Effects of Light Intensity on the Morphology and Productivity of *Caulerpa racemosa* (Forsskal) J. Agardh[1]

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Abstract

Six varieties and three additional growth forms of *Caulerpa racemosa* (Forsskal) J. Ag. were found on Guam's fringing reef flat. Variation in length and number of both assimilators and rhizoids and in the spacing of ramuli with light intensity was demonstrated for the varieties *uvifera* and *lamourouxii*. Characteristics of other varieties developed on specimens collected in the field after they were placed under altered laboratory light intensities. Seasonality of *C. racemosa* was correlated with the number of midday minus tides.

Productivity data suggested the adaptation of var. *uvifera* and var. *lamourouxii* f. *requienii* to habitats of high and low light intensity, respectively. Variety *lamourouxii* f. *requienii* had a lower compensation point, larger P/R (gross photosynthesis/respiration) value, and higher net photosynthetic rate than var. *uvifera* at low light intensities. When exposed to full sunlight the P/R value and net photosynthetic rate of var. *uvifera* exceeded those of var. *lamourouxii* f. *requienii*, which dropped at that intensity. Chlorophyll a and carotenoid concentrations of field-collected var. *lamourouxii* specimens were approximately twice those of var. *uvifera*. Chlorophyll a, but not carotenoid content, was found to decrease with exposure to increasing light intensity for specimens originally classified as var. *lamourouxii*.

The relationships of morphologic and productivity factors to light intensity provide evidence for their environmental rather than genetic control. Reference to *C. racemosa* growth forms as ecophenes is suggested.

INTRODUCTION

Caulerpa racemosa (Forsskal) J. Agardh is a siphonaceous green alga exhibiting an extreme degree of variation in its growth form. It is circumtropical in distribution (Eubank, 1946) and is characterized by having a prostrate cylindrical rhizome with rhizoids below and upright assimilators bearing protuberances termed ramuli. The original descriptions of various forms of this taxon were often based on few or single specimens, and it was believed that each form represented a distinct species (Børgesen, 1907). Subsequent examinations of more extensive collections by Weber-van Bosse (1898), Svedelius (1906), Børgesen (1907), Gilbert (1942),

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Eubank (1946), Cribb (1958) and Taylor (1950, 1960) have led to a recognition of the gradation between these morphologic forms and normally a reduction of their taxonomic level to varieties of the single species *C. racemosa*.

Bøgesen (1907) and Eubank (1946) suggest that environmental factors may control the development of *C. racemosa* varieties. The ability of transplanted specimens of *C. racemosa* to develop characteristics of other varieties has been demonstrated by Tandy (1933), although he did not hypothesize any specific environmental cause. Rehm (1969) also observed changes in the morphology of *C. racemosa* specimens brought into the laboratory and suggested that changes in ramuli shape were initiated by reduced light. The possibility of a genetic basis for separation of varieties is also suggested by Eubank (1946) and by Taylor (1950). Recommendations for more critical field and laboratory studies have appeared in the literature (Bøgesen, 1907; Gilbert, 1942; Eubank, 1946).

Studies involving *C. racemosa* varieties collected during floristic surveys or observed in the field often include limited descriptions of the environmental conditions under which these specimens were collected or observed. Light intensity has been mentioned in this respect. For example, Bøgesen (1907) refers to the extremely high light intensity of the reef flat habitat occupied by var. *uvifera*. Reduced light intensity can also be associated with the deep water habitat reported for var. *lamourouxii* (Bøgesen, 1907; Taylor, 1950). The agitated water and bubbles formed by braking waves at the reef margin would serve to reduce the light intensity at the substrate as compared to the calmer reef flat. Bøgesen (1907) has reported var. *clavifera* from such areas.

The tendency for *C. racemosa* varieties to be somewhat restricted to specific habitat situations raises the question of environmental versus genetic control of the morphologic characteristics used in their classification. The presence of intermediate forms (Weber-van Bosse, 1898; Bøgesen, 1907; Eubank, 1946; Taylor, 1950, 1960) tends to weaken the argument for genetic control as does the fact that characteristics of two or more varieties are occasionally found on the same specimen (Tandy, 1934; Eubank, 1946; Taylor, 1960; Rehm and Almodovar, 1971).

Bøgesen (1907) mentioned a general tendency for radial development of ramuli around the assimilator axis in shallow water versus bilateral development in deeper water for the species *C. racemosa* and *C. cupressoides* (West) J. Agardh. He also stated his belief that this tendency represents an ecological adaptation. Such an adaptation could be related to the water depth effect on light intensity and the vital requirement of light for plant growth. Round (1968) has reviewed some effects of light intensity on the morphology of algae and Daubenmire (1959) summarized such effects for higher terrestrial plants.

The phenomena of intermediate forms and multiple varietal characteristics were observed on Guam and strengthened my own speculation that many, if not all, of the *C. racemosa* varieties are the result of environmental variation within and between habitats. References to light in past literature plus my own observation that differences in light intensity occurred between many of these habitats suggested

that this environmental factor may influence the morphology of *C. racemosa*. Morphologic characteristics which have been used in identifying and describing *C. racemosa* varieties include concentration of rhizoids, length and concentration of assimilators, and especially the spacing and shape of ramuli.

The purpose of this study was to test two hypotheses: 1) that the morphology of *C. racemosa* is influenced by light intensity and 2) that characteristics of the morphology of *C. racemosa* function adaptively to ensure optimum productivity for the light intensity under which they develop.

MATERIALS AND METHODS

All specimens used during the course of this study were collected from the reef flat and margin on the eastern coast of Guam in an area extending from the University of Guam Marine Laboratory on Pago Bay 10 km south to Asanite Bay. Specimens were kept in plastic aquaria supplied with water from the Marine Laboratory seawater system.

Experiments in this study utilized specimens of the varieties *uvifera* and *lamourouxii*. One practical reason for using these varieties was that var. *uvifera* was relatively abundant on the reef flat and var. *lamourouxii*, though not always as available on the reef, was easily maintained in the laboratory. Another more important reason was that the varieties *uvifera* and *lamourouxii* were characteristically found in habitats of high and low light intensity, respectively. This allowed the consideration of pre-experimental light conditions and growth form in analyzing the effects of light intensity.

Most of the var. *uvifera* specimens on Guam had crowded, spherical ramuli conforming to Bøergesen's (1907) description of this variety in the Danish West Indies and to a drawing by Weber-van Bosse (1898) of var. *uvifera* f. *intermedia* (Pl. XXXIII, Fig. 24a). The more typical form, described by Taylor (1960), has ramuli which are crowded but slightly compressed tangentially with the surface of the assimilator. This latter form was present, though rare, on Guam and all references to var. *uvifera* in this paper, unless otherwise stated, refer to the former morphologic condition.

