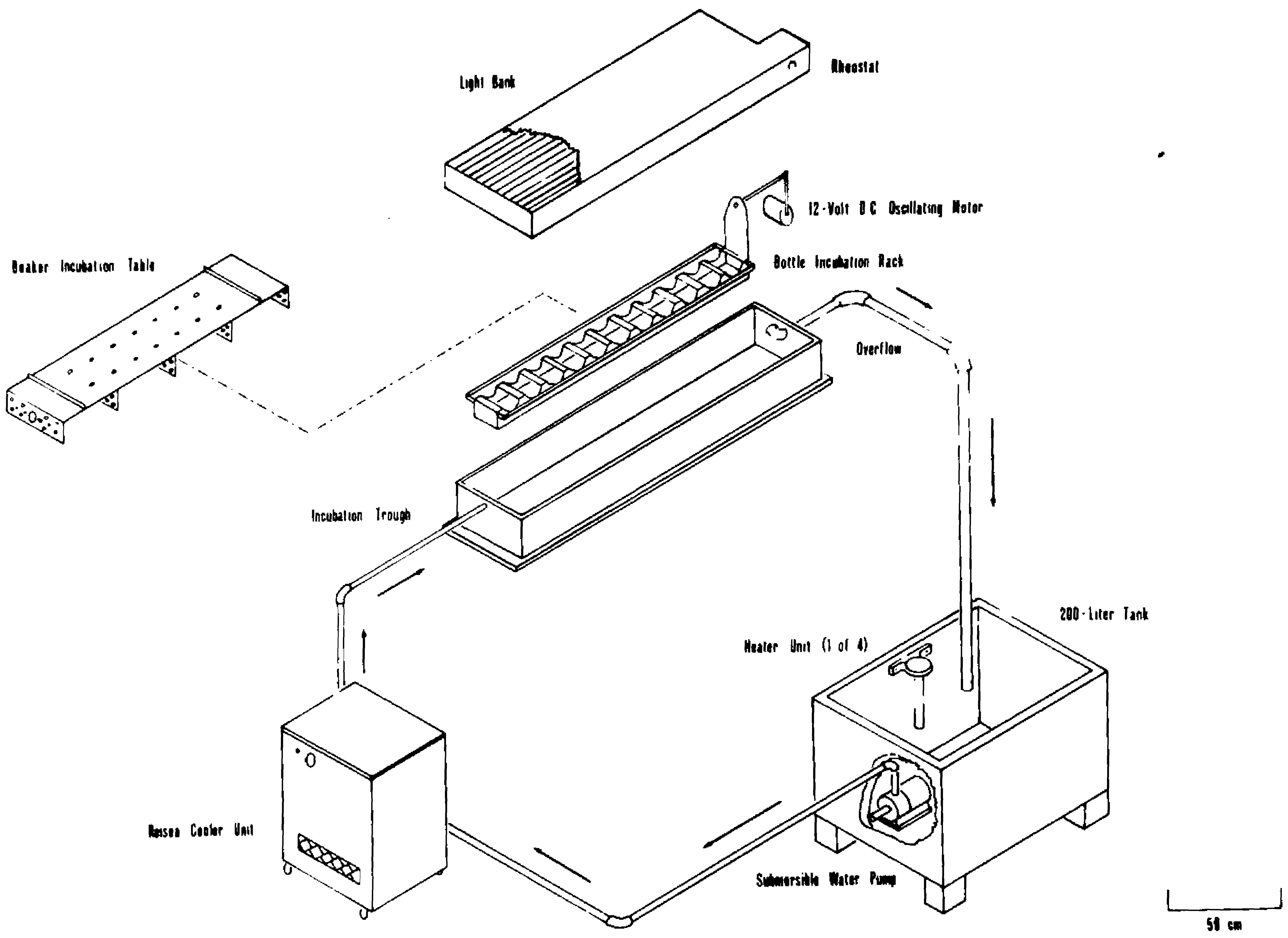

Fig. 2. Apparatus used for the incubation of *Enteromorpha clathrata* during salinity, temperature, nutrient, and mass growth experiments.