FIELD AND TRANSPLANT OBSERVATIONS

Observations of the distribution of *C. racemosa* varieties on the reef flats of Guam were made between December, 1970 and November, 1971. Seasonal abundance and degree of exposure to light and low tide were noted. A number of specimens collected in the field were transplanted into the laboratory at various light intensities. Photographs were taken to record changes in their morphologic characteristics.

GROWTH APPARATUS EXPERIMENTS

To quantify the effects of light intensity on the morphology of *C. racemosa*, specimens were maintained in the apparatus shown in Fig. 1. This trough-like construction was made with pieces of clear plexiglas joined with epoxy glue. The partitions had double coats of white epoxy paint, and the entire outer surface was first painted white to allow a uniform white interior background. The outer surface was then painted black to further limit the entrance of light from outside the trough.

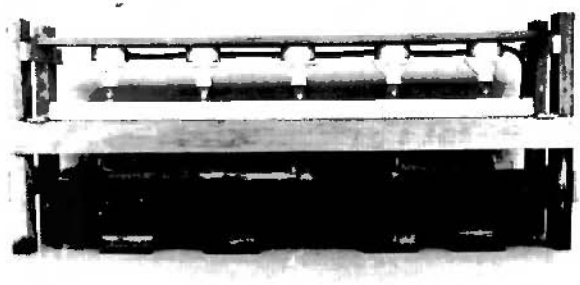

Fig. 1. Growth apparatus.

The light sources were four 40-watt cool-white fluorescent tubes and five 150-watt incandescent bulbs. To vary light intensity, 1/32 inch mesh nylon screen stapled to wooden frames was placed over the tops of sections A (eight layers of screen), B (four layers) and C (two layers). Section D had a wooden frame without screen attached. This created light intensities of 0.5, 3.5, 8.3 and 21 kilolux in sections A through D, respectively. Light intensity measurements were made at the level of the algae with a submarine photometer. The microampere readings of the submarine photometer were converted to foot candle values after comparative readings were made with a GE Type 213 light meter. Kilolux values were then determined by the conversion factor: 1 lux equals 0.093 foot candle. A 12/12 hour light/dark cycle was controlled automatically for all experiments by a timer wired into the lighting system. This light cycle closely simulated the photoperiod on Guam (U.S.D.C., 1967).

Water from the seawater system ran into an aquarium where suspended material was allowed to settle. Water was siphoned from a flask within that aquarium through plastic hoses into the influent section at the extreme left of the trough at a rate of 1.3 liters per minute. Water flowed through the trough beneath and at the sides of the partitions. These slight openings allowed some light to pass between sections but were necessary for water movement. Water flowed out of the trough over the lower end wall of the effluent section at the extreme right of the trough.

Aluminum foil was placed between the incandescent bulbs and the two fluorescent light fixtures to prevent overheating. Foil was also placed over the influent section to prevent light from passing between it and section A. Radiant heat from the lights caused an increase in water temperature, especially at the surface. This factor was reduced by the continuous flow of water, and when in use the average midday temperatures at the level of the algae were 29.8, 29.8, 30.6, and 31.2°C for sections A through D, respectively.

Before specimens were placed in the growth apparatus they were checked for foreign material which, if present, was removed. Two-pound test monofilament line was tied to the rhizome of each specimen and to pieces of embossing tape marked to identify the specimen number and section of the trough in which it was maintained. Pieces of plexiglas with grooves cut by a coping saw were attached at their ends to the sides of each section. The embossing tape was slid into these grooves to hold the specimens approximately eight cm below the water surface.

Growth which occurred during six to 10 day exposure periods was measured by the following procedures:

1. Specimens were removed from the apparatus and placed in a seawater-filled tray. A millimeter rule was used to measure rhizoid length and distance from rhizome tip, and assimilator length and distance from rhizome tip. Number of ramuli per assimilator was also determined.
2. Specimens were removed from the apparatus and placed into a seawater-filled tray at the bottom of which was a sheet of two mm ruled graph paper fused between two pieces of plexiglas. A millimeter rule was also placed next to each specimen before it was photographed with slide film. When these slides were later projected onto a screen the above-mentioned measurements were made.

Growth responses of 17 var. *uvifera* specimens freshly collected from the same shallow reef flat depression were made with a millimeter rule. Measurements were made after a six day exposure period within the growth apparatus. Data for seven other var. *uvifera* specimens which did not appear in good condition during or at the end of this period are not included. The growth responses of 26 var. *lamourouxii* specimens within the growth apparatus were also determined. These specimens had flattened blade-like assimilators with very few ramuli at the start of the experiments and were developed in the laboratory at approximately 1.6 klux from material collected two months prior at the reef margin and identified as var. *clavifera*. The rarity of this form prohibited the use of field-collected specimens. Measurements were made by millimeter rule for 14 var. *lamourouxii* specimens after seven days of growth. The remaining 12 specimens were measured photographically after 10 days of growth. Mature assimilators and rhizoids were defined as those occurring on rhizome segments more than three days old. During a preliminary study it was determined that the majority of growth occurs during that period of time. When two or more rhizoids occurred less than two mm apart the longest length was recorded.

PRODUCTIVITY AND PIGMENTS

Oxygen production and consumption of specimens freshly collected from high, var. *uvifera*, and low, var. *lamourouxii* f. *requienii*, light intensity habitats were determined over a wide range of light intensities. The intensities and sources of light were (1) 0.8 klux, fluorescent tubes 1.5 m from specimens; (2) 3.7 klux, fluorescent tubes 0.6 m from specimens; (3) 13 klux, fluorescent tubes 10 cm from specimens; (4) 50 klux, 200 watt incandescent bulbs 7.5 cm from specimens; and (5) 100 klux, natural sunlight at midday with a clear sky.

The light- and dark-bottle oxygen technique (Gaardner and Gran, 1927) was used to measure photosynthesis and respiration. Results of this type of experiment are considered to represent biological activity (Hedgepeth, 1957) or food produced (Odum, 1959). Seawater samples were analyzed for dissolved oxygen according to the alkali-azide modification of the Winkler technique (A.P.H.A., 1965). Titrations were completed within eight hours after the samples were "fixed".

Twenty freshly collected specimens were used, two of each variety at each light intensity. Prior to use, specimens were checked for epiphytic algae and invertebrates which, if present, were removed. The following sequence of steps was performed to get replicate oxygen exchange data for each specimen in both light and dark bottles.

1. Specimens were exposed to the experimental light condition for one hour before testing.
2. Each specimen was individually incubated for 60 to 75 minutes in a 300-ml BOD bottle made light-tight with two layers of aluminum foil.
3. Specimens were placed in uncovered BOD bottles for three successive 30- to 38-minute incubation periods.
4. Same as step two.
5. Wet weight of each specimen was determined as described below.

During both light and dark incubation periods BOD bottles were kept under identical light conditions. An incubation medium of continuously running seawater was used to prevent heating. Seawater used to fill the BOD bottles was first filtered through 0.45-micron membrane filters to remove plankton that could otherwise affect the results. This water was always used within 30 hours of filtering. To eliminate possible effects of periodicity of oxygen production, all trials began at approximately 1100 hours. Such a periodicity has been demonstrated for phytoplankton (Doty and Oguri, 1957).

An average of two seawater samples taken at the start of each incubation period gave an initial oxygen value. The two dark period values were subtracted from their corresponding initial values before averaging to give a measure of each specimen's respiration rate. Net photosynthesis rates were obtained from an average of the differences between each of the three light period measurements and their initial values. Adding the rates of respiration and net photosynthesis gave a gross photosynthesis value for each specimen.

Percent dry weight was determined for 10 specimens each of the varieties

uvifera and *lamourouxii*. Wet weight was first determined for each specimen on a Mettler Model H 10 Balance after foreign material was removed and surface water had been absorbed by wrapping and blotting in absorbent tissue for 10 seconds. Specimens were then dried overnight at 100–105°C. After removal from the oven specimens were placed in a desiccator and allowed to cool for a few minutes before their dry weight values were recorded.

Chlorophyll a and carotenoid contents were measured for six specimens each of var. *lamourouxii*, collected in shaded areas of a reef flat depression, and var. *uvifera*, collected at the outer reef flat. These measurements were also made after 24 days of exposure for 12 specimens originally identified as var. *lamourouxii* which were randomly placed three each in sections A through D of the growth apparatus.

Wet weight value was recorded for each specimen before it was ground, under reduced lighting, in 100% acetone with a mortar and pestle. The acetone and plant residue, plus 100% acetone used to rinse the mortar and pestle, were poured into 50 ml centrifuge tubes. Additional acetone was added to make a total of 30 ml of 100% acetone. The tubes were covered and placed overnight in a refrigerator at 8°C. When removed they were again protected from light and allowed to return to room temperature. The percent dry weight values described above, were used to estimate the amount of water in each specimen and additional distilled water or 100% acetone was added to make a final acetone concentration of 90% for each sample. The tubes were then centrifuged at 3100 rpm for 10–15 minutes.

Samples of the acetone solution were individually placed in a cuvette to record optical density at 665, 645, 630, 510 and 480 millimicrons with a Beckman Model B spectrophotometer. The formulas developed by Richards and Thompson (1952) were used to calculate pigment concentrations. Twelve samples were also measured at 750 millimicrons as a turbidity check.

RESULTS AND DISCUSSION

FIELD AND TRANSPLANT OBSERVATIONS

Nine *Caulerpa racemosa* growth forms were found on Guam's fringing reef flat. Varietal and form names, and general habitat descriptions are given below. Observations of abundance are indicated by the terms rare, common and abundant. These are subjective estimates based on the relative number of times each form was encountered during the period of maximum *C. racemosa* growth.

1. Var. *clavifera* (Turner) Weber-van Bosse; Børgesen, 1907: p. 47, Fig. 25 and 26.

   Outer reef flat to margin, growing in tangled mats. Subjected to breaking waves, low tide exposure and high light intensity. Abundant.

2. Var. *clavifera* (Turner) Weber-van Bosse f. *reducta* Børgesen, 1907: p. 48, Fig. 27.

   Outer reef flat to margin over raised rocks and at edges of depressions. Subjected to breaking waves, low tide exposure and high light intensity. Rare.

3. Var. *uvifera* (Turner) Weber-van Bosse, 1898: Pl. XXXIII, Fig. 6.

Thick mats within 0.5 m deep, outer reef flat depressions, protected from breaking waves and low tide exposure. Isolated specimens on outer reef flat, exposed to breaking waves and low tide exposure. All exposed to high light intensity. Rare.

4. Var. *uvifera* (Turner) Weber-van Bosse f. *intermedia* Weber-van Bosse, 1898: Pl. XXXIII, Fig. 24.

Outer reef flat singly or in patches up to 3 m wide. Subjected to extreme high light intensity and low tide exposure. Occasionally in shallow reef flat depressions. Abundant.

5. Var. *macrophysa* (Kutzing) Taylor; Eubank, 1946: p. 428, Fig. 2n.

Outer reef flat, subjected to high light intensity and low tide exposure. Others within semi-shaded reef flat depressions and under rocks protected from high light intensity and low tide exposure. Common.

6. Var. *lamourouxii* (Turner) Weber-van Bosse, 1898: Pl. XXXII, Fig. 1–4 and 6.

Shaded areas within reef flat depressions. Protected from breaking waves, high light intensity and low tide exposure. Rare.

7. Var. *lamourouxii* (Turner) Weber-van Bosse f. *requienii* (Montagne) Weber-van Bosse, 1898: Pl. XXXII, Fig. 5 and 7.

Beneath ledges and thick mats of other var. *lamourouxii* thalli within reef flat depressions. Protected from breaking waves and low tide exposure. Minimum light intensities. Rare.

8. Var. *occidentalis* (J. Agardh) Bøergesen, 1907: p. 49, Fig. 29.

Within slight to one m deep reef flat depressions and splash pools. Protected from breaking waves and low tide exposure. Subjected to high light intensity. Common.

9. Var. *peltata* (Lamx.) Eubank, 1946: p. 428, Fig. 2r and Ss.

Reef flat and margin. Usually within an articulated coralline algae mat on the reef flat or within slight reef flat depressions. One specimen on side of rock. Occasionally within mats of var. *clavifera*. Sometimes subjected to breaking waves and low tide exposure. Protected from high light intensity. Common.

There was a definite seasonality in the abundance of *C. racemosa* on the reef flats of Guam. Seasonality of *C. racemosa* has also been reported for Bermuda (Bernatowicz, 1952; Taylor and Bernatowicz, 1969). Development of *C. racemosa* began in October and November, and maximum growth occurred between December and May. Growth declined and specimens were increasingly difficult to find during the period June through September.

Seasonality of *C. racemosa* may be controlled by minus tides occurring at midday. Midday minus tides, whose lowest levels occurred between 0900 and 1500 hours, were absent from the latter part of October through February. They began in March and peaked at 12 per month during July and August before again declining

in number. Exposure to midday minus tides has also been suggested to explain seasonality of *Sargassum duplicatum* J. Ag (=*S. cristaefolium* C. Ag.) on Guam (Tsuda, in press).

The correlation between decreased abundance of *C. racemosa* and midday minus tides may be due to desiccation damage resulting from atmospheric exposure during periods of high light intensity. Rehm (1969) found that *C. racemosa* assimilators could survive one hour of atmospheric exposure in the shade but not one hour 15 min. Assimilators exposed to direct sunlight did not recover after a 15 min. exposure.

The possibility that low tide exposure controls seasonality is further evidenced by the fact that areas of most prolonged growth were reef flat depressions not exposed at low tide, and the reef margin area which is periodically washed by breaking waves even during the lowest tide levels. In addition, laboratory specimens never exposed to the atmosphere showed no seasonal effect and var. *chemnitzia* (Esper) Weber-van Bosse was abundant at a depth of approximately 30 m off Guam in September.