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 (Welsher 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 additional 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 distilled 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 phosphorus was added in amounts which matched the ratio of N to P measured in the alga (Tab. I). To simplify the enrichment procedure the concentration of phosphate in normal sea water was assumed to be zero. To limit experimental bias due to possible variation in physical condition in the trough, a randomization scheme was used to arrange the samples in the trough. The medium in each of the enrichment beakers was

Tab. I. 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
1 ×	5	0	0.358	0.592
10 ×	50	14	0.358	0.592
30 ×	150	38	0.358	0.592
70 ×	350	89	0.358	0.592
130 ×	750	189	0.358	0.592

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 ‰). 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 *E. clathrata* is present throughout the year in Tumon Bay; however, there are large quantative variations from month to month (Fig. 3). The mean standing crops at Sites 1, 2, 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 levles tested (4, 5, and 6 ft.) a highly significant negative correlation (−0.7254, −0.7611, and −0.7166) between standing crop and surf heights.

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 longshore 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 II). 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.

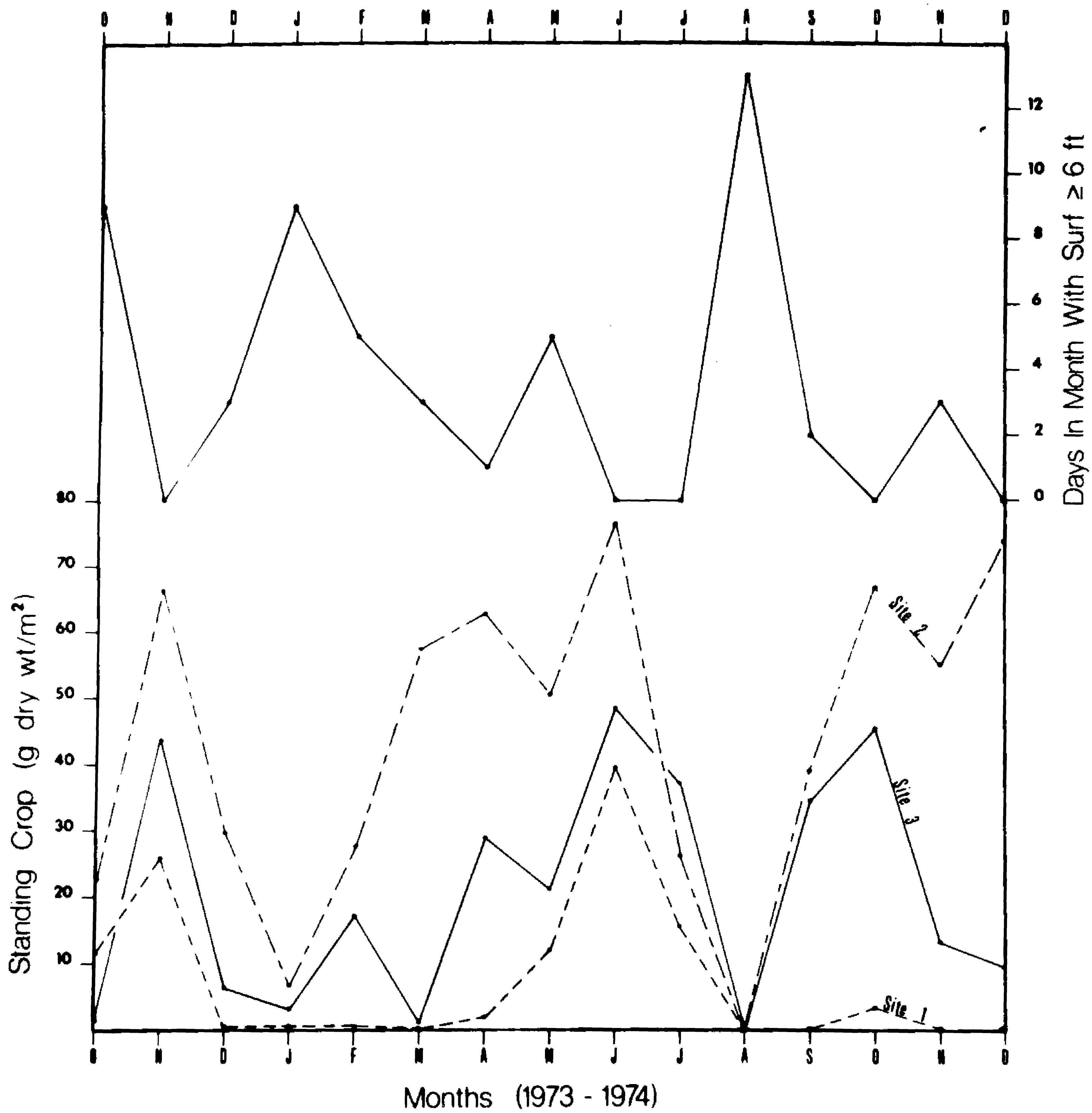

Fig. 3. Standing crop of *Enteromorpha clathrata* at Tumon Bay during the period of October 1973 through December 1974

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 occured (Kami 1976), 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) and a dirunal 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). Bieble (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 reamining sites (Tab. II), 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.