Maturity may have an effect on *C. racemosa* morphology, but it is difficult to separate its effect from that of light intensity. For example, var. *peltata* was relatively common during the developmental period of the growth season, in some areas showing an almost continuous transition from small specimens with flattened ramuli growing within a substrate of mat-like articulated coralline algae (Fig. 2) to larger specimens with hemispherical ramuli growing over the top of this substrate (Fig. 3). This latter form was classified as var. *macrophysa*. While the specimen with enlarged ramuli (Fig. 3) may be a more mature form of var. *peltata*, it is also

Fig. 2. A var. *peltata* specimen growing within an algal mat of articulated coralline algae.

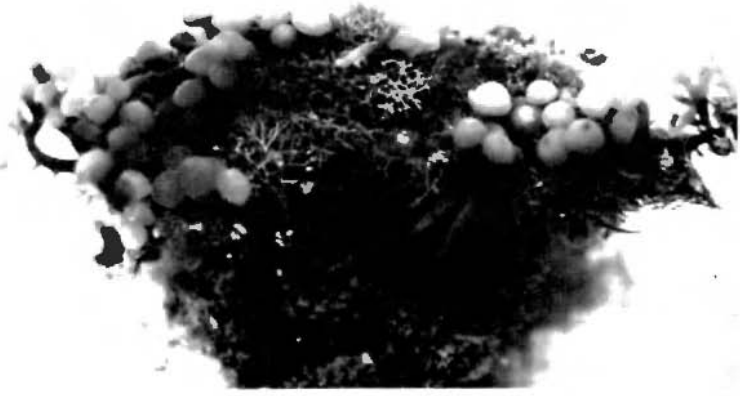

Fig. 3. A var. *macrophysa* specimen growing at the top of an algal mat of articulated coralline algae.

possible that it results from increased light intensity at the surface of the substrate. Possibly the conditions within the algal mat, including a reduced light intensity, are favorable for germinating zygotes with var. *peltata* being an early developmental form. Carrying this speculation further, a positive phototropic response of this form may eventually bring it closer to the surface of the substrate where increased light intensity results in form changes. Undisturbed *Zonaria farlowii* specimens kept at low light intensity have shown a phototropic growth response, with curving of their apical region resulting in a perpendicular orientation to the light source (Dahl, 1971).

Børgesen (1925) referred to var. *peltata* as a separate species but recognized the presence of forms transitional to *C. racemosa*. Svedelius (1906) also classified var. *peltata* as a separate species but noted swollen ramuli at the upper part of some assimilators. These reminded him of var. *clavifera* (which he also categorized as a species) except that they had a border as evidence that they were originally flat. No such border was observed on Guam specimens. Taylor (1950) in placing specimens into var. *macrophysa* referred to them as an extension of the variation shown by his var. *clavifera* specimens. If var. *clavifera* is a more mature form, developing from var. *macrophysa*, the question again arises whether such a form change results from aging or from increased light intensity. Variety *macrophysa* was observed on the surface of the outer reef flat mostly during the developmental period indicating it had not been there long. Thus, such specimens (i.e., Fig. 3) may have just reached the top of their somewhat protective coralline algae substrate and with further growth at this more exposed position would develop a var. *clavifera*-like form.

Variety *clavifera*, in occurring close to the reef margin in tangled mats, would be somewhat shaded both by especially agitated water in that area and by other var. *clavifera* thalli within these mats. Variety *uvifera*, on the other hand, was very abundant on the reef flat at some distance from the margin and occasionally near the margin during the maximum growth period. Although it often occurred in patches up to three meters in diameter, the thalli were not nearly as tightly packed as they were in the var. *clavifera* mats. It thus seems a reasonable speculation that var. *uvifera* results from growth in areas exposed to higher light intensities than genetically identical thalli which develop into var. *clavifera* at the reef margin.

A number of the specimens transplanted into the laboratory developed characteristics associated with other varieties. These form changes occurred in the areas of new apical growth at the tips of both assimilators and rhizomes. The form of this alga, once completely developed, was never observed to change.

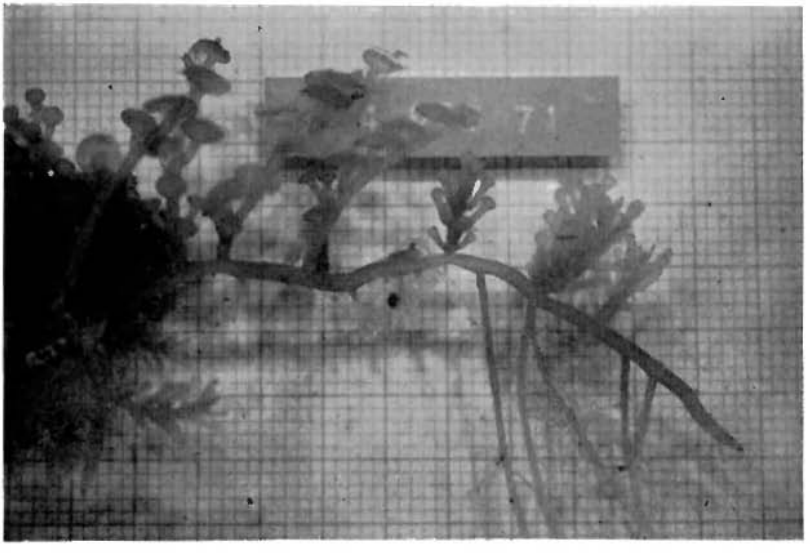

Fig. 4. Single specimen showing characteristics of var. *uvifera* (left), var. *peltata* (center), and var. *laetevirens* (right) when exposed to different light intensities.

Figure 4 is a photograph of a specimen classified as var. *uvifera* when collected. During five days of growth at 3.8 klux in a laboratory aquarium the ramuli became extremely flattened and fewer in number as is characteristic of var. *peltata*. When placed in the 21 klux section of the growth apparatus for another five days the number of new ramuli increased and they became gradually expanded to cylindrical in form, closely resembling var. *laetevirens* (Mont.) Weber-van Bosse.

During three weeks in section A of the growth apparatus, another var. *uvifera* specimen had rhizome growth without assimilator development. When the specimen was placed in an aquarium at 1.6 klux for one week, assimilators developed both from the rhizome and from two ramuli (Fig. 5). These new assimilators had

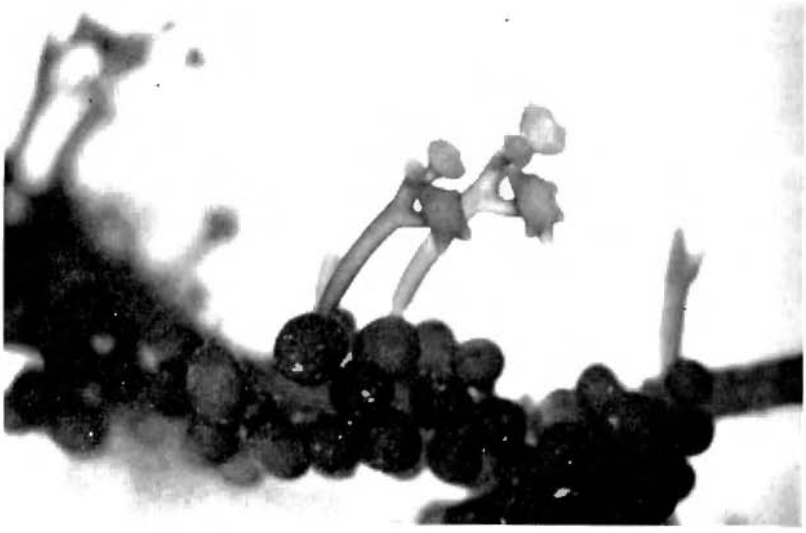

Fig. 5. Variety *uvifera* developing new assimilators from ramuli of its original growth with new ramuli characteristic of var. *exigua*.

flattened ramuli as does var. *peltata,* however, some had indented margins characteristic of var. *exigua* (Weber-van Bosse) Eubank. This same specimen was next transferred to section D with a light intensity of 21 klux. Ramuli developed at this intensity increased in number but were not crowded, and they gradually expanded to a rounded but still somewhat flattened end. This new form was identified as var. *macrophysa.*

The var. *uvifera* specimen shown in Fig. 6 was collected on the reef flat and had crowded, spherical ramuli. During approximately six weeks at 3.5 klux the newly developed assimilators were flattened with bilaterally arranged ramuli, similar to those of var. *lamourouxii.* A number of var. *clavifera* specimens made this same form change.

A var. *peltata* specimen (Fig. 7) collected from a shaded position near the reef margin was placed in section D at 21 klux for six days. During the first three days at that intensity the ramuli became slightly spherically expanded at their tips. Ramuli developed during the following three days were more expanded but somewhat flattened and like the form taken by the Fig. 5 specimen at the same intensity, this specimen was identified as var. *macrophysa.*

These obvious changes in *C. racemosa* morphology under altered light intensities serve as evidence for environmental rather than genetic control of varietal differences. The flattened ramuli and blade-like assimilators developed at reduced light intensities result in increased surface area. This may have an adaptive function at low light intensities in allowing increased light absorption in comparison with forms having enlarged ramuli.

Transplanting specimens to extreme light conditions sometimes resulted in

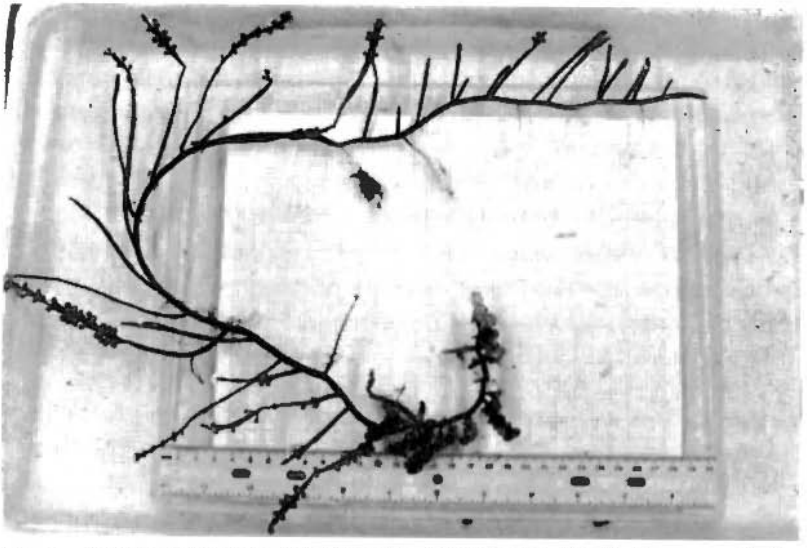

Fig. 6. Variety *uvifera* (center), changing form to that of var. *lamourouxii* when when exposed to 3.5 klux for six weeks.

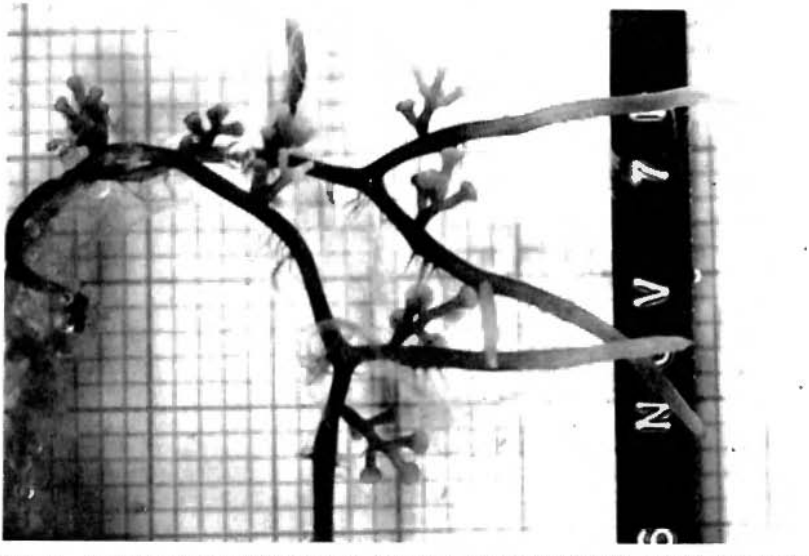

Fig. 7. Variety *peltata* (extreme left, bleached appearance) with ramuli becoming increasingly enlarged, to form var. *macrophysa*.

injury. For example, four var. *lamourouxii* specimens became flaccid and colorless, except for a short segment of the rhizome near the apex, after nine days exposure to full sunlight in an outdoor aquarium. Four similarly treated var. *uvifera* specimens remained healthy. Another group of specimens placed in an outdoor aquarium screened to receive 10% sunlight responded quite differently. All four var. *la-*

mourouxii specimens remained healthy whereas only one of four var. *uvifera* specimens survived. Dahl (1971) reported injury to *Zonaria farlowii* specimens collected or maintained at reduced light intensity upon exposure to full sunlight and suggested a cellular adaptation to the prevailing light intensity. *C. racemosa* may have a similar means of adaptation.

GROWTH APPARATUS EXPERIMENTS

Light intensity values were transformed logarithmically and statistical tests performed to test the effects of light intensity on all growth factors. An increase in the number of ramuli per unit length of assimilator with increasing light intensity

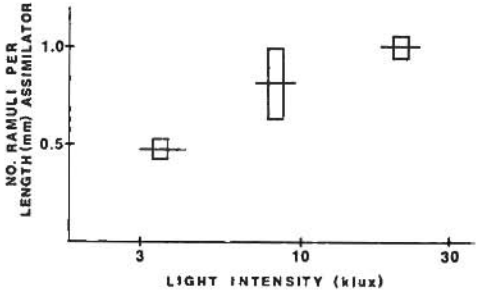

Fig. 8. Variety *uvifera*, number of ramuli per unit length of assimilator for assimilators developed on specimens maintained in sections B through D of the growth apparatus. The vertical bar represents two standard errors on either side of the mean, which is indicated by the horizontal line.