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 marine 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 (Tab. III). Nitrate showed up to a 13.2 times increase

Tab. II. 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

while phosphate did not show a significant increase over reef flat water. Marsh (in press) 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.

Drainage water from the hotels also adds high levels of nitrate and occasionally high levels of phosphate (12.5 μg–at/l) (Marsh 1977). However, drainage water

Tab. III. Water analysis results. The samples were collected on a −0.4 tide at 2300. 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 ‰
Runoff Site							
Groundwater	0	179.40	0.042	0.225	0	0.182	1.0
Groundwater	0	137.10	0.089	0.225	0	0.364	0
Drain Water	0	88.50	1.189	0.238	0	0.182	0
Transect (Perpendicular to the Shoreline)							
Groundwater	0	114.9	0.021	0.325	0	0.182	3.0
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

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.

Tab. IV. 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						
	10‰	*40‰*	20‰	*35‰*	*25‰*	30‰
Respiration n = 10						
	30‰	*25‰*	*20‰*	*35‰*	10‰	40‰
Temperature						
Photosynthesis n = 10						
	10 °C	40 °C	*20 °C*	*30 °C*	25 °C	
Respiration n = 10						
	10 °C	25 °C	20 °C	30 °C	40 °C	
Nutrients n = 15						
	1 x	*130 x*	*10 x*	70 x	30 x	
Salinity (mass Growth Indicator) n = 18						
	40‰	10‰	20‰	30‰		

Salinity

The optimum P/R quotient for salinity was found at 30‰. Both the net photosynthesis and dark respiration showed significant differences (Table IV) among the salinity levels ($p < 0.05$). The findings are similar to those of Conover (1964), who found an optimum growth between 25 and 35‰ for *E. clathrata*. Kapraun (1970)