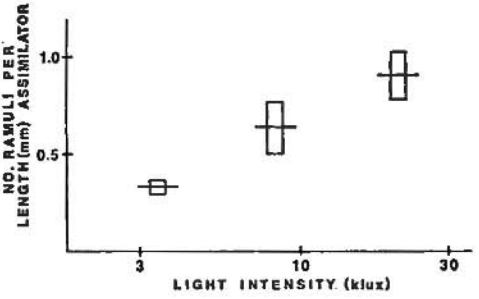

Fig. 9. Variety *lamourouxii*, number of ramuli per unit length assimilator for assimilators developed on specimens maintained in sections B through D of the growth apparatus. The vertical bar represents two standard errors on either side of the mean, which is indicated by the horizontal line.

was found to be significant for both var. *uvifera* (Fig. 8; $P < .005$) and var. *lamourouxii* (Fig. 9; $P < .025$) when tested by regression analysis. Although never presented as a ratio value, the spacing of ramuli is frequently referred to in keys and descriptions of *C. racemosa* varieties. For example, Taylor (1960) refers to

the ramuli of var. *lamourouxii*, found in reduced light intensity habitats, as "... alternate, sub-opposite, or few and widely scattered."; var. *laetevirens*, reported from both semi-exposed and sheltered habitats (Cribb, 1958), as "... densely imbricate to widely spaced..."; var. *clavifera*, found in high but less than maximum intensity due to agitated water and possibly self-shading within mats, as "... generally not crowded..."; and var. *uvifera*, exposed to extreme high intensities, as "... crowded and imbricate...". Thus, there seems to be a general tendency for varieties found in habitats of reduced light intensity to have more widely spaced ramuli. The fact that the spacing of ramuli, a characteristic used in classifying *C. racemosa* varieties, can be significantly related to light intensity for the varieties *uvifera* and *lamourouxii* indicated environmental rather than genetic control of this factor.

Assimilators did not initiate development at 0.5 klux, indicating that a minimum light intensity between 0.5 and 3.5 klux is required for initiation of assimilator development. Most of the ramuli of var. *lamourouxii* specimens developed at 3.5 klux were bilaterally arranged. Those developed at the higher intensities were generally radially arranged, as were those of the experimental var. *uvifera* specimens at all intensities. Most of the ramuli were gradually expanded from their base to a spherical tip and would be classified as var. *clavifera*, although some var. *uvifera* specimens developed more abruptly expanded ramuli at the highest intensity resulting in their classification as var. *occidentalis*.

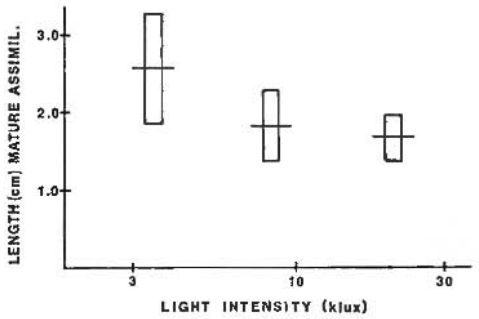

Fig. 10. Variety *uvifera*, length of mature assimilators developed on specimens maintained in sections B through D of the growth apparatus. The vertical bar represents two standard errors on either side of the mean, which is indicated by the horizontal line.

Length of assimilators did not vary significantly for either var. *uvifera* (Fig. 10) or var. *lamourouxii* (Fig. 11) when tested by analysis of variance. Both varieties did, however, show a tendency to develop shorter assimilators as light intensity increased. When analyzed separately by Student's t-test the assimilators developed at 3.5 klux were found to be significantly longer than those developed at 21 klux for var. *uvifera* ($P < .05$) but not var. *lamourouxii*. Decreased growth of terrestrial plant leaves, both coniferous (Whittaker and Garfine, 1962) and deciduous (Murray

and Nichols, 1966), has also been related to increased light intensity.

Assimilator length is used by Taylor (1960) in his descriptions of *C. racemosa* varieties. For example, the varieties *clavifera*, *macrophysa* and *uvifera*, all found in relatively high light intensity habitats, are reported to have assimilator lengths

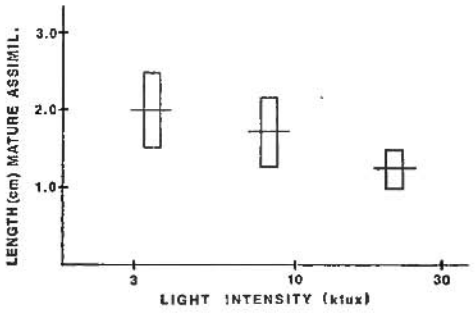

Fig. 11. Variety *lamourouxii*, length of mature assimilators developed on specimens maintained in sections B through D of the growth apparatus. The vertical bar represents two standard errors on either side of the mean, which is indicated by the horizontal line.

of 1 to 11 cm, 3 to 6.5 cm and 1.5 to 2.5 cm, respectively. This is contrasted with the varieties *laetevirens* and *lamourouxii* whose lengths Taylor reported to be 12 to 30 cm and up to 16 cm, respectively. I have found var. *lamourouxii* only in shaded reef flat depressions on Guam. Bøergesen (1907) reported var. *lamourouxii* to inhabit lagoon bottoms where the depth of water (20 to 30 m) would also reduce light intensity. Variety *laetevirens* was not found on Guam's reef flat although it developed from specimens originally classified as var. *uvifera* after being transplanted into reduced laboratory lighting. The fact that assimilator length, a morphological characteristic used in identifying *C. racemosa* varieties, can be related to light intensity is another indication that the form of this alga is under environmental rather than genetic control.

In analyzing the effect of light intensity on the ratio of number of assimilators developed per unit length of rhizome, a significant effect ($P < .001$) was found by analysis of variance for both var. *uvifera* (Fig. 12) and var. *lamourouxii* (Fig. 13). However, only the var. *lamourouxii* data from sections B through D showed a significant regression relationship ($P < .05$). The decrease in number of assimilators per unit of rhizome length with increases in light intensity for var. *lamourouxii* suggests an inhibitory effect and indicates its adaptation to low light intensity. Number of assimilators per unit of rhizome length was highest for var. *uvifera* at 8.3 and 21 klux, in this case suggesting adaptation to higher light intensities.

Both varieties demonstrated increased rhizoid development as light intensity increased. Variety *uvifera* had a significant regression relationship ($P < .05$) between increasing light intensity and an increase in its ratio of number of rhizoids per unit length of rhizome (Fig. 14). Length of rhizoids of var. *uvifera* did not vary

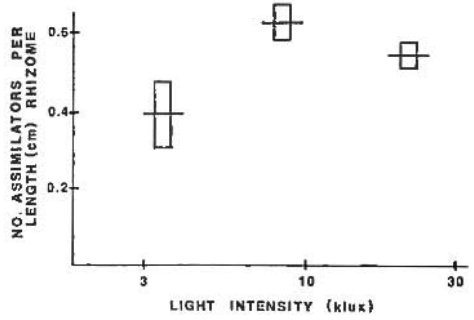

Fig. 12. Variety *uvifera*, number of assimilators per unit length of rhizome developed on specimens maintained in sections B through D of the growth apparatus. The vertical bar represents two standard errors on either side of the mean, which is indicated by the horizontal line.

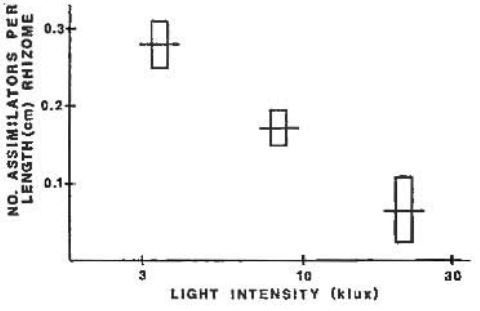

Fig. 13. Variety *lamourouxii*, number of assimilators per unit length of rhizome developed on specimens maintained in sections B through D of the growth apparatus. The vertical line represents two standard errors on either side of the mean, which is indicated by the horizontal line.

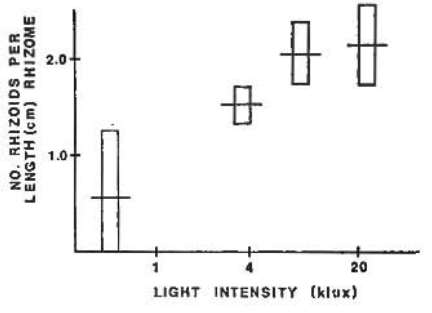

Fig. 14. Variety *uvifera*, number of rhizoids per unit length of rhizome developed on specimens maintained in sections A through D of the growth apparatus. The vertical bar represents two standard errors on either side of the mean, which is indicated by the horizontal line.

significantly between intensities. Variety *lamourouxii* (Fig. 15) did show increased rhizoid length with increasing light intensity when tested by linear regression analysis ($P < .01$), although its number of rhizoids per unit length of rhizome did not vary significantly. Because of focus and contrast problems the photographic rhizoid

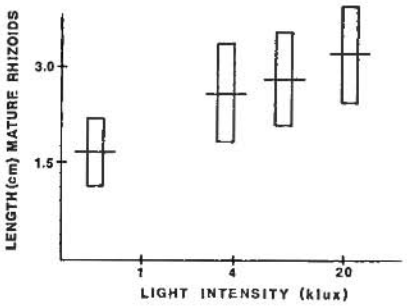

Fig. 15. Variety *lamourouxii*, length of mature rhizoids developed on specimens maintained in sections A through D of the growth apparatus. The vertical bar represents two standard errors on either side of the mean, which is indicated by the horizontal line.

data for var. *lamourouxii* were not included. Although not quantified, rhizoids seemed to develop more diffuse branching at the higher intensities.

Svedelius (1906), in separating the varieties *clavifera* and *uvifera* into distinct species, stated that var. *uvifera* has more highly developed rhizoids; however, I do not believe this difference is genetic. Light intensity and perhaps other environmental factors now seem a more likely explanation for varietal differences in rhizoid development.

Rhizome growth rate data showed no significant difference between varieties and no effect by light intensity over the range of intensities tested. Average rhizome growth rates for varieties *uvifera* and *lamourouxii* were 8.4 mm and 11.9 mm per day, respectively. Continued development of *C. racemosa* is dependent on rhizome growth. Perhaps other growth factors, including ramuli, assimilator and rhizoid development, are adjusted through some unknown equilibration process to ensure continued rhizome growth over a wide range of light intensities.

PRODUCTIVITY AND PIGMENTS

Net photosynthetic rate (Fig. 16) varied significantly between light intensities for both var. *uvifera* and var. *lamourouxii* f. *requienii* when tested by analysis of variance ($P < .001$). The net photosynthetic rate of var. *uvifera* dipped below the compensation point at approximately 1.6 klux whereas var. *lamourouxii* f. *requienii* required about half that intensity for compensation. At 3.7 klux var. *lamourouxii* f. *requienii* produced oxygen at three times the rate of var. *uvifera*. This gap narrowed with increasing light intensity and at 100 klux the rate for var. *uvifera* was slightly

higher. Net photosynthesis increased approximately with the $\log_{10}$ of light intensity through 100 klux for var. *uvifera* and to 50 klux for var. *lamourouxii* f. *requienii*. Similar relationships have been reported for other plants (Blackman and Wilson, 1951; Marsh, 1970). The decrease, though small, in net photosynthesis of var. *lamourouxii* f. *requienii* at the highest intensity is suggestive of the photosynthetic inhibition which occurs with phytoplankton at intensities beyond their "light saturation" value (Strickland, 1960).

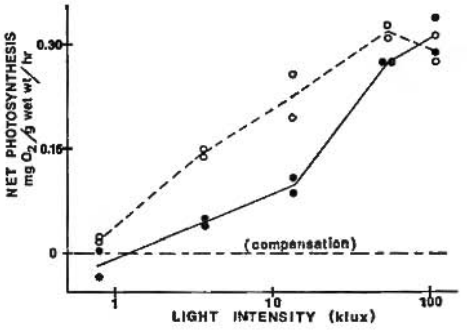

Fig. 16. Rates of net photosynthesis for var. *uvifera* (—●—) and var. *lamourouxii* f. *requienii* (—○—) at five light intensities. The point of zero oxygen exchange is the compensation intensity.

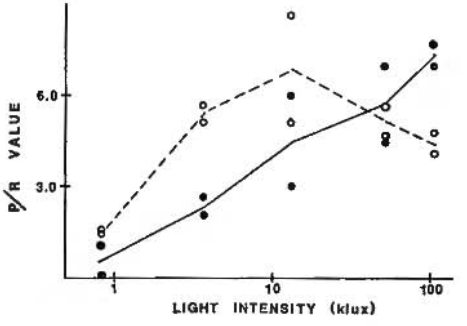

Fig. 17. P/R values (gross photosynthesis/respiration) for var. *uvifera* (—●—) and var. *lamourouxii* f. *requienii* (—○—) at five light intensities.

The P/R ratio (gross photosynthesis/respiration) of both varieties tested is plotted against light intensity in Fig. 17. This value rose steadily for var. *uvifera* and peaked at 100 klux, suggesting the adaptation of this form to high light intensities. The P/R value of var. *lamourouxii* f. *requienii* rose up to 13 klux and dropped at higher intensities, in this case suggesting adaptation to low light intensity.