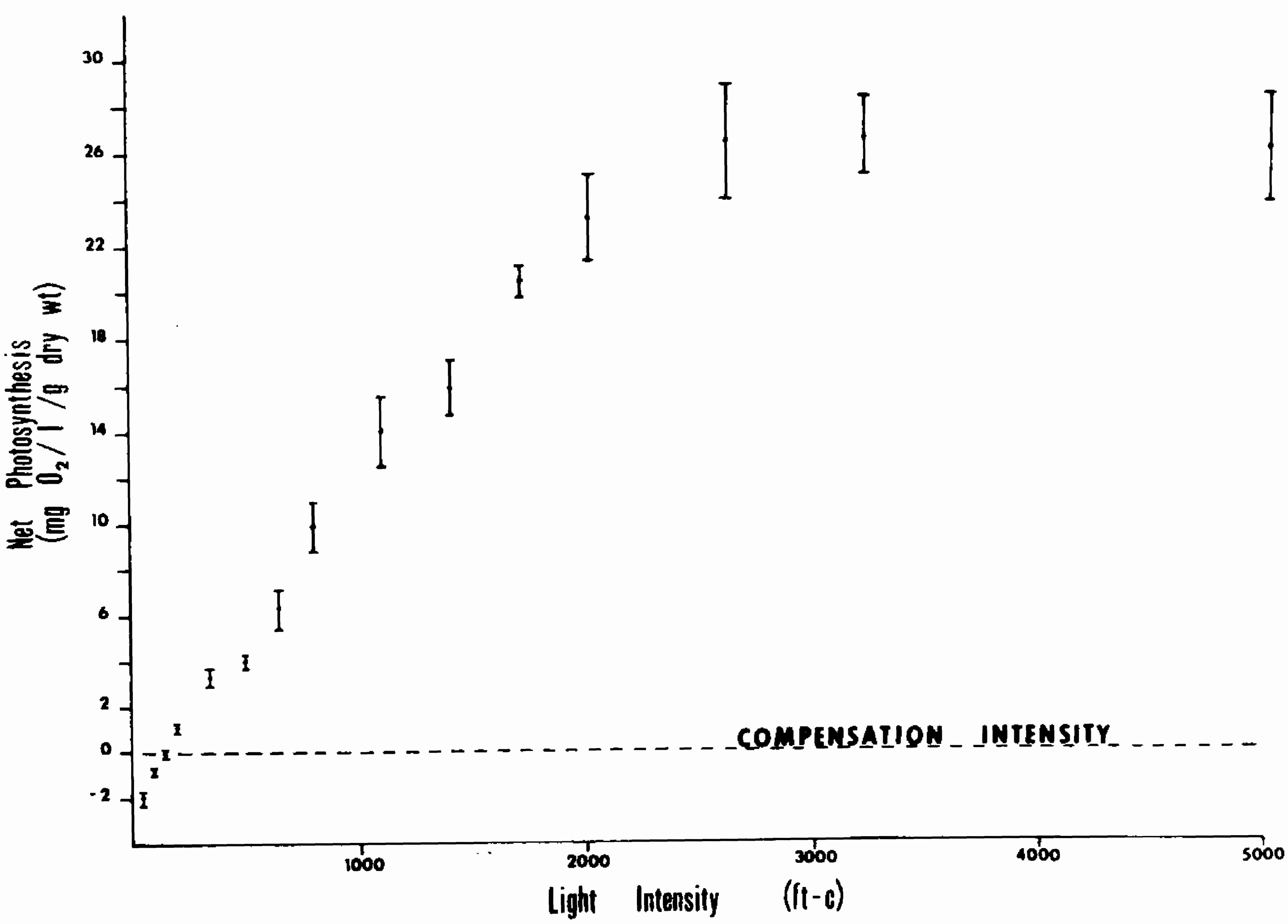

Fig. 5. Light saturation curve of *Enteromorpha clathrata* (28 °C ± 0.5 °C, 34‰ ± 1‰)

found the development of *E. clathrata* to be best in a salinity of 27 ± 2.5‰, and sporulation at 15–30‰. Kjeldsen and Phinney (1972) recorded an optimum growth for *E. linza* at 30–35‰. 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‰ 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/l) under conditions of high (45‰) or low (12‰) salinity.

Temperature

The optimum P/R quotient for temperature (at 30‰) 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).