Part of the reduction in the P/R value of var. *lamourouxii* f. *requienii* at 50 and 100 klux can be attributed to its higher respiration rates determined immediately after exposure to these intensities (Fig. 18). It is generally assumed that respiration remains stable under variable light conditions and that rapid increases in oxygen

utilization at high light intensities result from photo-oxidation accompanied by pigment bleaching (Kinne, 1970). Regardless of whether it is increased respiration or photo-oxidation, the results again suggest the adaptation of var. *lamourouxii* f. *requienii* to low light intensity. The respiration data for var. *uvifera* were relatively stable at all light intensities, indicating that this form is not significantly affected by photo-oxidation.

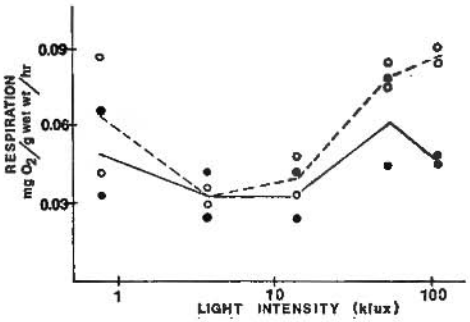

Fig. 18. Rates of respiration for var. *uvifera* (—●—) and var. *lamourouxii* f. *requienii* (--○--) measured after exposure to five light intensities.

Percent dry weight values of the varieties *uvifera* and *lamourouxii* were 4.8 and 5.5, respectively. The combined data gave a percent dry weight value of 5.2 for *C. racemosa*, similar to that reported for other *Caulerpa* species (Santos and Doty, 1971).

None of the pigment extracts tested at 750 millimicrons differed by more than 0.05 units from the optical density value of the 90% acetone standard. Most differed by a factor less than 0.01.

Chlorophyll a and carotenoid concentrations were found to be significantly higher ($P < .001$) for six specimens of var. *lamourouxii* than six specimens of var. *uvifera*, when analyzed by the t-test. Average chlorophyll a contents for var. *lamourouxii* and var. *uvifera* were 135.0 and 76.3 micrograms per gram wet weight, respectively. Corresponding carotenoid values were 43.8 and 24.1 MSPU per gram wet weight.

The results of 24 days of exposure to the light intensities within the growth apparatus on pigment contents are given in Fig. 19. The unusually high chlorophyll a value at 8.3 klux may have resulted from damage to the specimen or from an error in the weighing or pigment extraction technique. If that value is removed the decrease in chlorophyll a content shows a significant regression relationship with increasing light intensity ($P < .005$). The fact that chlorophyll a content varied with light intensity further indicates that characteristics which differ between *C. racemosa* varieties can result from environmental variables. The chlorophyll content of phytoplankton, both marine (Marshall, 1965) and freshwater (Sargent, 1940), and of higher terrestrial plants (Whittaker and Garfine, 1965; Murray and

Nichols, 1966) has also been demonstrated to decrease in environments exposed to higher light intensities.

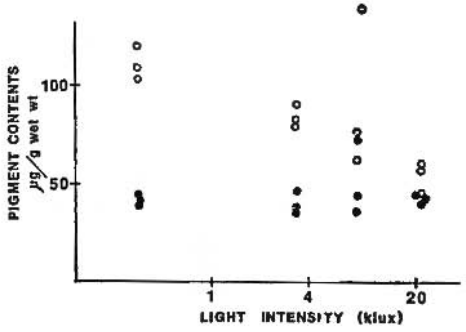

Fig. 19. Chlorophyll a (○) and carotenoid contents (●) of 12 specimens originally classified as var. *lamourouxii* after 24 days of exposure in sections A through D of the growth apparatus.

The larger chlorophyll a content of var. *lamourouxii* may be responsible for its higher photosynthetic rate at lower light intensities where photochemical processes are a limiting factor as opposed to enzymatic limitation at high light intensity (Steeman Nielsen, 1962). The lower chlorophyll a content of var. *uvifera* could be considered an adaptation to high light intensity. Its low level would allow increased transmission of light which, if excessively absorbed and converted to heat, could damage the internal water balance (Daubenmire, 1959) as well as result in photo-oxidation.

Although carotenoid content was significantly greater for var. *lamourouxii* than var. *uvifera*, this factor did not vary with light intensity (Fig. 19) after 24 days of exposure in the growth apparatus. Apparently factors other than light intensity control carotenoid content.

CONCLUSIONS

Caulerpa racemosa demonstrated a remarkable ability to change its growth form under altered light conditions. Growth factors for both var. *uvifera* and var. *lamourouxii* were shown to be related to the $\log_{10}$ of light intensity. The observation that net photosynthesis similarly increased with the $\log_{10}$ of light intensity, for var. *uvifera* to 100 klux and var. *lamourouxii* to 50 klux, suggests a close relationship. However, this similarity is not necessarily causal.

Growth forms such as var. *lamourouxii* f. *requienii* and var. *peltata* appear to develop as adaptations to low light intensity. Both are flattened and present a greater surface area in proportion to size than forms occupying more exposed habitats. For this reason they should be able to use available light more effectively. The lower compensation point, larger gross P/R value and higher net photosynthetic

rate at lower light intensities of var. *lamourouxii* f. *requienii* when compared to var. *uvifera* serve as evidence for such an adaptation. The observation that var. *uvifera* rose steadily in both rate of photosynthesis and P/R value as light intensity increased, whereas both of these factors dropped for var. *lamourouxii* f. *requienii* at the highest intensity, can also be considered an indication of its adaptation to high light intensity.

The increased chlorophyll a content of *C. racemosa* at low light intensity could be considered a means of adaptation for var. *lamourouxii* to occur in darkened habitats. An increased chlorophyll content would be able to absorb a greater percentage of the light available for photosynthesis. This would be especially beneficial at lower intensities where photosynthesis is limited by the rate of photochemical processes. The lower chlorophyll content of var. *uvifera* may also have an adaptive function. It allows increased transmission of light which, if absorbed and converted to heat, could damage the internal water balance or result in photo-oxidation.

Seasonality of *C. racemosa* within reef flat environments on Guam appears to be controlled by midday minus tides. The period of new development of *C. racemosa* corresponded with the cessation of midday minus tides and the period of maximum growth with their absence and low number. Minimum growth of *C. racemosa* occurred during months having the greatest number of midday minus tides.

The ability of *C. racemosa* to change growth form in altered light environments and the relationships of both morphologic and productivity factors to light intensity provide evidence for their environmental rather than genetic control. This information suggests that the classification of *C. racemosa* varieties as separate species, such as *C. peltata* (Gilbert, 1942; Taylor, 1960) and *C. lamourouxii* (Santos and Doty, 1971), is indeed in error and that these growth forms would more properly be referred to as ecophenes (ecological phenotypes) of the single species *C. racemosa*.

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