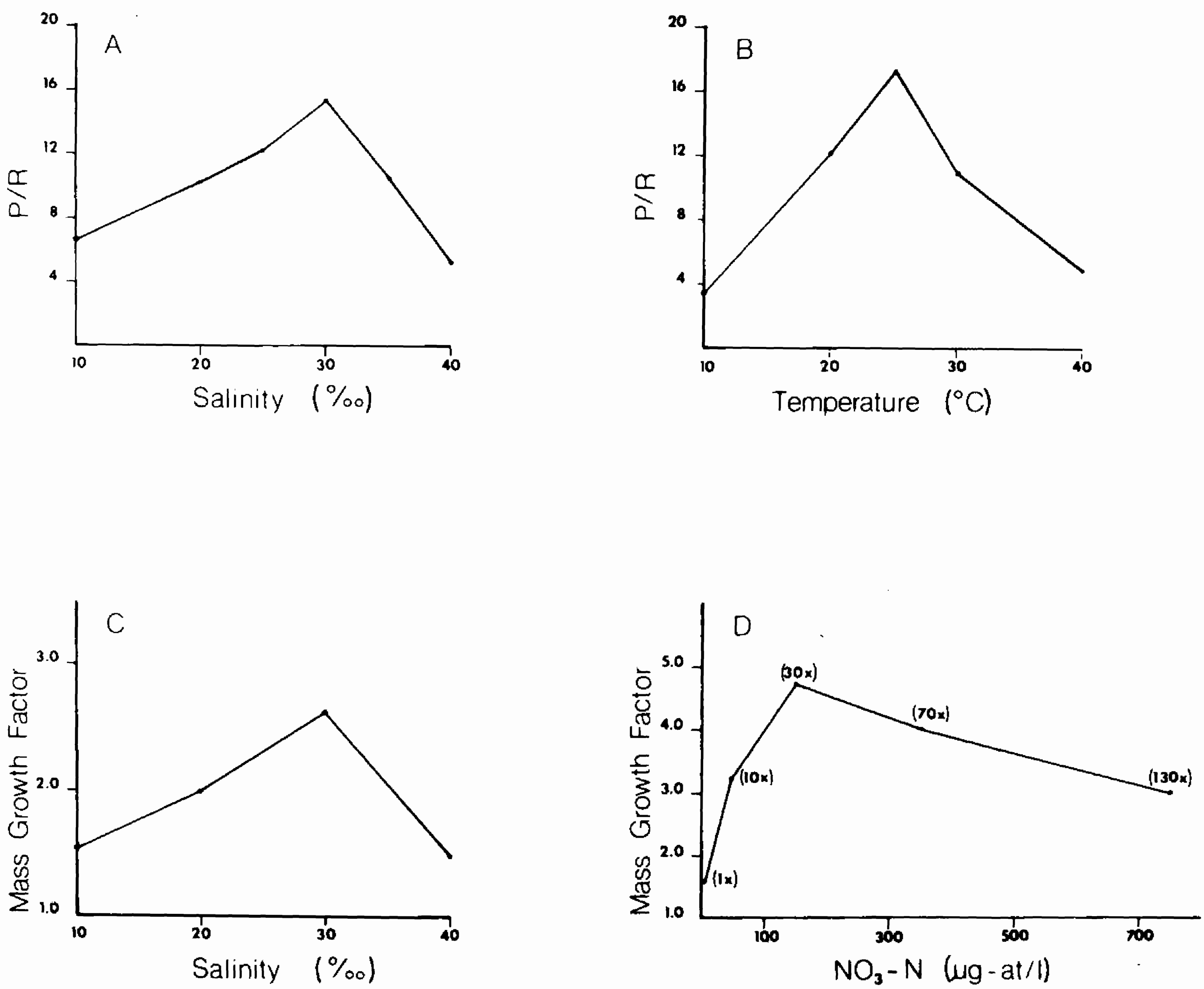

Fig. 6. Optimum growth conditions for *Enteromorpha clathrata*.
A. Optimum salinity at ambient temperature 28 °C ± 1 °C,
B. Optimum temperature at 30‰,
C. Optimum salinity at 25 °C using the mass growth factor,
D. Optimum nutrient enrichment at N/P = 4:1, 30‰, and 25 °C. All experiments were carried out at light saturation (2600 ft-c).

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 phosphorous 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 influencing 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, Tab. IV) showed an optimum at 30 x (150 μg-at/l) the nitrate level found in 1x seawater 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 predominante 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 (Tab. 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-Dieters 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 culturing *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 maganese to further promote the utilization of the available nitrate. The ill-defined and varying constituents of earth extracts 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 or water.

Salinity Mass Growth Factor Indicator

Cultivation of *E. clathrata* at 10, 20, 30, and 40 ‰ showed a significant difference between consecutive salinities in volume change (Table 4). The maximum volume increase occurred at 30‰ (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‰/10‰ 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 possible 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‰ as compared to that at 10‰. 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, temperature, and nutrient levels. The optimum conditions for growth are at a light saturation of 2600 ft-c or higher, 25 °C, 30‰, 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‰, and 2600 ft-c) demonstrate 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‰), 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.

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